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ALLEN'S
COMMERCIAL ORGANIC ANALYSIS

VOLUME VI

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ALLEN'S COMMERCIAL

ORGANIC ANALYSIS

A TREATISE ON

THE PROPERTIES, MODES OF ASSAYING, AND PROXIMATE ANALYTICAL EXAMINATION OF THE VARIOUS ORGANIC CHEMICALS AND PRODUCTS EMPLOYED IN THE ARTS, MANUFACTURES, MEDICINE, Etc.

WITH CONCISE METHODS FOR

THE DETECTION AND ESTIMATION OF THEIR IMPURITIES, ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

VOLUME VI

Amines and Ammonium Bases, Aniline and its Allies, Naphthylamines, Pyridine, Quinoline and Acridine Bases, Vegetable Alkaloids, Volatile Bases of Vegetable Origin, Nicotine and Tobacco, Aconite Alkaloids, Atropine and its Allies, Cocaine, Opium, Strychnos Alkaloids, Cinchona Alkaloids, Berberine, Caffeine, Tea and Coffee, Cocoa and Chocolate

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LONDON

J. & A. CHURCHILL

7 GREAT MARLBOROUGH STREET

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PREFACE.

It is now nearly twenty years since the last edition of this volume was issued. During this period, the chemistry of the organic bases and alkaloids has been greatly extended so that very far-reaching alterations have been necessary in the course of revision of this volume. As far as possible, however, the original arrangement of subject-matter has been retained, although considerable changes of order have in some instances been made, as, for example, in dealing with the cinchona alkaloids. It has, too, been found advisable to split up the section on *Caffeine and Its Allies* in the last edition into two separate portions, so as to deal with tea and coffee in one section and cocoa and chocolate in another.

As in the last edition, it has not been found possible to include all the principal vegetable alkaloids in one volume; those dealt with in Vol. III, part iii, of the last edition will be found in Vol. VII of the new edition.

CONTENTS.

AMINES AND AMMONIUM BASES.

W. A. DAVIS.

Separation of Amines from Tetralkylammonium Salts, 3; Separation of Primary, Secondary and Tertiary Amines, 4; Generic Character of Monamines, 10; Methyl Amines, 12; Hydrazines, 23; Azoimide, 127; Hydrazines, 28; Phenyl hydrazine, 30; Pyrazolenes, 35; Antipyrine, 36.

ANILINE AND ITS ALLIES.

S. S. SADTLER.

Aniline, 51; Aniline Sulphonic Acids, 61; Nitraniline, 62; Toluidines, 63; Xylidines, 71; Aniline Oils, 74; Acetanilide, 83; Benzanilide and Phenylmethane, 87; Substituted Anilines, 88; Aminophenols, 97; Phenacetin, 99; Diaminophenols, 105; Phenylenediamines, 105; Benzidine, 108.

NAPHTHYLAMINES, PYRIDINE, QUINOLINE AND ACRIDINE BASES.

W. H. GLOVER.

Naphthylamines, 112; Naphthylene Diamines, 117; Sulphonic Acids of Naphthylamines, 117; Aminonaphthols, 125; Aminonaphtholsulphonic Acids, 126; Pyridine Bases, 128; Pyridine, 131; Picolines, 142; Dimethylpyridines, 143; Pyridinecarboxylic Acids, 145; Pyrrole, 148; Methylpyrroles, 149; Quinoline and Allies, 150; Kairines, 157; Thalline, 157; Quinazolines, 160; Acridine, 162; Phenanthridine, 165.

VEGETABLE ALKALOIDS.

THOMAS A. HENRY.

General, 167; Isolation and Purification of Alkaloids, 171; Estimation of Total Alkaloids, 178; Properties, 183; Reactions, 185; Classification, 206.

VOLATILE BASES OF VEGETABLE ORIGIN.

FRANK O. TAYLOR.

Areca or Betel Nut Alkaloids, Coniine, 211; Assay of Hemlock, 221; Lobeline, 223; Lupine Alkaloids, 225; Piturine, 229; Pomegranate Alkaloids, 230; Sparteine, 232; Spigeline, 235.

NICOTINE AND TOBACCO.

R. W. TONKIN.

Nicotine, 237; Poisoning by Nicotine, 241; Tobacco, 242; Insecticides, and Germicides, 250.

ACONITE ALKALOIDS.

FRANCIS H. CARR.

Constitution and Character of the Aconite Bases, 256; Aconitine, 258; Derivatives of Aconitine, 262; Benzoaconitine, 263; Pyraconitine, 265; Japaconitine, 266; Indaconitine, 268; Lycaconitine and Myoctonine, 273; 168; Lapaconite, Sepentrionaline and Cynoctonine, 277; Assay of Aconite Root and its Preparations, 279; Toxicology of Aconite, 283; Pharmacology of Aconite, 287.

ATROPINE AND ITS ALLIES.

FRANCIS H. CARR.

Tropic Acid and Analogues, 292; Atropine, 294; Hyoscyamine, 297; Hyoscine, 299; Artificial Tropeines, 301; Detection and Estimation of Tropeines, 303; Plants Yielding Mydriatic Alkaloids, 311; Hyoscyamus, 316; Datura, 319.

COCAINE.

SAMUEL P. SADTLER.

Cocaine, 321; Estimation, 328; Decomposition Products of Cocaine, 336; Bases Allied to Cocaine, 338; Amorphous Bases of Coca, 341; Coca Leaves, 344.

OPIUM ALKALOIDS.

FRANK O. TAYLOR.

Opium Bases, 354; Action of Solvents, 362; Colour Reactions, 366; Estimation and Separation of Bases, 370; Morphine, 374; Detec-

tion and Estimation of Morphine, 379; Apomorphine, 387; Codeine, 390; Basic Associates of Morphine, 395; Opium, 407; Meconates, 414; Action of Solvents, 414; Assay of Opium, 416; Toxicology of Opium and Morphine, 434.

STRYCHNOS ALKALOIDS.

CHARLES E. VANDERKLEED.

Strychnine, 441; Analytical Reactions, 444; Toxicology of Strychnine, 455; Preparations, 459; Bruceine, 464; Nux Vomica, 467; Curare, 474;

CINCHONA ALKALOIDS.

OLIVER CHICK.

Cinchona Barks, 479; Composition of Barks, 481; Assay of Barks, 486; Separation of Cinchona Bases, 490; Cinchona Alkaloids, 498; Constitution of Alkaloids, 501; Properties of Cinchona Bases, 503; Quinine, 507; Detection and Estimation of Quinine, 510; Quinine Preparations, 531; Amorphous Cinchona Bases, 543; Alkaloids of Remijia Barks, 546; Cupreine, 548.

BERBERINE AND ITS ASSOCIATES.

EDWARD HORTON.

Berberine, 551; Hydrastine, 564; Alkaloidal Associates of Berberine, 574.

CAFFEINE, TEA AND COFFEE.

J. J. FOX AND P. J. SAGEMAN.

Caffeine, 579; Theobromine, 591; Diuretin, 594; Tea, 597; Analysis of Tea, 602; Estimation of Caffeine, 606; Adulterations of Tea, 629; Lie Tea, 639; Mate, 641; Coffee, 642; Caffetannic Acid, 645; Analysis of Coffee and Mixtures, 651; Caffeine in Coffee, 656; Chicory, 674; Kola, 680; Guarana, 683.

COCOA AND CHOCOLATE.

R. WHYMPER.

Cocas, 685; Chocolate, 693; Milk Chocolate, etc., 695; Cocoa Butter, 701; Analysis of Cocoa and Chocolate, 705.

AMINES AND AMMONIUM BASES.

By W. A. DAVIS.

The amines are substituted ammonias of the type

NH_2R	NHR_1R_2	$NR_1R_2R_3$
Primary.	Secondary.	Tertiary.

derived from ammonia by the replacement of its hydrogen atoms by alkyl groups, *R*. When more than 1 hydrogen atom is replaced, the alkyl groups introduced may be identical, as methyl for example, in trimethylamine, NMe_3, or different, as in methyl-ethyl-propylamine, NMeEtPr. In the latter case the amines are known as *mixed* amines.

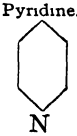
From the commercial point of view the most important amines are those of the aromatic series, such as aniline, naphthylamine, etc. It is usual, however, to distinguish these from the aliphatic amines, from which they differ in certain important respects, by giving them the name of *aromatic amino-compounds*, the true aromatic amines being substances, such as benzylamine, $NH_2.CH_2.C_6H_5$, which bear a much closer relationship to the aliphatic amines than the coal-tar bases, of which aniline is the most important representative.

Diamines are derived from hydrocarbons by replacing 2 hydrogen atoms by 2 amino-groups, *e. g.*, *ethylene-diamine*, $NH_2.CH_2.CH_2.NH_2$; *phenylene-diamine*, $NH_2.C_6H_4.NH_2$; *tetramethylene-diamine* (*putrescine*), $NH_2.CH_2.CH_2.CH_2.CH_2.NH_2$.

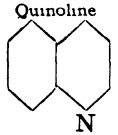
Triamines or *triamin-compounds*, *tetramines* or *tetramino-compounds* are also known but are relatively unimportant.

Ring nitrogen compounds such as pyridine and quinoline have the

Pyridine.

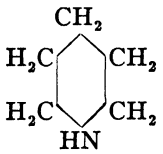


Quinoline



properties of tertiary bases. The reduced pyridine, hexahydropyridine

or *piperidine*,



is a secondary base; coniine and sarcosine (methylglycine) are also secondary bases.

When one hydrogen atom of ammonia is replaced by an acid radical such as acetyl or benzoyl, an *amide* is obtained, *e. g.*, acetamide, $CH_3.CO.NH_2$. Mixed compounds such as methylacetamide, $NHMe.CO.CH_3$, and acetanilide, $NHPh.CO.CH_3$, are formed by the replacement of one hydrogen atom of ammonia by a hydrocarbon radical and one by an acid radical. Urea or carbamide is the diamide of carbonic acid, and has the constitution $NH_2.CO.NH_2$. Guanidine (Vol. 7) is the corresponding *imine*, $NH:C(NH_2)_2$.

Of these various compounds the monamines may with advantage be considered at the present stage, but the majority of the amines will be dealt with in other sections.

MONAMINES.

These bases are derived from 1 molecule of ammonia by the substitution of 1 or more of the hydrogen atoms by an equivalent number of alkyl radicals. The first substance obtained of this class was ethylamine, $C_2H_5.NH_2$, prepared by Wurtz in 1848 by distilling ethyl cyanurate with potassium hydroxide. Methylamine, $CH_3.NH_2$, was obtained by the same chemist in the following year, by the distillation of methyl isocyanate with alkali hydroxide: $2KOH + CH_3.N.CO = K_2CO_3 + CH_3.NH_2$.

Hofmann obtained the monamines by the action of an alkyl iodide on an alcoholic solution of ammonia. The action is not a simple one, all three monamines being formed together with a tetra-alkyl-ammonium base. Thus, when ethyl iodide is heated with alcoholic ammonia to 100° in a sealed tube, there are obtained:

Hydriodide of ammonia,	$H_3N,HI.$
Hydriodide of ethylamine,	$(C_2H_5)H_2N,HI.$
Hydriodide of diethylamine,	$(C_2H_5)_2NH,HI.$
Hydriodide of triethylamine,	$(C_2H_5)_3N,HI.$
Tetra-ethyl-ammonium iodide,	$(C_2H_5)_3N,C_2H_5I.$

Similar products result when bromide or chloride of ethyl is substi-

tuted for the iodide, except as to the relative proportions of the amines obtained. Thus chloride of ethyl produces almost exclusively $EtNH_2$,-HCl, with small quantities of Et_2NH,HCl and NEt_3,EtCl; ethyl bromide gives chiefly $EtNH_2$,HBr with very appreciable quantities of $NHEt_2$HBr and NEt_3,HBr, but very little NEt_4Br; while ethyl iodide produces NH_2Et,HI, $NHEt_2$,HI, and NEt_3,HI in about equal proportions, as well as very appreciable quantities of Et_4NI (Groves, *J. Chem. Soc.*, **13**, 331).

A similar series of products is obtained by heating iodide, bromide, or nitrate of methyl with a solution of ammonia in methyl alcohol. When the methyl nitrate and ammonia solution are used in equivalent proportions for the action—$MeNO_3 + H_3N = NH_2Me,HNO_3$, methyl-amine is the chief product, though more or less of each of the more highly substituted products is also formed. With excess of methyl nitrate, tetramethyl-ammonium nitrate, $Me_4N.NO_3$, is produced in large excess, and the same quartary compound is formed if methyl bromide or iodide be substituted for the nitrate.

The complex nature of the products obtained by treating alkyl iodides, etc., with alcoholic ammonia is due to the tendency of the amines first produced to act on the remaining portions of the alkyl iodide or other salt to form ammonium iodide and more highly substituted amines. The hydriodides of the amines similarly react with alkyl iodides in presence of ammonia to form ammonium iodide and more highly substituted amines.

From these reactions it follows that the hydriodide of diethylamine, for instance, may be obtained by heating ethyl bromide or iodide with a calculated amount of ethylamine in a sealed tube. A great variety of mixed amines may be obtained by precisely similar means.

Separation of Amines from Tetralkylammonium Salts.

In the preparation of an amine a mixture of the salts of primary, secondary and tertiary bases is obtained together with the quartary or quaternary ammonium salt. The product of the action is filtered from ammonium iodide, which is nearly insoluble in the alcoholic liquid, and is evaporated to dryness to get rid of excess of alcohol, free ammonia, and unchanged alkyl iodide. The residue is then distilled with potassium hydroxide, when the hydriodides of the amines are decomposed, the bases volatilising, while the tetra-alkyl ammonium iodide remains in the retort unchanged by, and insoluble in, the

strong potassium hydroxide solution. The mixture of amines is conducted over calcium hydroxide, and then condensed by passage through a well-cooled tube.

Detection and Separation of Primary, Secondary and Tertiary Amines.

1. **Hinsberg's Method.**—Probably the most useful method of separating or distinguishing the three classes of amines is that of Hinsberg (*Ber.*, 1890, **23**, 2963, and *Annalen.*, 1891, **265**, 178). It depends on the fact that whereas tertiary amines are not affected by aromatic sulphochlorides, such as benzenesulphochloride, primary and secondary amines yield sulphonamides, *e. g.*, $C_6H_5.SO_2.NHR$ and $C_6H_5.SO_2.NR_1R_2$, when shaken with the sulphochloride in presence of alkali. The sulphonamides of the first type, moreover, differ from those of the type $C_6H_5.SO_2.NR_1R_2$, in forming alkali salts which are soluble in water. After the action of the sulphochloride is completed the excess of alkali is nearly neutralised and the product subjected to steam distillation which generally serves to remove the trtiary base. The sulphonamide of the primary base can then be separated from that of the secondary base by taking advantage of its solubility in aqueous alkali, the sulphonamide of the secondary amine remaining undissolved. The amines are regenerated from their sulphonamides, after separation, by heating the latter with hydrochloric or sulphuric acid in a sealed tube at 130–150° (Hinsberg); or by warming with chlorosulphonic acid, SO_3HCl, in an open vessel at 130–150°, followed by boiling with dilute alkali (Marckwald and von Droste-Huelshoff, *Ber.*, 1898, **31**, 3261). In some cases the action is not strictly normal, alkali-insoluble dibenzene-sulphonyl compounds, $RN(SO_2.C_6H_5)_2$, being formed from the primary amine along with the normal alkali-soluble monobenzenesulphonyl derivative, thus causing confusion. In the case of primary amines with more than 6 carbon atoms, as well as with certain amino-compounds of the terpene series, the sodium compounds of the true monobenzenesulphonamides are easily hydrolysed by water and insoluble in an excess of alkali, and a false conclusion as to the presence of secondary bases may thus be arrived at. According to Hinsberg and Kessler (*Ber.*, 1905, **38**, 906), however, these difficulties may be overcome by the following procedure: 1. The abnormal dibenzene-sulphonyl compounds can be hydrolysed to the normal alkali-soluble forms by warming with sodium ethoxide dissolved

in alcohol. 2. The abnormal insoluble monobenzenesulphonamides can be converted by sodium in ethereal solution into sodium salts which are *insoluble* in ether, while the sulphonamide-derivatives of secondary bases are without exception soluble in ether and unaffected by sodium.

2. **Acetylation.**—Primary and secondary amines are readily attacked, generally at the ordinary temperature, more rapidly on warming, by acetyl chloride or acetic anhydride (a sufficiency to wet the compound thoroughly, generally 1 1/2 to 5 times the theoretical quantity required for acetylation). Primary bases form acetyl derivatives of the type RNHAc, and secondary bases compounds of the type R_1R_2.NAc. Tertiary bases do not form acetyl derivatives, but, when treated with acetyl chloride or acetic anhydride and subsequently with water, dissolve in the form of chloride or acetate respectively. Thus, on acetylating a mixture of a primary, secondary and tertiary amine, as the acetyl derivatives formed from the first two are as a rule sparingly soluble in water, on diluting the reaction product with a large volume of water, the tertiary base dissolves and leaves the acetyl derivatives undissolved; on adding alkali hydroxide to the solution of the soluble salt of the tertiary amine, the free base is separated and can be suitably dealt with. The mixture of acetyl compounds is hydrolysed by heating with concentrated hydrochloric acid and the secondary base separated from the primary amine in the form of nitrosamine (see page 8) The primary base is converted by this treatment into the corresponding alcohol.

According to Menschutkin (*Chem. Soc. Abstr.*, 1900, *i*, 335) the different rate of acetylation of primary and secondary amines can be used as a means of distinguishing these bases. The amine is sealed in a glass tube with 1 equivalent of acetic acid and heated in a bath of nitrobenzene during 30 minutes, after which it is quickly cooled, the tube broken and the contents mixed with a few c c. of 96% alcohol and titrated with alcoholic potassium hydroxide using phenolphthalein as indicator. Primary amines are as a rule acetylated under these conditions to the extent of 87.5 to 97.5%, and secondary amines to the extent of only 40 to 50%. As the rule given is empirical it is liable to variation with alterations of structure, etc., and the method should be used therefore only with caution.

3. **Hofmann's Method.**—*a*. If an amine be heated to 100°, under pressure, with an excess of alkyl iodide, a quaternary iodide will at length be formed, and the problem whether the original base was a

primary, secondary, or tertiary amine will be solved by comparing the composition of the ultimate product with that of the original base or its hydriodide. Thus, if methyl iodide has been the alkylating agent employed, the iodide ultimately obtained will differ from the hydriodide of the original base by $3CH_2$ if the amine was primary; by $2CH_2$ if secondary, and by CH_2 if tertiary.

b. The following is an outline of the method devised by Hofmann for the separation of mixed ethylamines.

The bases are treated in a flask with one and a half times their weight of ethyl oxalate (previously dried over calcium chloride), which is added gradually through a tap funnel. This has no action on triethylamine or other tertiary bases, but converts diethylamine into liquid ethyl diethyloxamate, and ethylamine into solid diethyloxamide,[1] according to the following equations:

1. $$\begin{matrix} CO_2Et \\ | \\ CO_2.Et \end{matrix} + 2EtNH_2 = \begin{matrix} CO.NHEt \\ | \\ CO.NHEt \end{matrix} + 2EtOH$$

Ethyl oxalate. Ethylamine. Diethyloxamide. Alcohol.

2. $$\begin{matrix} CO.OEt \\ | \\ CO.OEt \end{matrix} + Et_2NH = \begin{matrix} CO.OEt \\ | \\ CO.NEt_2 \end{matrix} + EtOH$$

Ethyl oxalate. Diethyl-amine. Ethyl diethyl-oxamate. Alcohol.

The liquid gets very hot, but for the completion of the action the mixture should be heated to 100° for several days in a closed vessel. The triethylamine, which has taken no part in the change, is then distilled off on the water-bath. The residue is well cooled, and the solid oxamide separated from the liquid oxamate by pressure.[2] On subsequent distillation with potassium hydroxide these compounds yield the primary and secondary amines respectively:

1. $C_2O_2(NH.Et)_2 + 2H.(OK) = C_2O_2(OK)_2 + 2H(NH.Et)$

2. $$\begin{matrix} CO.OEt \\ | \\ CO.NEt_2 \end{matrix} + 2HOK = \begin{matrix} CO.OK \\ | \\ CO.OK \end{matrix} + NHEt_2 + HOEt.$$

The foregoing process, with certain modifications in detail, is of

[1] Diethyloxamide may also be separated from the ethyl diethyloxamate by cold water in which the former dissolves easily, the latter very sparingly. If hot water be used, the separation is more perfect and the residual oxamate quite pure, but some of it suffers hydrolysis and goes into solution as diethyloxamic acid.

[2] Some ethyl monoethyloxamate, $C_2O_2\begin{cases} O\,C_2H_5 \\ NH\,C_2H_5 \end{cases}$, is always formed from the primary amines in this reaction.

general application for the separation of primary, secondary, and tertiary amines; the first class forming oxamides, the second oxamic esters, and the third being unacted on.

An important modification in the foregoing method has been made by Duvillier and Buisine (*Ann. Chim. Phys.* [*v*], **23**, 289), who operated on an aqueous solution of the bases. Under these conditions, the primary amines are converted by ethyl oxalate into insoluble or sparingly soluble oxamides, while the secondary and tertiary bases are unchanged, or at any rate remain wholly in solution. After separating the oxamides by filtration, the mother-liquor[1] [is boiled for some time, which causes the hydrolysis of the ethyl diethyloxamate with formation of diethyloxamic acid, $\begin{vmatrix} \text{CO.OH} \\ \text{CO.NEt}_2 \end{vmatrix}$, and the further change of this into the acid oxalate of diethylamine, $(C_2H_5)_2HN.H_2C_2O_4$. This salt separates on cooling, and yields the free base on distillation with alkali. The filtrate] is distilled with potassium hydroxide, the bases dried by potassium hydroxide, and dissolved in absolute alcohol. On adding ethyl oxalate to this solution the secondary amines are converted into oxamic esters, while any remaining primary amines are converted into the corresponding oxamides. After allowing the mixture to stand for 24 hours to complete the action, the alcohol and unchanged *tertiary bases* are distilled off on the water-blath. The oxamates remaining in the retort may be converted into calcium salts by treatment with milk of lime, or the *secondary bases* at once liberated and recovered by distillation with potassium hydroxide.[2] Duvillier and Buisine have applied this method to the analysis of the complex mixture of amines present in commercial trimethylamine from *vinasses* (page 15). A. Muller (*Bull. Soc. Chim.*, 1884, **42**, 202) has described a method for the separation of amines based on much the same principle.

4. **Delépine's Method** (*Compt. Rend.*, 1896, **122**, 1064, 1272; *Ann. Chim. Phys.*, 1896 [*vii*], **8**, 1439).—Formaldehyde condenses in alka-

[1] The treatment described in the brackets is optional, and chiefly of advantage in the separation of ethylamines

[2] The conversion into calcium salts is especially suitable for the treatment of the ethylamines. The precipitated calcium diethyloxamate and monoethyloxamate are filtered off, and the filtrate treated with alcohol, which precipitates the remainder of the calcium salts. The precipitates are treated with boiling water, when the monoethyloxamate dissolves, and is deposited again on cooling in large crystals, which on distillation with potassium hydroxide yield *ethylamine* On concentrating and cooling the mother-liquors, calcium diethyloxamate separates It is recrystallised from alcohol, washed with ether to remove any adhering oxamide, and distilled with potassium hydroxide, when it yields pure *diethylamine*.

line solution with primary amines giving polymeric methylene derivatives of the type $(CH_2{:}NR)_x$ or $OH.CH_2.NR_1R_2$. No action occurs with tertiary amines. The methylene compounds can be separated by fractional distillation and then decomposed into their components by alcoholic hydrogen chloride. The process has been suggested as a means of separating the methylamines.

The primary, secondary, and tertiary monamines may also be distinguished by the following.

5. If a primary monamine be boiled with alcoholic potassium hydroxide and chloroform, the characteristic and highly disagreeable odour of the corresponding carbamine or isonitrile is evolved, according to the equation: $MeNH_2 + CHCl_3 + 3KHO = MeNC + 3H_2O + 3KCl$.

6. If a primary monamine be dissolved in a mixture of equal volumes of alcohol and carbon disulphide, and the liquid then boiled down to one-half, a thiocarbamate will be formed thus: $2MeNH_2 + CS_2 = MeNH.CS.S.HNH_2Me$.

If the resultant liquid be boiled with a solution of mercuric or ferric chloride, a pungent odour of mustard oil will be produced owing to the formation of an alkyl iso-thiocyanate:[1]

$$MeNH.CS.S.NMeH_3 + HgCl_2 = HgS + MeNCS + NMeH_3Cl + HCl.$$

Secondary amines combine with carbon disulphide under the same conditions but give alkylthiocarbamic acids which are not convertible into a "mustard-oil."

$$CS_2 + 2NHEt_2 = NEt_2.CS.SH,NHEt_2.$$

7. Nitrous acid converts *primary fatty monamines* into the corresponding alcohols: $MeH_2N + NO.OH = Me.OH + OH_2 + N_2$.

Aromatic amino-compounds (*e. g.*, aniline) are converted by nitrous acid into diazo-compounds. $PhNH_2 + NO.OH + HCl = Ph.N{:}N.Cl + 2H_2O$, which on boiling with water yield phenols.

Secondary amines, whether fatty or aromatic, are converted by nitrous acid into nitrosamines, thus: $Me_2NH + NO.OH = Me_2N.NO + H_2O$. The nitrosamines are yellow liquids, of neutral character and aromatic odour, volatile without decomposition in a current of steam. Weak reducing agents (zinc dust and acetic acid) convert them into hydrazines; but by more powerful hydrogenising agents, or by warming with alcohol and hydrochloric acid, they are reconverted into the original secondary amines.

[1] In the case of aromatic primary amines, the product is usually a thio-urea, which requires to be treated with phosphorus pentoxide to obtain the iso-thiocyanate.

These nitrosamines give an intensely blue or bluish-violet colouration when they are warmed with phenol and concentrated sulphuric acid and the mixture diluted with water (*Liebermann's nitroso-reaction*).

Nitrous acid has no action on *tertiary fatty amines*. It converts most *tertiary aromatic amines* into nitroso-derivatives which contain the nitroso-group in the benzene nucleus.

In practice, the action of nitrous acid on the amines is best effected by distilling their hydrochlorides with a strong solution of potassium or sodium nitrite after adding the necessary quantity of hydrochloric acid. If a mixture of the 3 methylamines be thus treated, the *methylamine* is destroyed (with formation of methyl alcohol, which will be found in the distillate), *dimethylamine* is converted into dimethyl-nitrosamine, which distils,[1] while the hydrochloride of *trimethylamine* remains in the retort and on distilling it with alkali hydroxide the free base can be obtained.

This method, however, loses some of its quantitative value owing to the fact that a portion of the tertiary amine may undergo conversion into the secondary amine by an alkyl group being split off in the form of aldehyde (Bannow, Meyer-Jacobson's *Lehrbuch*, 1906, 345); the amount of nitrosamine is thus increased.

8. Both *primary* and *secondary monamines* react with aldehydes to form neutral compounds. The action between œnanthal and mono- and di-methylamine respectively is as follows:

$$H_2.N.CH_3 + C_6H_{13}.CHO = H_2O + C_6H_{13}.CH\,NCH_3; \text{ and}$$
$$2HN(CH_3)_2 + C_6H_{13}.CHO = H_2O + C_6H_{13}.CH[N(CH_3)_2]_2.$$

This reaction has been utilised by Schiff (*Annalen*, 1871, **159**, 158) for the volumetric assay of amines. The base is dissolved in benzene, fused calcium chloride added, and then a standard solution of œnanthal in benzene dropped in from a burette so long as water continues to separate. Each addition of the œnanthal solution produces a turbidity from separation of water, but this is absorbed by the calcium chloride on gentle agitation. As a primary amine reacts with twice as much œnanthal as the corresponding secondary amine, the proportions of the two in a mixture can be estimated from the result of the titration, provided the mean combining weight of the mixture be known, or ascertained in a separate experiment by titration with standard acid.

[1] On separating the nitrosamine, which forms a yellow oil, from the aqueous distillate, treating it with aqueous hydrochloric acid, and then passing hydrochloric acid gas till the liquid is homogeneous, the hydrochloride of the secondary amine is formed, and may be obtained by evaporation of the solution.

9. The acid ferrocyanides of the *tertiary amines* are remarkably insoluble in water. They are precipitated on adding potassium ferrocyanide to the solutions of the amines acidified with hydrochloric acid. The bases can be recovered from their ferrocyanides by treating the precipitate with solution of cupric sulphate, filtering, and removing the sulphuric acid and excess of copper from the filtrate by barium hydroxide. (Fischer, *Annalen*, 1878, **190**, 185; Chrétien, *Compt. Rend.*, 1902, **135**, 901.)

Generic Characters of Monamines.

The monamines, as a class, are readily volatile liquids, of lower sp. gr. than water. Their b. p. rise with the number of carbon atoms in the molecule. The lower members dissolve with great facility in water, forming strongly alkaline liquids of an ammoniacal odour. The higher members are, however, without odour and do not dissolve in water. From their solutions, ethylamine and the higher homologues can be separated by saturating the liquid with potassium hydroxide. By boiling the aqueous solutions of the free bases, or of their salts after adding excess of lime or alkali hydroxide, the monamines can be completely volatilised, and condensed again in water or acid, and titrated in the same manner as ammonia. The monamines are all powerful bases, closely resembling ammonia in their general characters. They are, however, distinguished from ammonia by their inflammability, a fact which led to their discovery; they burn with a yellow flame. They form crystallisable salts and well defined double-salts, such as the *aurichlorides* and *platinichlorides*, which are very useful for their identification and analysis; the *picrates* are also generally well defined. The monamines precipitate magnesium salts, but the precipitated magnesium hydroxide dissolves in the amine hydrochloride, forming a double salt from the solution of which phosphate of sodium precipitates an amino-magnesium phosphate. The amines thus behave exactly in the same manner as ammonia.

The only amines (not described in other chapters) requiring detailed consideration are the primary, secondary, and tertiary monamines of methyl and ethyl. These substances are typical of the amines generally, and most of the statements made respecting them would be true of all the compounds of this class. Their aqueous solutions dissolve silver chloride, and behave in much the same manner as ammonia with metallic salts; but there are some interesting differences, as shown in

the table below, from which is will be seen that certain of the precipitates which are soluble in excess of ammonia are undissolved by the amines, and *vice versa*.[1]

Metallic salt	Ammonia H_3N	Ethylamine $(C_2H_5)H_2N$	Methylamine $(CH_3)H_2N$	Dimethylamine $(CH_3)_2HN$	Trimethylamine $(CH_3)_3N$
Aluminium	Insoluble (nearly)	Soluble	Soluble	Soluble	Soluble
Cobalt	Blue precipitate, soluble in excess to brown solution	Insoluble	Blue, insoluble in excess, turned brownish on heating	Blue, insoluble in excess, turned brownish on heating	Blue; insoluble in excess; turned brownish on heating
Nickel	Soluble in excess to violet-blue solution	Insoluble	Apple-green, insoluble in excess	Apple-green, insoluble in excess	Apple-green, insoluble in excess
Zinc	Very soluble	Soluble	Soluble in large excess, reppted on heating	Soluble in large excess, reppted on heating	Soluble in very large excess, reppted on heating
Cadmium	Soluble	Insoluble	Insoluble	Insoluble	Insoluble
Silver	Brownish, very soluble in excess.		Brownish, soluble in large excess, reppted on warming	Brownish, soluble in large excess, reppted on warming	Dirty brown ppt changing to black, sol large excess to dark solution, reppted on warming
Cupric	Blue; soluble in excess to deep blue solution	Soluble with difficulty in excess	Blue, soluble in large excess to deep blue solution, reppted dirty brown on boiling	Blue, partly soluble in large excess, reppted dirty brown on boiling	Blue, partly soluble in large excess, reppted dirty brown on boiling
Mercuric	White		White, insoluble	White; soluble in much water	Yellow, changing to very pale yellow
Stannic	Insoluble	Very soluble in excess		Soluble	Soluble
Antimonic				Soluble	Soluble in large excess.
Gold	Insoluble	Soluble	Brownish yellow ppt, readily soluble in excess to orange-red liquid	Yellow precipitate, soluble in excess to brown liquid	
Ruthenium	Insoluble	Soluble			
Lead	Insoluble		Insoluble	Insoluble	Insoluble

[1] Allen was indebted to Leo Taylor for repeating and enlarging on the experiments of Vincent, on whose observations the table is chiefly founded. Several blanks in the observations of Vincent have been filled by Taylor.

In all cases a solution of aluminium phosphate in hydrochloric acid behaves similarly to a solution of aluminium chloride (Taylor).

PHYSICAL PROPERTIES OF AMINES.

The following table is taken from Meyer-Jacobson's *Lehrbuch* (1906).

Alkyl radical R	Primary amine NH_2R			Secondary amine NHR_2		Tertiary amine NR_3	
	M p	B p	Sp gr	B p	Sp gr	B p	Sp gr
Methyl		−6 7°	o 699(−11°)	+7°	o 686(−6°)	+3 5°	o 662(−5°)
Ethyl	−83 8°	+19	o 708(−2°)	56°	o 711(+15°)	90°	o 735(+15°)
Propyl		49°	o 728(0°)	110°	o 738(20°)	156°	o 771(0°)
iso-Propyl		32°	o.690(18°)	84°	o 724(15°)		
n-Butyl		77 8°	o 742(15°)	160°		216 5°	o 791(0°)
iso-Butyl		66°	o 735(15°)	136°		187°	o 785(21°)
sec-Butyl		63°	o 718(20°)				
tert-Butyl		43 8°	o 698(15°)				
n-Amyl		104°	o 766(19°)				
iso-Amyl		95°	o 750(18°)	187°	o 782(0°)	235°	
tert-Butyl-methyl		82–83°					
sec-*n*-Amyl (Methyl-n-propyl-carbinamine)		92°	o 738(20°)				
sec-*n*-Amyl (Diethylcarbinamine)		90–91°	o 749(20°)				
Methyl *iso*-propyl carbinamine		83–84°	o 757(18 5)°				
tert-Amyl (Dimethyl-ethyl carbinamine)		78 5°	o 748(15°)				
n-Hexyl		129°				260°	
n-Heptyl		153°	o 777(20°)				
n-Octyl		175–177°	o 777(26 8°)	297°		366°	
n-Nonyl		190–192°					
n-Decyl	+17	216–218°					

Methylamine, $NH_2.CH_3$.

Methylamine exists ready-formed in *Mercurialis annua* and *M. perennis*, and, as obtained (in an impure state) from these plants, was formerly known as mercurialine. It also exists in herring-brine, coal

tar, bone-oil, and the products of the distillation of wood, beetroot molasses (*vinasses*), and certain alkaloids (*e. g.*, morphine, codeine). It is also produced when caffeine is boiled with baryta-water, and by heating trimethylamine hydrochloride to 285°, when methyl chloride and trimethylamine volatilise, and methylamine hydrochloride (mixed with some ammonium chloride) remains. Methylamine is best obtained pure by treating one equivalent of acetamide with 2 equivalents of bromine, and then adding a 10% solution of potassium hydroxide till the colour of the bromine has nearly disappeared:

$$C_2H_3O.NH_2 + Br_2 + 2KHO = C_2H_3O.N.BrK + KBr + 2H_2O.$$

Three additional equivalents of potassium hydroxide are now dissolved to a 10% solution, and heated in a retort to 70°. The product of the first action is then gradually added through the tubulure. The gases evolved are collected in hydrochloric acid, and on evaporating the solution a mixture of the hydrochlorides of ammonia and methylamine is obtained,[1] from which the latter only is dissolved by absolute alcohol.[2] On distillation with alkali hydroxide or slaked lime the salt yields the base, quite free from di- or tri-methylamine.

Methylamine boils at —6.7°, and hence is a gas at ordinary temperature. 1 volume of water at 12.5° dissolves 1,150 volumes of the gas, and hence it is more soluble even than ammonia, which methylamine closely resembles in odour and general characters; methylamine is distinguished by its ready inflammability—a property even possessed by its concentrated aqueous solution. It burns with a yellow flame, forming carbon dioxide water, nitrogen, and hydrocyanic acid.

On passing a succession of electric sparks through methylamine, hydrocyanide of methylamine is produced, and this is decomposed by a continuation of the treatment, with formation of a tarry deposit. When passed through a red-hot tube, methylamine is decomposed with formation of hydrogen and ammonium cyanides, methane, and hydrogen.

Methylammonium chloride, NH_2Me,HCl, melts at 225–226°. *Methylammonium picrate*, $NH_2Me,C_6H_3O_7N_3$, melts at 215°. The *platinichloride*, $(MeH_3N)_2PtCl_6$, is insoluble in alcohol, but soluble in boiling water, crystallising on cooling in beautiful golden-yellow scales.

[1] The reaction which occurs is very complex (A. W. Hofmann, *Ber.*, 1882, 15, 765), but the main decomposition may be expressed as follows

$$CH_3CO\,NKBr + 2KOH = CO(OK)_2 + KBr + CH_3.NH_2.$$

[2] See also H. Quantin, *Ann Chim. Anal.*, 1901, 6, 125.

A method for the proximate analysis of the bases present in crude methylamine, based on the principles of the process described on page 7, has been described by A. Muller (*Bull. Soc. Chim.*, 1884, **42**, 202).

Dimethylamine, $NH(CH_3)_2$.

Dimethylamine occurs in Peruvian guano and pyroligneous acid, and is also present in the products of the distillation of *vinasses*. It is formed when fish undergo putrefaction. It often forms a large percentage of commercial "trimethylamine." It is best separated from methylamine and trimethylamine by the action of nitrous acid, but, as already pointed out (page 8), a small proportion of trimethylamine is converted by this treatment into the secondary base. Dimethylamine may also be obtained pure by boiling 35 parts of nitroso-dimethylaniline hydrochloride with a solution of 15 parts of potassium hydroxide in 400 of water:

$$C_6H_4(NO).NMe_2,HCl + KHO = KCl + C_6H_4(NO).OH + HNMe_2.$$

Dimethylamine boils at 7°, has a sp. gr. 0.686 at −6°, and closely resembles the primary and tertiary methylamines. From the former it is at once distinguished by the non-formation of a precipitate on the addition of ethyl oxalate to the aqueous solution of the base (page 6), and the non-production of an isonitrile on treatment with alcoholic potassium hydroxide and chloroform. From trimethylamine it is distinguished by the formation of a nitrosamine on treating it with nitrous acid.

The *platinichloride*, $(Me_2H_2N)_2PtCl_6$, crystallises in very long needles.

Dimethylnitrosamine boils at 149°.

Dimethylamine hydrochloride is remarkable in being soluble in chloroform, a fact which permits of its ready separation from ammonium chloride, which is insoluble in this solvent.

Trimethylamine, $N(CH_3)_3$.

Trimethylamine is found notably in herring-brine, and has been detected in urine, unputrefied blood of the calf, cod-liver oil, and other animal fluids. It occurs in the *Chenopodium vulvaria* (stinking goosefoot), from the leaves of which it constantly exudes; *Arnica montana; Mercurialis annua;* the blossoms of the pear, white-thorn (*Cratægus oxyacantha*), hawthorn, and wild cherry; and in ergot and other para-

sites of the vegetable kingdom. Trimethylamine is also a product of the dry distillation of certain alkaloids, wood, etc., but especially of the *vinasses* or residue left after the distillation of the spirit from fermented beet-root mollasses. The bases obtained by the destructive distillation of this product are derived from the betaine.

The products of the destructive distillation of the "vinasses,"[1] left after the distillation of the fermented beetroot-molasses, vary with the concentration of the liquid. As the proportion of water decreases, the quantity of ammonia increases, and the trimethylamine is replaced by the primary and secondary methylamines. The *vinasses* from different localities yield varying proportions of gaseous and liquid products on distillation, the nitriles and methyl alcohol appearing to be the most variable constituents.[2]

[1] The *vinasses*, or spent wash from the stills, is evaporated till it acquires a sp gr of 1 31 when it is subjected to dry distillation in cast-iron retorts The aqueous portion of the distillate contains Ammonium carbonate, sulphydrate and cyanide, methyl alcohol, methyl sulphide, and methyl cyanide; various other substances of the fatty series, and a large proportion of salts of trimethylamine The tar yields, on distillation ammoniacal liquor, various oils, pyridine bases, solid hydrocarbons, phenols, and pitch of superior quality The aqueous liquid is neutralised with sulphuric acid and concentrated, when crystals of ammonium sulphate are deposited, and vapours of methyl alcohol are evolved together with methyl cyanide and other nitriles The methyl cyanide is converted into ammonia and acetate by treatment with an alkali· $CH_3NC + NaHO + H_2O = H_3N + CH_3COONa$ The dark-coloured mother-liquors retain the trimethylamine sulphate, which is decomposed by distillation with lime, the vapours being passed into hydrochloric acid The resultant solution is boiled down till the temperature reaches 140° Ammonium chloride crystallises out on cooling, and the mother-liquor is separated and concentrated till the b p rises to 200°, the product forming commercial trimethylamine hydrochloride, from which the free base may readily be obtained by treatment with lime or alkali hydroxide

[2] In a specimen of "commercial trimethylamine," prepared from vinasses, Duvillier and Buisine found only from 5 to 10% of trimethylamine and some 50% of dimethylamine, while the remainder consisted of methylamine, propylamine, and isobutylamine in about equal proportions, the ethylamine being estimated at about 2%, and ammonia being absent (*Compt Rend*, 1879, **89**, 48) The method employed by these chemists for the separation of the amines in question was as follows (*Ann Chim Phys* 1881, **23**, 289) The aqueous solution of the free bases was treated with ethyl oxalate, the dense white precipitate of oxamides filtered off, the filtrate concentrated by distillation, and the further precipitate added to that previously obtained By treating the precipitate with hot water it was separated into 3 fractions The most insoluble portion (1) consisted of dibutyloxamide (or possibly di-*iso*-butyloxamide), which melted and floated on the hot water, and on cooling formed a solid waxy mass When recrystallised from alcohol, it was obtained in pearly needles The *butylamine*, obtained by distilling the oxamide with potassium hydroxide, had a faintly aromatic odour, and yielded a slightly soluble platinichloride, crystallising in orange-coloured plates Of the oxamides soluble in boiling water, the dipropyl compound (2) was first deposited It crystallised from alcohol in pearly needles melting at 110°, and the *propylamine*, obtained from it gave an orange platinichloride When the proportion of butylamine and propylamine was small, the authors preferred to utilise the comparative insolubility of their sulphates in alcohol to separate them from the other amines The most soluble portion of the mixed oxamides (3) was deposited in opaque white needles or grains, and consisted of dimethyloxamide The base obtained by distilling it with potassium hydroxide was converted into the sulphate, which on treatment with boiling absolute alcohol was obtained quite pure, and yielded pure *methylamine* on treatment with potassium hydroxide

The mother-liquor separated from the oxamides of the primary amines was distilled with potassium hydroxide, and the dried gas collected in absolute alcohol A portion of the solution was then titrated with standard acid, and the remainder gradually added to a quantity of ethyl oxalate sufficient for the action· $Me_2NH + Et_2C_2O_4 = (MeHN)_2C_2O_2 + 2EtOH$, assuming the alkalinity to be wholly due to dimethylamine The operation was conducted in a flask, which was surrounded with ice and continually shaken When the action was completed, the flask was heated on the water-bath, and the alcohol and unchanged *trimethylamine* distilled off and collected in hydrochloric acid. It yielded a platinichloride in large orange-red crystals, and was the only tertiary amine found in the mixture of bases under examination

The syrupy residue left in the flask after the distillation of the alcohol and trimethylamine consisted of the ethyl dialkylated-oxamates, with traces of ethyl monalkylated-oxamates

Pure trimethylamine is best prepared in the laboratory by heating ammonium chloride (50 grm.) with 40% formaldehyde solution (440 grm.) at 120° in an autoclave. The action is finished when the internal pressure has reached a value of 35–40 atms. (Eschweiler and Naeppen, *Ber.*, 1905, **38**, 882).

Physical Properties (see table, page 12).

When pure and concentrated, trimethylamine is stated to have a purely ammoniacal odour; but when highly diluted, the vapour has at the same time a smell of ammonia and a peculiar fishy odour suggestive of herring-brine. The latter odour is gradually developed by adding lime to a solution of the base, but requires some time to reach its maximum intensity (L. Taylor).

Trimethylamine is apparently soluble in all proportions of cold water.[1]

A mixture of equal volumes of trimethylamine and water is inflammable.

Trimethylamine is employed for preparing pure potassium carbonate from the chloride by a method analogous to the ammonia-soda process. Ammonia is not available, because of the nearly equal solubility in water of ammonium chloride and potassium hydrogen carbonate, whereas the hydrochloride of trimethylamine is much more soluble.

Trimethylamine might, *prima facie*, be supposed the active agent in Wollheim's process of treating sewage with herring-brine and lime (*Eng. Patent* No. 15321, 1888); but those who have investigated the matter incline to the opinion that the bactericide is a hitherto unisolated sudstance they term aminol, produced by the action of lime on one of

and oxamides of primary amines It was treated with water, which caused hydrolysis, and, on neutralising the liquid with milk of lime, calcium ethyloxamate and propyloxamate were thrown down, which on distillation with potassium hydroxide yielded *ethylamine*, and *propylamine*. On treating the filtrate from the calcium oxamates precipitate with an equal volume of alcohol, a precipitate was formed from which warm water extracted calcium dimethyloxamate, yielding *dimethylamine*, on distillation with potash, while the less soluble portion consisted of calcium monomethyloxamate, yielding *methylamine* under similar treatment.

Ethylamine, which escaped detection on Duvillier and Buisine's first examination of the bases from vinasses, owing to the small proportion present, was subsequently detected by distilling with potassium hydroxide the mother-liquors obtained by treating the oxamides with water, and converting the bases into sulphates On treating these with absolute alcohol, methylamine sulphate remained On distilling the soluble portion with alkali, collecting the bases in absolute alcohol, and treating the solution with ethyl oxalate, as already described, the ethylamine was converted into a monoethyloxamate, from which the calcium salt was prepared and decomposed by alkali

[1] According to Guthrie, the solubility of trimethylamine in water is notably diminished by heating, the liquid becoming distinctly turbid (compare nicotine) from partial separation of the base. Thus a 10% solution of trimethylamine in water became turbid at 22°; an 8% at 24 5°, and a 4% solution at about 42° Leo Taylor failed to confirm Guthrie's observations, which were not improbably made on impure material (see, however, under *Triethylamine*).

the amines of herring-brine. Pure trimethylamine employed without lime has not the same effect.

Trimethylamine is distinguished from the primary and secondary methylamines by its negative reaction with alcoholic potash and chloroform, ethyl oxalate, and nitrous acid, and by its solution in excess of hydrochloric acid being precipitated by potassium ferrocyanide.

Trimethylamine has been employed in medicine, and is said to have proved of value in the treatment of gout and acute rheumatism. (A description of its therapeutic effects will be found in the *Year-Book of Pharmacy* for 1873, pages 197–262.)[1]

Trimethylamine combines with carbon disulphide at the ordinary temperature with great evolution of heat, according to the equation $CS_2+(CH_3)_3N=N(CH_3)_2.CS.S.CH_3$. The product, perhaps trimethyl-thiocarbamic acid, is prepared more readily by passing gaseous trimethylamine into a mixture of carbon disulphide and alcohol. It is obtained on evaporating the solvent, in white rhombic needles, m. p. 125°, and decomposes gradually at the ordinary temperature. It is soluble in dilute alcohol and water, but nearly insoluble in absolute alcohol, ether, chloroform, or benzene. Dilute acids combine with it to form salts, but strong acids and alkalies decompose it into carbon disulphide and trimethylamine.

Trimethylamine Hydrochloride, hydrochlorate of trimethylamine, $(CH_3)_3HNCl$, is obtained by neutralising trimethylamine with hydrochloric acid. It differs from ammonium chloride in being extremely deliquescent, and soluble in absolute alcohol. The fishy odour of the base liberated on treating the salt with lime or alkali hydroxide further distinguishes it from ammonium chloride. With platinum tetrachloride it unites to form the platinichloride, $(Me_3HN)_2PtCl_6$, which crystallises in orange octahedra, sparingly soluble in absolute alcohol.

When heated to 260–285°, trimethylamine hydrochloride is decomposed with formation of free trimethylamine, ammonia, and methyl chloride· $3Me_3HNCl=2Me_3N+H_3N+3MeCl$. This reaction has been utilised by Camille Vincent for the manufacture of methyl chloride. The vapours are passed through hydrochloric acid, which absorbs the bases, while the gaseous methyl chloride passes on. It is

[1] The solution of trimethylamine for medicinal use should be clear, colourless, and of 1.124 sp. gr It should have a peculiar odour, recalling that of ammonia and herring-brine, be miscible in all proportions with water and alcohol, and contain 20% of the base 1 volume of hydrochloric acid, of 1 170 sp gr , should neutralise 3 volumes of the solution of the base, and the salt obtained on evaporating the resultant solution should be completely soluble in absolute alcohol

washed by dilute sodium hydroxide and dried by strong sulphuric acid, after which it is collected in a gas-holder, from whence it is pumped into strong wrought-iron cylinders, in which it is condensed to liquid. The vapour of liquid methyl chloride has a tension of 2.5 atmospheres at 0° and 4.8 at 20°.

Separation and Estimation of the Three Methylamines and Ammonia.

A method has been recently described by Bertheaume (*Compt. Rend.*, 1910, **150**, 1251) which is based on the insolubility of ammonium chloride and methylamine hydrochloride in chloroform. 1 to 2 grm. of the mixed hydrochlorides, dried at 110°, are dissolved in a small quantity of water acidified with hydrochloric acid and the solution thoroughly mixed with at least 20 grm. of quartz sand. The mixture is thoroughly dried in a vacuum desiccator, extracted with pure warm chloroform, the extract evaporated to dryness and the residue weighed and dissolved in 2,000 times its weight of water. A known quantity of the solution (200 to 300 c c. at most) is cooled to 0° and a solution of iodine in potassium iodide (127 grm. iodine, 150 grm. potassium iodide in 1,000 c.c. water) also cooled to 0° is added in such proportion that at least 30 c.c. are used per 100 c c. of the methylamine solution. The mixture is left at 0° during 1 hour, the crystals of trimethylamine periodide are filtered off in a funnel plugged with glasswool, drained, and washed with a cold mixture of the above iodine solution and water (1:3). The crystals are then dissolved in normal sodium sulphite solution, the solution distilled with excess of sodium carbonate in a Schloesing apparatus and the trimethylamine estimated volumetrically in the distillate. The dimethylamine is similarly estimated in the mother liquor from the trimethylamine periodide. The residue insoluble in chloroform is dried to remove chloroform and then extracted with hot water. In the solution the methylamine and ammonia are separated by François' method (*Compt. Rend.*, 1907, **144**, 857) as follows:

Five-tenths grm. of the carefully dried hydrochloride is placed in a 250 c.c. flask and 7 c.c. of a 30% solution of sodium hydroxide, 10 c c. of a 20% solution of sodium carbonate and 5 grm. of yellow mercuric oxide added. The mixture is diluted to the mark with water and agitated for 1 hour. The freedom of the supernatant liquid from ammonia should then be ascertained by adding a few c.c. to a Nessler

solution made by dissolving 22.7 grm. of mercuric iodide, 33 grm. of potassium iodide and 35 grm. of sodium hydroxide in 1 litre of water. The Nessler solution, when heated to boiling, gives a reddish-brown precipitate if a liquid containing as little as 0.002% of ammonium chloride is added, but gives no precipitate with methylamine. The methylamine in the supernatant liquid free from ammonia is then estimated by Schloesing's method using litmus as indicator and barium hydroxide as standard alkali. The ammonia remains in combination with mercuric oxide, and may be obtained by washing the latter with water containing sodium hydroxide and carbonate, placing it in a Schloesing's apparatus and adding 50 grm. of potassium iodide which liberates ammonia. The latter is then estimated in the usual manner.

In presence of large quantities of ammonia and only relatively small proportions of the amines, the above described process of separating the methylamines needs modifying, Jarry's method of eliminating ammonia being employed (Bertheaume, *Compt. Rend.*, 1910, **151**, 146). The liquid is placed in the first of a series of 4 Durand wash bottles, (600–800 c c. capacity) with about 6 times the quantity of hydrochloric acid necessary to neutralise the estimated quantity of amines present. A similar quantity of hydrochloric acid, diluted to 50–100 c.c. with water, is placed in each of the other 3 wash bottles. 2 large wash bottles (each of 1,000 c.c. capacity) containing 1:1 hydrochloric acid sufficient to neutralise the whole of the ammonia, complete the series. A rapid current of air is aspirated through the vessels until the contents of the first 4 are neutral: these 4 now contain the whole of the mono- and dimethylamines together with a little ammonium chloride. The united liquids are evaporated to a few c.c., mixed with quartz sand and treated by the method already given for separating methylamine and dimethylamine. The other 2 wash bottles contain the trimethylamine and the whole of the ammonia save the small portion remaining with the other amines. The liquid is evaporated to 500 c c , cooled to 0°, excess of ammonium chloride separated and the trimethylamine estimated as periodide.

Ethylamines.

The ethylamines are obtainable in the manner already described (page 2). A convenient source of the primary amine, $C_2H_5.NH_2$, is the crude ethyl chloride obtained as a by-product in the manufacture of

chloral (A. W. Hofmann, *Ber.*, **3**, 109, 776). When ethyl chloride is heated to 90° under pressure with an equivalent proportion of strong aqueous ammonia, a layer of triethylamine containing ammonia is formed, while the aqueous liquid contains the hydrochlorides of ethylamine and diethylamine. When a similar mixture of aqueous ammonia and ethyl chloride is heated under pressure to 150°, H_4NCl, EtH_3NCl, and Et_4NCl are the chief products, only traces of Et_2H_2NCl and Et_3HNCl being formed.

The ethyl amines can be separated by methods already described. They present the closest analogy to the corresponding methyl bases. Various differences between the 3 amines are described on pages 4, 5 and 6. The following table shows other of their characteristic properties.

	Ethylamine	Diethylamine	Triethylamine
Formula ...	$(C_2H_5)H_2N$	$(C_2H_5)_2HN$	$(C_2H_5)_3N$
Boiling-point,	19°	56°	90°
Specific gravity	$\overset{8}{x}$° 6964	$\overset{0}{x}$° 7062	$\overset{20}{x}$° 7277
	$\overset{2}{x}$° 708	$\overset{20}{x}$° 706	$\overset{15}{x}$° 735
Reaction with zinc sulphate	Precipitate soluble in excess	Precipitate insoluble in excess	Precipitate insoluble in excess
Product when boiled with nitrous acid (or a salt of the bases with sodium nitrite solution).	Alcohol and nitrogen	Diethylnitrosamine, a neutral oily liquid boiling at 177°, and distilling with steam	Unchanged
Hydrochloride . ..	Deliquescent laminæ and prisms	Non-deliquescent plates	Non-deliquescent laminæ
Platinichloride	Hexagonal rhombohedra, moderately soluble in water	Monoclinic, moderately soluble	Monoclinic, very soluble
Acid ferrocyanide .	Soluble . .	Soluble	Very sparingly soluble

Triethylamine mixes with water in all proportions below 18°, but on raising the temperature the solution becomes turbid and separates into two layers. For the mutual solubility of triethylamine and water see Rothmund (*Zeit. Phys. Chem.*, 1898, **26**, 433).

Tetralkylammonium Bases.

Tetralkylammonium *iodides* result from the action of alkyl iodides on tertiary amines; action generally takes place at the ordinary tempera-

ture, with evolution of heat. Trimethylamine combines in the same way with methyl chloride to form NMe_4Cl, but it does not combine with ethyl chloride at the ordinary temperature even under a pressure of 50 atm. The tetralkylammonium *chlorides* as a rule are best obtained by digesting the corresponding iodides with silver chlorides: $NMe_4I + AgCl = AgI + NMe_4Cl$. In a similar way the *sulphates* may be obtained with the aid of silver sulphate. All these salts are crystalline compounds which generally dissociate on heating, giving the tertiary amines $NMe_4I \rightleftarrows NMe_3 + MeI$. In the decomposition of mixed ammonium haloids containing methyl groups, the methyl radical generally separates from the nitrogen atom, *e. g.*, $NMeEt_3Cl = NEt_3 + MeCl$.

The tetralkylammonium iodides combine with iodine to form intensely coloured tri-iodides, penta-iodides, hepta- and ennea-iodides, *e. g*, NMe_4I, I_2; NMe_4I, I_4; etc.

The tetra-alkylammonium iodides cannot be decomposed by aqueous potassium hydroxide, even on heating, but react with freshly precipitated silver oxide to form silver iodide and the tetra-alkyl ammonium hydroxides. These hydroxides are non-volatile, syrupy or solid deliquescent substances, of highly caustic, alkaline character, presenting, as a class, a strong analogy with potassium hydroxide. Many of them have marked poisonous characters.

It is possible to liberate the tetralkylammonium bases from their halogen salts by potassium hydroxide if a solvent is used in which the potassium haloid is sparingly soluble. Thus, for example, in methyl or ethyl alcohol, the action $NMe_4Cl + KOH = NMe_4.OH + KCl$ takes place with precipitation of potassium chloride. (Walker and Johnson, *Trans.*, 1905, **87**, 955). On filtering, adding a little water, and concentrating *in vacuo*, crystalline hydrates are obtained. The *hydrate*, $NMe_4.OH,5H_2O$, has m. p. 62–63°; 100 parts water dissolve 151 parts at 0° and 220 parts at 15°. $NMe_4OH,3H_2O$ has m. p. 59–60° and when warmed in a vacuum at 35° gives NMe_4OH,H_2O, which decomposes when heated at 130–135°, forming trimethylamine.

It is noteworthy that the mixed tetralkylammonium bases containing methyl, *unlike the haloids*, generally retain methyl in combination with nitrogen when decomposed by heat while ethyl groups separate in the form of ethylene, *e. g.*,

$$NMe(C_2H_5)_2(C_5H_{11}).OH = NMe(C_2H_5)(C_5H_{11}) + C_2H_4 + H_2O.$$

Tetrethylammonium iodide, $(C_2H_5)_4NI$, is prepared by expos-

ing a mixture of equivalent proportions of triethylamine and ethyl iodide to a temperature of 100° for a few minutes in a flask fitted with a reflux apparatus, or preferably in a sealed tube. Violent action ensues, and, on cooling, the product sets to a dark mass of crystals. On dissolving in water, and allowing the solution to evaporate spontaneously, the iodide is obtained in extremely bitter crystals of considerable size, which, when pure, are colourless, but are apt to be mixed with reddish crystals of the tri-iodide, $(C_2H_5)_4NI,I_2$.

Tetrethylammonium iodide is not volatile at 100°, but when rapidly heated in a retort to a higher temperature melts and is decomposed into ethyl iodide and trimethylamine, which form separate layers in the receiver but re-unite to produce the original compound.

Tetrethylammonium iodide is not apparently decomposed by treatment with potassium or sodium hydroxide, but is much less soluble in caustic alkaline solutions than in water. Hence, on adding excess of potassium hydroxide to its concentrated aqueous solution, a solid crystalline mass is produced. This behaviour sharply distinguishes the iodide of tetrethylammonium (and of other compound ammoniums) from the compounds Et_3HNI, Et_2NHI, and EtH_3NI, which are at once decomposed by alkali hydroxide with liberation of the corresponding amines. The aqueous solution of tetrethylammonium iodide reacts with silver nitrate or sulphate to form a precipitate of silver iodide and a solution of the tetrethylammonium nitrate or sulphate.

Tetrethylammonium hydroxide, $(C_2H_5)_4N.OH$, is obtained in solution by adding freshly-precipitated oxide of silver to a dilute and warm solution of tetrethylammonium iodide, until the brown colour of the silver oxide ceases to change into the lemon-yellow of the iodide. The solution is then filtered, and may be evaporated to a considerable extent at a gentle heat, but further concentration must be conducted *in vacuo*, at the ordinary temperature, over sulphuric acid and lime. Long, hair-like, deliquescent needles of the base are deposited, but these subsequently disappear, and the liquid ultimately dries up to a semi-solid mass.

Tetrethylammonium hydroxide presents the closest analogy to potassium hydroxide. It is highly deliquescent, absorbs carbon dioxide from the air, and the aqueous solution has a strong alkaline reaction. It has an alkaline, caustic, and extremely bitter taste, and in a concentrated state burns the tongue and acts on the skin like potassium hydroxide. With metallic solutions it behaves like the

alkali hydroxides except that aluminium hydroxide is soluble with difficulty in excess of the base and chromium hydroxide is insoluble.

A moderately concentrated solution of tetrethylammonium hydroxide may be boiled without decomposition; but in a concentrated state, even at 100°, the liquid froths strongly, and the base is resolved gradually but completely into triethylamine, ethylene, and water: $(C_2H_5)_4N.OH = (C_2H_5)_3N + C_2H_4 + H.OH$. This action affords a convenient means of obtaining triethylamine unmixed with the primary and secondary amines.

When a solution of tetrethylammonium hydroxide is boiled with a slight excess of ethyl iodide for 24 hours, under a reflux condenser, the solution becomes perfectly neutral, the following action occurring: $(C_2H_5)_4N.OH + C_2H_5I = (C_2H_5)_4NI + C_2H_5.OH$.

Tetrethylammonium hydroxide also hydrolyses ethyl oxalate, and saponifies fats as readily as potassium hydroxide.

On adding potassium hydroxide and potassium iodide to a strong solution of tetrethylammonium hydroxide, a white crystalline mass of tetrethylammonium iodide is produced.

The *salts* of tetrethylammonium are mostly crystallisable and readily soluble.

Tetrethylammonium chloride, $(C_2H_5)_4NCl$, obtained by neutralising the hydroxide with hydrochloric acid, is crystalline and highly deliquescent. If forms double salts with auric, mercuric, and platinic chlorides. *Tetrethylammonium platinichloride*, $(Et_4N)_2PtCl_6$, is thrown down immediately as an orange-yellow precipitate, consisting of microscopic octahedra, on adding platinic chloride to a solution of tetrethylammonium chloride. It is slightly soluble in water, and less soluble in alcohol and ether.

For the resolution of asymmetric tetralkylammonium compounds into optically active components by means of *d*-camphorsulphonic acid, see Pope and Peachey, *Trans.*, 1899, **75**, 1127, and Pope and Harvey, *Trans.*, 1901, **79**, 828.

HYDRAZINES.

Hydrazine. Diamidogen. Diamide. N_2H_4 or $H_2N.NH_2$.

Hydrazine is obtained by the decomposition of triazo-acetic acid by heating it with water or mineral acids, when the following action occurs:

$$\underset{\text{Triazo-acetic acid.}}{C_3H_3N_6(COOH)_3} + \underset{\text{Water.}}{6H_2O} = \underset{\text{Hydrazine.}}{3N_2H_4} + \underset{\text{Oxalic acid.}}{3C_2H_2O_4}.$$

The oxalic acid is more or less split up, according to the temperature and the strength of the acid employed, into carbonic and formic acids, so that when only water is used the hydrazine separates as a formate; but if a mineral acid be present it forms the corresponding salt.

Hydrazine hydrate (infra) is best prepared (Curtius and Schulz) by distilling a mixture of 11 parts of hydrazine sulphate with 4 of potassium hydroxide and 1 of water in a silver retort provided with a silver condenser. When the last drop has passed over, the distillate is fractionated. After four fractionations the last portions boil constantly at 119°. Curtius and Jay (*Jour. Prakt. Chem.*, **39**, [*ii*], 27) prepare hydrazine hydrate by heating the hydrochloride of the base with calcium oxide in a silver retort, and passing the vapours through a heated silver tube containing anhydrous lime.

Free hydrazine, $NH_2.NH_2$, is obtained by decomposing the hydrate with barium oxide or from its hydrochloride by the action of sodium in absolute ethereal or methyl alcoholic solution (Lobry de Bruyn, *Rec. Trav. Chim.*, 1894, **13**, 433; 1896, **15**, 174). It has b. p. 113.5°/761.5 mm.; 56°/71 mm.; sp. gr. 1.014 at 15°.

A rapid means of preparing hydrazine sulphate for laboratory purposes is given by Raschig (*Ber.*, 1907, **40**, 4588), based on the action of ammonia on monochloramine, NH_2Cl, prepared by the interaction of ammonia and sodium hypochlorite.

Hydrazine has an extraordinary affinity for water, readily forming the hydrate N_2H_4,H_2O. This is a liquid which fumes in the air, b. p. 119°.

The solution of hydrazine turns reddened litmus-paper a deep blue, and gives white fumes with acid vapours. In a concentrated state it has a very peculiar odour, only slightly resembling that of ammonia. It powerfully affects the nose and throat, has an alkaline taste, and leaves a burning sensation on the tongue. When boiling, the solution attacks glass, and quickly destroys corks and india-rubber. Hydrazine, like hydroxylamine, is a strong poison of universal character.

Hydrazine reduces Fehling's solution and ammoniacal silver nitrate in the cold. With copper sulphate it yields a red precipitate, with mercuric chloride a white precipitate, and precipitates alumina from a solution of alum. With aromatic aldehydes and ketones it yields sparingly soluble crystalline compounds.

Salts of Hydrazine.

Hydrazine combines with 1 or 2 molecules of monobasic acids to form very stable salts, which are usually crystalline and isomorphous with the corresponding ammonium salts. The salts Hz,2HR crystallise in the cubic system and are readily soluble in water, but nearly insoluble in alcohol. The mono-acid salts, HzHR, are easily soluble in water and warm alcohol, from which they crystallise well. The salts of both classes are insoluble in ether, benzene, etc. In acid solution, the salts of hydrazine possess remarkably strong reducing properties, and are powerfully toxic toward the lower organisms. Peptone solutions containing 0.1% of hydrazine sulphate are unable to support bacterial life.

Hydrazine dihydrochloride, $N_2H_4,2HCl$, crystallises from hot water in large glassy octahedra that are freely soluble in water, but less so in alcohol. On treatment with platinum tetrachloride it does not yield a platinichloride, but is decomposed with evolution of nitrogen. It melts at 198°, with evolution of hydrochloric acid, to a clear glass consisting of the *monohydrochloride*, N_2H_4HCl, and this on further heating to 240° is decomposed into ammonium chloride, nitrogen, and hydrogen.

Hydrazine sulphate, N_2H_4,H_2SO_4, is somewhat sparingly soluble in water.

Salts of hydrazine in solution are decomposed by sodium nitrite, with evolution of gas, attended by much frothing. The reaction is analogous to the decomposition of ammonium salts by a nitrite, with the difference that whereas in the latter case (*a*) nitrogen is formed, in the case of hydrazine (*b*) azoimide, HN_3, is found among the products of the action:

a) $NH_3,HCl+NaNO_2=NaCl+2H_2O+N_2$.

b) $N_2H_4,HCl+NaNO_2=NaCl+2H_2O+HN_3$.

Detection of Hydrazine.

1. Benzaldehyde gives with its solutions, alkaline or acid, dilute or concentrated, yellow flocks of *benzalazine*, CHPh:N.N:CHPh, m. p. 93. 2. In solutions more dilute than 1:2,000, copper sulphate gives a sparingly soluble blue double salt, $CuSO_4,(N_2H_5)2SO_4$ (Curtius and

Schrader). 3. Hydrazine reduces gold chloride in acid solution, a fact which distinguishes it from hydroxylamine.

Estimation.

1. By measuring the iodine absorbed by a known volume of its solution (Curtius and Schulz). This is obviously practicable only in the absence of other substances which absorb iodine. According to Stolle (*J. Pr. Chem.*, 1902, **66**, 332) hydrazine or its salts can readily be titrated with iodine solution in presence of sodium hydrogen carbonate: the action is $N_2H_4 + 2I_2 = N_2 + 4HI$. The sodium hydrogen carbonate is added and the solution immediately titrated with standard iodine in presence of starch as indicator. As the action finishes slowly the final colouration should persist at least 2–4 minutes. Rupp (*J. Pr. Chem.*, 1903, **67**, 140) advises dissolving the hydrazine sulphate in aqueous potassium hydrogen carbonate, leaving for 15 minutes with excess of N/10 iodine and then estimating the excess of iodine with sodium thiosulphate. The use of potassium tartrate or sodium acetate in place of potassium hydrogen carbonate is said to give better results

2. By measuring the nitrogen evolved when shaken with Fehling's solution.

3. By measuring the potassium permanganate required for its oxidation in sulphuric acid solution (6–12%). (Petersen, *Zeit. anorg. Chem.*, **5**, 1)

4. **Rimini's method** (*Gazzetta*, 1899, **29** [*i*], 265) is based on the following reaction:

$$5(NH_2.NH_2,H_2SO_4) + 4KIO_3 = 5N_2 + 12H_2O + 2K_2SO_4 + 3H_2SO_4 + 2I_2.$$

A known volume of a standard solution of potassium iodate is added to the solution of hydrazine sulphate so as to be in slight excess. The liquid is then boiled over a flame until colourless, showing that the iodine is expelled, and after cooling and acidifying with dilute sulphuric acid the remaining excess of iodate is titrated with thiosulphate in the usual way.

5. According to Stollé (*loc. cit.*) hydrazine sulphate can be titrated with potassium hydroxide using methyl orange as indicator; the action which occurs is as follows:

$$2N_2H_4,H_2SO_4 + 2KOH = (N_2H_4)_2,H_2SO_4 + K_2SO_4 + 2H_2O.$$

6. **Ebler's gasometric method** (*Zeit. anorg. Chem.*, 1905, **47**, 377)

is based on the reduction of mercuric salts, for example mercuric chloride, according to the equation: $N_2H_4 + 2HgCl_2 = 4HCl + 2Hg + N_2$. The mercuric salt is dissolved in 10 c.c. of dilute hydrochloric acid, 5 grm. of sodium acetate dissolved in 10 c c. of water are added and the whole introduced into a flask (500–700 c.c.) fitted with a ground stopper carrying (1) a tap funnel, (2) a tube passing to the bottom of the flask through which CO_2 can be introduced, and (3) a reflux condenser the inner tube of which can be connected with a Schiff's nitrometer. Carbon dioxide is passed through the flask until all air is expelled, the liquid being maintained just below 100°. The solution of the hydrazine salt is then added very gradually, and CO_2 is passed continuously until the volume of gas in the nitrometer ceases to increase. The volume of nitrogen may be measured direct in the nitrometer if graduated in the usual way, or the gas may be transferred to a Hempel's gas burette.

7. Hofmann and Kuspert (*Ber.*, 1898, **31**, 64) measure the nitrogen evolved from the hydrazine salt when oxidised by vanadic acid, thus, $N_2H_4 + O_2 = N_2 + 2H_2O$; or the amount of vanadic acid used in the oxidation may be estimated by means of permanganate.

Azoimide. Iminazoic Acid. $HN_3 = HN\langle \begin{matrix} N \\ \| \\ N \end{matrix}$

Azoimide is obtained in the form of its sodium derivative, with a yield of 50%, by the action of nitrous oxide on sodamide: $N_2O + NH_2Na = NaN_3 + H_2O$ (Wislicenus). It can also be obtained by passing nitrous fumes into a solution of hydrazine sulphate at 0°. $NH_2.NH_2 + HNO_2 = N_3H + 2H_2O$ (Angeli). Curtius, who discovered it, prepared it (*Ber.*, 1890, **23**, 3023) by decomposing nitroso-hippurylhydrazine, $NHBz.CH_2.CO.N(NO).NH_2$, with dilute sodium hydroxide, which splits it up into hippuric acid and the sodium salt of azoimide:

$$NHBz.CH_2.CO.N(NO).NH_2 + 2NaHO = NHBz.CH_2.COONa + 2H_2O + NaN_3.$$

On distilling the compound NaN_3 with dilute sulphuric acid, azoimide volatilises with the steam, which when passed into a neutral solution of silver nitrate gives a precipitate of the silver salt. This is washed and decomposed by dilute sulphuric acid, this solution being used instead of silver nitrate to absorb the vapours of azoimide. By

repeating this process, a solution containing 27% of the new acid is obtainable. It can be obtained by several other methods.

In the anhydrous state, azoimide is a colourless gas of a peculiarly nauseous odour, and condensible on cooling to an extremely explosive liquid which boils at 37°. It is very soluble in water, and on distillation of the liquid a concentrated acid passes over, the distillate gradually becoming weaker until an acid of constant composition and b. p. distils. The solution reddens litmus, and gives white fumes with ammonia, of the salt $NH_3.HN_3$ or N_4H_4, which sublimes completely at 100°, but does not crystallise in the cubic system like ammonium chloride. Iron, zinc, copper, aluminium and magnesium dissolve readily in dilute iminazoic acid (7%) with evolution of hydrogen, and gold is dissolved with formation of a red salt. The *silver* (AgN_3) and *mercurous salts* of iminazoic acid are insoluble, the former closely resembling silver chloride, but not blackening in the light. Both the silver and the mercurous salts are extraordinarily explosive, 0.001 grm. of the former indenting an iron plate on which it is heated to 250°. *Barium azoimide*, BaN_6, separates from concentrated solutions in short shining anhydrous crystals, which explode with a green flash when heated or exposed to a strong green light. The solution of *cupric azoimide* deposits cuprous oxide on boiling. The free acid is liberated from any of the iminoazoates by treatment with dilute sulphuric acid. By concentrated sulphuric acid, the azoimide is itself decomposed. *Esters* of iminazoic acid have been prepared, phenyl iminazoate, PhN_3, being identical with the diazobenzolimide previously described by Griess.

SUBSTITUTED HYDRAZINES.

Hydrazine is the parent of a large and important class of bases generally called hydrazines, one member of which, phenylhydrazine, $(C_6H_5)NH.NH_2$, has proved, in the hands of E. Fischer and others, a reagent of the highest importance. By replacing a second atom of hydrogen by (*e. g.*) phenyl, secondary hydrazines may be obtained either symmetrical like hydrazobenzene, $(C_6H_5)HN.NH(C_6H_5)$, or unsymmetrical like diphenylhydrazine, $(C_6H_5)_2N.NH_2$. The latter class resemble the tertiary amines in their power of reacting with the haloid salts of the alkyl radicals (*e. g.*, ethyl iodide) to form hydrazonium compounds, $R_2N.NH_2 + AlkI = IAlkR_2N.NH_2$.

The hydrazines containing fatty alkyl-radicals are liquids boiling without decomposition; those of the aromatic series are readily fusible solids or oily liquids, and are partially decomposed on distillation. Hydrazine itself and some of the fatty derivatives are di-acid bases; but the hydrazines of the benzene series have all monobasic functions.

The hydrazines closely resemble the amines, but are distinguished from the latter by their capacity of reducing Fehling's copper solution, in many instances at the ordinary temperature. The product of the oxidation of the hydrazine is the corresponding amine. Thus, diethyl-hydrazine, $(C_2H_5)_2N.NH_2$, is oxidised to diethylamine, $(C_2H_5)_2HN$.

The general and special characters of the hydrazines are sufficiently exemplified by two typical species, ethylhydrazine and phenylhydrazine.

Ethylhydrazine. $(C_2H_5)HN.NH_2$.

On treating diethyl-carbamide with nitrous acid, a nitroso-compound is formed, which on reduction with zinc-dust and acetic acid is converted into diethyl-semicarbazide.

$CO\langle^{NH(C_2H_5)}_{NH(C_2H_5)}$ Diethyl-carbamide. $CO\langle^{NH(C_2H_5)}_{N(NO)(C_2H_5)}$ Nitroso-compound $CO\langle^{NH(C_2H_5)}_{N(NH_2)(C_2H_5)}$ Diethyl-semicarbazide

This carbamize decomposes, on heating with strong hydrochloric acid, into ethylhydrazine, ethylamine, and carbon dioxide:

$$NH(C_2H_5).CO\ N(NH_2)C_2H_5 + H_2O = HN.(NH_2)(C_2H_5) + C_2H_5NH_2 + CO_2.$$

Primary hydrazines can also be obtained by heating the potassium salts of alkylsulphuric acids with hydrazine hydrate (Stollé, 1902) and by the reduction of nitroamines.

Ethylhydrazine hydrochloride is less soluble than the corresponding salt of ethylamine, and may be separated from it by crystallisation.

Ethylhydrazine is a colourless, mobile liquid of ethereal and faintly ammoniacal odour. It boils at 99 5° under 709 mm., and distils undecomposed. It is very hygroscopic, forming white fumes with moist air, dissolves in water and alcohol with evolution of heat, and corrodes cork and caoutchouc.

Ethylhydrazine gives Hofmann's isonitrile reaction for primary amines with chloroform and alcoholic potassium hydroxide. Bromine decomposes it with evolution of nitrogen, and it is also decomposed by nitrogen trioxide.

Ethylhydrazine is a very powerful reducing agent. It reduces Fehling's copper solution at the ordinary temperature and liberates silver from its oxide. It yields a black precipitate with Nessler's solution.

Ethylhydrazine reacts with aldehydes, with evolution of heat, to form ethylhydrazones, $R.CH:N_2H(C_2H_5)$.

Potassium pyrosulphate, $K_2S_2O_7$, acts on ethylhydrazine to form potassium ethylhydrazine-sulphonate, $(C_2H_5)HN.NH(SO_3K)$, which, on treatment with mercuric oxide, gives potassium diazo-ethane-sulphonate, $C_2H_5.N:N.(SO_3K)$, a substance which explodes violently when warmed, and otherwise resembles the diazo-benzene-sulphonates.

Asymm. diethylhydrazine, $(C_2H_5)_2N.NH_2$, is obtained by the reduction of the nitroso-derivative of diethylamine: $(C_2H_5)_2N\,NO + 2H_2 = (C_2H_5)_2N.NH_2 + H_2O$. It boils at 96–99°, and closely resembles ethylhydrazine, but does not reduce Fehling's solution unless the liquid is heated. It unites with ethyl iodide to form the compound $(C_2H_5)_3N_2H_2I$, which on treatment with oxide of silver yields a strongly alkaline solution of triethylazonium hydroxide, $(C_2H_5)_3N_2$-H_2OH, a powerful base analogous to tetrethylammonium hydroxide (page 22); when heated with water, this decomposes into ethylene, diethylhydrazine, and water Mercuric oxide, even in the cold, converts asymm. diethy hydrazine into tetraethyltetrazone, $(C_2H_5)_2$-$N.N:N.N(C_2H_5)_2$, a co ourless, strongly basic oil, volatile with steam, which yields a metallic mirror with ammoniacal silver nitrate.

Sym. diethylhydrazine, NHEt NHEt, boils at 84–86°, and is converted by oxidation with mercury oxide into mercury ethyl, $HgEt_2$, nitrogen being evolved; nitrous acid transforms it into ethyl nitrite, and concentrated hydrochloric acid splits it up into ethyl chloride and ammonium chloride.

Methylhydrazine, $NHMe.\dot{N}H_2$, has b. p. 87°; asymm. *dimethylhydrazine* boils at 63° and has sp. gr. 0.801/11°; sym. *dimethylhydrazine* has b. p. 50–60.°

Phenylhydrazine. $C_6H_8N_2 = (C_6H_5)HN.NH_2$.

Phenylhydrazine is prepared by the action of reducing agents on diazobenzene salts, $C_6H_5N:NX$. Thus benzenediazonium chloride may be reduced by the calculated amount of stannous chloride and hydrochloric acid; or the potassio-sulphite with zinc-dust and acetic

acid, the product being subsequently decomposed by boiling with hydrochloric acid:

$$C_6H_5.HN.NH.SO_3K + HCl + H_2O = KHSO_4 + C_6H_5.HN.NH_2,HCl.$$ [1]

Phenylhydrazine is generally a colourless crystalline mass which melts at 23° to a slightly yellow oil and boils, with slight change and evolution of ammonia, at 241–242°. It distils unchanged at 120° under 12 mm. pressure. It volatilises in a current of steam, but not very readily. Phenylhydrazine dissolves sparingly in cold water, more readily in hot, and very readily in alcohol, ether, chloroform, and benzene.

Phenylhydrazine is readily oxidisable, and becomes red and ultimately dark brown on exposure to air, from absorption of oxygen.

Phenylhydrazine has well-marked antiseptic properties, and a 0·1% solution of the hydrochloride has been recommended as a substitute for one of mercuric chloride of equal strength (*Pharm. Jour.* [*v*], **19**, 608).

Under certain undetermined conditions, contact of phenylhydrazine with the skin produces troublesome sores.

Phenylhydrazine has well-marked basic properties, and forms well-crystallised salts. The *hydrochloride* crystallises from hot water in small, thin, lustrous plates, and is almost completely precipitated from its aqueous solution by concentrated hydrochloric acid, a reaction by which phenylhydrazine may be readily separated from aniline and several other bases.

Solutions of the hydrochloride and other salts of phenylhydrazine act as powerful reducing agents. They reduce the salts of silver, mercury, gold, and platinum in the cold. Freshly-precipitated mercuric oxide is reduced, a salt of diazobenzene being reproduced. Fehling's solution is reduced in the cold, with evolution of nitrogen and precipitation of cuprous oxide, aniline and benzene being simultaneously formed.

Phenylhydrazines as a class are converted by aqueous copper sulphate into the corresponding aromatic hydrocarbon. This reaction affords

[1] Phenylhydrazine is best obtained, as described by V Meyer, by dissolving 1,000 parts of aniline in 2,000 parts of concentrated hydrochloric acid, cooling the solution by means of ice, and then slowly adding an ice-cold solution of 75 parts of sodium nitrite in 400 c c of water To the cold solution of benzenediazonium chloride, $C_6H_5 N N Cl$, so obtained, a solution of 450 parts of stannous chloride in an equal weight of hydrochloric acid is then added The mixture soon sets to a white crystalline pulp of phenylhydrazine hydrochloride, $C_6H_5N_2H_3,HCl$, which is filtered or strained off, and washed with a mixture of alcohol and ether The free base is obtained by dissolving the hydrochloride in water, adding sodium hydroxide, and agitating with ether, which is separated and evaporated. The product is purified by distillation.

a ready means of replacing an amino-group in an aromatic nucleus by hydrogen, the aromatic base being first converted into the corresponding hydrazine, which in the form of its hydrochloride or sulphate is added to a boiling solution of copper sulphate.

If phenylhydrazine hydrochloride be treated with a cold solution of potassium nitrite, a nitroso-compound, $C_6H_5(NO)N.NH_2$, separates in yellow flocks, which, on treatment with phenol and strong sulphuric acid, yield a brown solution, changing to green and blue. (Liebermann's test for nitroso-derivatives.)

Phenylhydrazine combines directly with carbon dioxide, carbon disulphide, and cyanogen. The sulphonic acid (para) is employed for the preparation of *tartrazin* and other dyes.

Phenylhydrazides.—The acetyl-derivative of phenylhydrazine, $C_6H_5.HN.NH(C_2H_3O)$, which may be regarded as acetphenylhydrazide, has powerful antipyretic properties, and has been introduced into German pharmacy under the name of "hydracetin." The same substance is said to be the active ingredient of the preparation known as "pyrodine" (*Pharm. Journ.* [*iii*], **19**, 425, 508, 1049). Both substances seem to be uncertain in their action and dangerous in use; in fact, hydracetin is reported by Renvers to be a direct blood-poison, the antithermic properties of which are really due to destruction of the red corpuscles.

"Orthine" is the name given by R. Kobert to orthohydrazino-parahydroxy-benzoic acid:

$$C_6H_3\begin{cases}(OH)^{(1)}\\(HN.NH_2)^{(2)}\\(CO.OH)^{(4)}\end{cases}$$

The free base is very unstable; but the hydrochloride is stable, reduces the persalts of the heavy metals, and possesses a marked antiseptic action.

Phenylhydrazine in aqueous solution reacts very readily with the hydroxy-acids of the sugar group (*e. g.*, gluconic and galactonic acids, $C_5H_6(OH)_5.COOH$; arabinose-carboxylic acid, $C_6H_{12}O_7$) with elimination of water, to form crystalline phenylhydrazides, $R.CO.HN.NH(C_6H_5)$. They are prepared by treating a 10 % solution of the acid or its lactone with a moderate excess of phenylhydrazine and an equal quantity of 50% acetic acid, and heating the mixture to 100° for 80 to 120 minutes. The hydrazide sometimes crystallises from the hot solution, but more usually separates on cooling. Any free mineral acid

should be neutralised by sodium carbonate before adding the hydrazine, and bromides, chlorides and sulphates should be got rid of by adding acetate of lead. If a sugar be present, the osazone formed can usually be separated from the hydrazide by crystallisation from hot water. The products are beautifully crystalline, those derived from monobasic acids being but little soluble in cold, and only with difficulty soluble in hot water, while those from polybasic acids (*e. g.*, saccharic, metasaccharic, and mucic) are still less readily soluble. The compounds from isomeric acids usually present a close resemblance in their physical properties, but the acids from which they are derived can be regenerated (in a pure state) by boiling the hydrazide for half an hour with 30 volumes of 10% baryta water, which treatment hydrolyses them completely. From the product, the phenylhydrazine is extracted by agitation with ether, and the aqueous liquid, with any precipitate which may have been formed, is boiled and treated with sulphuric acid in quantity sufficient to precipitate the barium as $BaSO_4$. The filtered liquid yields the free acid or lactone on evaporation (Fischer and Passmore, *Ber.*, 1889, **22**, 2728).

The hydrazides are colourless and readily hydrolysed by alkalies and baryta. They can be readily distinguished from the hydrazones by the reddish-violet colouration they give when dissolved in strong sulphuric acid and treated with a drop of ferric chloride solution.

Detection of Phenylhydrazine.

Simon (*Compt. Rend.*, 1898, **126**, 483 and *Bull. Soc. Chim.*, 1898, **19**, 299) has given the following test for detecting phenylhydrazine and its substituted derivatives, which behave in the same way: The test is capable of detecting phenylhydrazine in a solution of 1 in 50,000. The solution is momentarily warmed with a few drops of aqueous trimethylamine and several drops of a solution of sodium nitroprusside are then added. A colour varying from blue to green is produced which becomes more pronounced on adding a little concentrated potassium hydroxide solution. If a little acetic acid be added, either before or after the potassium hydroxide, the colour is of a sky-blue shade. Added in excess, acetic acid causes the colour to disappear. Ether and alcohol do not affect the test but chloroform and benzene interfere with it. Acetone gives its own colouration (the red of Legal's test). Mineral or organic acids retard the production of

colour until after the addition of potassium hydroxide. Ammonia does not interfere with the test. On heating the blue coloured liquid the tint becomes red when potassium hydroxide is present and clear yellow when it is absent. Simon's reaction is not given by hydrazones and seems to be characteristic of phenylhydrazine and its substituents. Negative results are given by formyl and benzoylphenylhydrazine (*i. e.*, substituted compounds of the type Ph.NH.NH.Ac.).

The blue colour is easily distinguishable from that given by aldehyde with the same reagents by its persistence in the presence of potassium hydroxide, ammonia and acetic acid.

Rimini (*Ann. Farm.*, 1898, 102) states that *pure* trimethylamine does not give the above reaction but that it is due to the presence of formaldehyde which can be substituted with advantage in the test (see Vol. 1, p. 258).

Estimation of Phenylhydrazine.

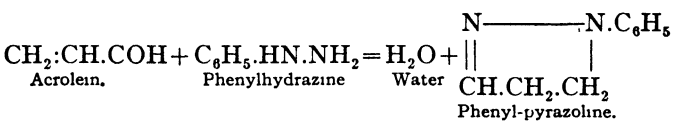
Causse's method is as follows (*Compt. Rend.*, 1898, **125**, 712). It is based on the reduction of arsenic acid according to the equation $As_2O_5 + C_6H_8N_2 = N_2 + H_2O + C_6H_6O + As_2O_3$. The solutions required are: (1) 125 grm. of arsenic acid is dissolved as 125 c.c. of concentrated hydrochloric acid, the cooled solution filtered and made up to 1 litre with glacial acetic acid; (2) N/10 iodine solution; (3) 200 grm. of sodium hydroxide (free from sulphides) dissolved in 1 litre of water; (4) cold saturated solution of sodium hydrogen carbonate. 0.2 grm. of the sample of phenylhydrazine or its hydrochloride is placed in 500 c.c., 60 c.c. of the arsenic acid solution added and the liquid boiled under a reflux condenser, using a spiral of platinum wire to prevent bumping. When action has ceased, that is, after about 40 minutes, the liquid is cooled, 200 c.c. of water is added and then sodium hydroxide until the liquid is alkaline, and finally a drop or two of hydrochloric acid. 60 c.c. of the sodium hydrogen carbonate are then added and the arsenious acid estimated by iodine solution and starch. The method can be applied also to aromatic phenylhydrazones, but in the case of fatty phenylhydrazones the aldehyde should be removed before titration on account of its action on arsenic acid.

Hydrazones.—Phenylhydrazine behaves in a highly interesting manner with aldehydes and ketones, with which it reacts with elimina-

tion of water to form hydrazones. Most of the substances of this class are solid and crystalline, and therefore well suited for the recognition of the aldehydes or ketones producing them. The action is general for substances containing the carbonyl group, CO, but is sometimes complicated by the presence of other reactive groups. Thus compounds containing the α-ketone-alcohol group—CH(OH).CO—react in the cold with only 1 molecule of phenylhydrazine to form colourless compounds containing the group CH(OH).C.(N.NHC$_6$H$_5$).

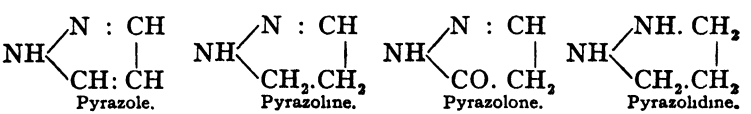
Osazones.—When the compound thus formed is heated with excess of phenylhydrazine, the alcohol group undergoes oxidation, reacting at the same time with a second molecule of phenylhydrazine and giving rise to a yellow compound containing the complex group—C(N.NHC$_6$H$_5$).C(N.NHC$_6$H$_5$) Compounds of this kind, in which 2 hydrazine-residues are attached to 2 contiguous carbon-atoms, are called osazones, and may be obtained directly by the action of phenylhydrazines on the di-ketones. They are of interest in connection with the carbohydrates, which may frequently be recognized by means of their characteristic osazones. A solution of phenylhydrazine hydrochloride containing sodium acetate can be used for the detection of sugar in urine.

Pyrazolines.—An unsaturated hydrocarbon group (*e g*, allyl, C$_3$H$_5$), if contiguous to the carbonyl group, may also react with phenylhydrazine:



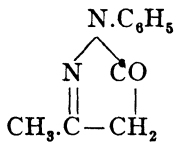
Pyrazolones.

The pyrazolones are derivatives of a substance of the formula C$_3$H$_4$N$_2$O, the synthesis of which has been affected by Balbiano (*Ber.*, 1890, **23**, 1103). The relationship of pyrazolone to pyrazole, pyrazoline, and pyrazolidine is shown by the following formula:



Phenylpyrazolones. Antipyrine.

1 :3-Phenyl-methylpyrazolone, $C_{10}H_{10}ON_2$;



When phenylhydrazine is added to ethyl aceto-acetate, $CH_3.CO.CH_2.CO.O(C_2H_5)$, the two substances interact in the cold, with elimination of water, to form $CH_3.C(N.NHPh)CH_2.CO.O(C_2H_5)$.[1] On heating, the hydrazone thus formed splits up into alcohol and phenyl-methyl-pyrazolone, a substance which was originally regarded by its discoverer, Knorr, as a methyl-oxyquinizine.

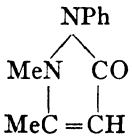
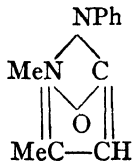
To prepare 1:3-phenyl-methylpyrazolone, 100 parts of phenyl-hydrazine are added to 125 of ethyl aceto-acetate, the water which forms is separated, and the oily product is heated for 2 hours on a water-bath, until a portion is found to solidify on cooling, or on the addition of ether. The warm mass is poured into and stirred with ether, which removes colouring matter, and the white crystalline product washed with ether, and dried at 100°. The yield is quantitative and the product pure. It is almost insoluble in cold water, ether, and petroleum spirit, more readily in hot water, and easily in alcohol. It crystallises from hot water or alcohol in hard brilliant prisms and melts at 127°.[2] The *hydrochloride*, $C_{10}H_{10}ON_2,HCl+H_2O$, melts at 96°, and the *platinichloride*, $(C_{10}H_{10}ON_2)_2H_2PtCl_6+4H_2O$, in prisms melting at 110.° Phenyl-methylpyrazolone yields crystalline precipitates with salts of many of the heavy metals. With silver nitrate an aqueous solution gives crystals of $C_{10}H_9AgON_2+$

[1] **Antithermin.**—When an aqueous solution of levulinic acid (aceto-propionic acid), CH_3 CO CH_2 CH_2 COOH, is added to an equivalent amount of phenylhydrazine, dissolved in dilute acetic acid, a yellow precipitate is produced of the hydrazone, $CH_3C(N$ - NHPh) $CH_2.CH_2$ COOH. When recrystallised from alcohol, this forms large colourless, odourless crystals of a slight bitter taste, melting at 108°, and nearly insoluble in water, but soluble in alcohol, ether, and dilute acid. It has met with a limited application as an antipyretic under the name of antithermin and also in cases of phthisis and Bright's disease. It is decomposed by alkalies with liberation of phenylhydrazine, to which fact it probably owes its physiological activity.

[2] When a mixture of phenyl-methylpyrazolone and phenylhydrazine is heated to boiling, bisphenyl-methylpyrazolone, $C_{20}H_{18}O_2N_4$, is formed Heated with methyl alcohol or methyl iodide it yields diantipyrine, $C_{22}H_{22}O_2N_4$, melting at 245°, and distinguished from antipyrine by its sparing solubility in water and the m p, of its picrate (161°). When the compound $C_{20}H_{18}O_2N_4$ is treated in alkaline solution with excess of sodium nitrite, and the mixture poured into dilute sulphuric acid, *pyrazole-blue* $C_{20}H_{16}O_2N_4$, separates in flocks. When crystallised from chloroform it forms blue needles, insoluble in water, dilute acids, and alkalies, and only sparingly soluble in alcohol and ether. Its solutions in chloroform and strong sulphuric acid has an indigo blue colour.

$C_{10}H_{10}ON_2$. The ultramarine cobalt compound and the orange-yellow uranium salt are especially characteristic.

1-Phenyl-2:3-Dimethylpyrazolone. Antipyrine. Phenazone.

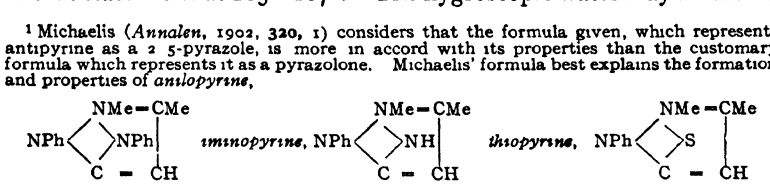
$C_{11}H_{12}ON_2 =$  (Knorr) or  (Michaelis[1])

When 1-phenyl-3-methylpyrazolone is heated with methyl iodide, a further action takes place, with formation of phenyl-dimethylpyrazolone, a substance known generally as "antipyrine," less commonly as "analgesin," and called in the additions to the *British Pharmacopœia* (1890), *phenazone*. It is official in the *German Pharmacopœia* under the name of *Antipyrinum*.

Antipyrine is prepared by heating equal parts of phenyl-methylpyrazolone, methyl iodide, and methyl alcohol to 100° in a closed vessel. The dark product is decolourised by boiling with sulphurous acid, the alcohol distilled off, and the residue shaken with strong sodium hydroxide, when the base separates as a heavy oil. This is separated and treated with ether, in which it is sparingly soluble. On separating the ether and evaporating off the solvent, the antipyrine is obtained as a mass of crystals which are purified by recrystallisation from toluene.

Antipyrine forms small, lustrous, rhombic needles or plates, which are odourless, but have a somewhat bitter taste. When perfectly anhydrous it melts at 114° (*British Pharmacopœia, German Pharmacopœia;* 113° *United States Pharmacopœia*, 8th Rev.), but on exposure to air takes up a small proportion (0.6 %) of water, and in that state melts at 105°–107°. The hygroscopic water may be driven

[1] Michaelis (*Annalen*, 1902, **320**, 1) considers that the formula given, which represents antipyrine as a 2 5-pyrazole, is more in accord with its properties than the customary formula which represents it as a pyrazolone. Michaelis' formula best explains the formation and properties of *antipyrine*,



(Compare *Ber*, 1899, **32**, 2398; 1901, **34**, 723.)

off by exposing the substance to a temperature of 100°, when the original m. p. is restored.

Antipyrine is soluble in about its own weight of cold water, and in less than half its weight of boiling water. It dissolves in twice its weight of absolute alcohol, but in little more than its own weight of rectified spirit (1 part of alcohol, *United States Pharmacopœia*, 8th Rev.). Antipyrine is soluble in an equal weight of amyl alcohol, and in one and a half times its weight of chloroform, but requires about 50 parts of ether for solution (30 parts of ether at 25°, *United States Pharmacopœia*), is difficultly soluble in benzene, and nearly insoluble in petroleum spirit.

On adding strong sodium hydroxide to an aqueous solution of antipyrine, the base separates as a milky precipitate, which speedily collects into oily globules. On adding a little ether, these immediately solidify to white crystals without appreciably dissolving, but they dissolve instantly on adding chloroform (J. C. Waterhouse).

An aqueous solution of antipyrine exhibits no alkaline reaction with litmus or phenolphthalein, but destroys the red colour of an acidified solution of methyl-orange. Free antipyrine may be estimated with accuracy by titration in aqueous or alcoholic solution with methyl-orange.

Antipyrine is a strong monacid base. Its salts, most of which are soluble, do not readily crystallise, with the exception of the *picrate* (m. p. 188°); the *ferrocyanide* $(C_{11}H_{12}ON_2)_2,H_4Cfy$, which forms a crystalline precipitate; the *platinichloride*, $(C_{11}H_{12}ON_2)_2,H_2PtCl_6+2H_2O$, which forms yellowish-red prisms m. p. about 200°; and the *salicylate* (page 45).

When antipyrine is heated with hydrochloric acid under pressure to 200°, it suffers complete decomposition, yielding much aniline and a small quantity of methylamine, besides other products. On distillation with zinc-dust is yields benzene, aniline, a base boiling at 86° to 87°, and other products.

Antipyrine is unchanged by treatment with reducing agents in the wet way, but with oxidizing agents it gives a series of interesting reactions (Gay and Fortuné, *Pharm. Jour.* [*iii*], **18**, 1066). Thus when boiled with potassium chlorate and hydrochloric acid, antipyrine gives a reddish-yellow liquid, which on cooling deposits bright-red oily globules which dissolve in chloroform with greenish-yellow colour. A solution of bleaching powder produces no change in the cold, but on

heating a brick-red precipitate is formed, and the liquid is coloured yellow. Sodium hypochlorite is said to give the yellow colouration on heating without any precipitate being formed. Chlorine-water produces no change, and bromine-water a light yellow precipitate, dissolving on heating. Potassium dichromate and permanganate are reduced by acid solutions of antipyrine.

When a solution of iodine in potassium iodide is added to a solution of antipyrine, a precipitate is formed which disappears on agitation, leaving the solution colourless; but on further addition of the reagent, a permanent brick-red precipitate is produced, perceptible in a dilution of 1 in 20,000. According to Manseau (*Pharm. Jour.* [*iii*], **20**, 162), the point at which a permanent precipitate is formed is perfectly definite, and he suggests that the purity of a sample can be ascertained by titration with a standard solution of iodine. Millard and Stark (*Pharm. Jour.* [*iii*], **20**, 863) find that the point of permanent precipitation depends to a marked degree on the dilution of the antipyrine solution. Thus in a 1% solution, 1 grm. of antipyrine gives a permanent precipitate after the addition of 3.9 c.c. of N/10 iodine, while with twice the volume of water 7 2 c.c. are required. The authors state that more concordant results are obtainable by using starch as an indicator. They dissolve 0.5 grm. of the sample of antipyrine in 200 c.c. of water, add plenty of starch solution, and then drop in N/10 iodine solution gradually until a distinct blue colouration is obtained, which does not disappear on vigorously shaking or stirring the mixture. E. Munzer has described an *iodo-antipyrine*, $C_{11}H_{11}ION_2$, which forms colourless, tasteless needles, m. p. 160°. (Compare page 43.)

An acid solution of mercuric nitrate gives a white precipitate with a solution of antipyrine. 2 c.c. of Millon's reagent and 4 c.c. of a 1% (neutral) solution of antipyrine give a white precipitate in a yellow liquid; in a solution acid with hydrochloric acid, a yellow precipitate in an orange-yellow liquid, the precipitate eventually becoming red. In a solution 10 times more dilute a yellow precipitate and green liquid results, and in an acid solution of 1 part of antipyrine in 20,000, a white precipitate and yellow liquid. 1 c.c. of a saturated solution of mercurous nitrate added to twice its volume of a 1% solution of antipyrine gives a yellow precipitate floating on a blood-red liquid.

If antipyrine be heated with strong nitric acid till action commences, and the liquid be then allowed to cool, a fine purple colouration is pro-

duced; on adding water a violet precipitate is thrown down, and the filtered liquid is purple-red.

Iso-nitroso-antipyrine.—Several of the foregoing indications are probably due to the presence of nitrous acid, which (if added in the form of red fuming nitric acid) gives with a 1% solution of antipyrine a beautiful green colouration, still perceptible when diluted to 1 in 20,000; when the liquid is heated it becomes purple-red. In strong solutions a copious formation of small, green, needle-shaped crystals occurs. These consist of isonitroso-antipyrine, $C_{11}H_{11}(NO)ON_2$, and are best obtained by adding a solution of sodium nitrite to a solution of antipyrine in acidified water. The liquid at once becomes bluish-green in colour, and an abundant formation of crystals speedily occurs. These may be washed with cold water, and dried at the ordinary temperature.[1] Nitroso-antipyrine explodes when heated to about 200°, is nearly insoluble in water and dilute acids, soluble in alkalies and in acetic acid, moderately soluble in alcohol, and sparingly in chloroform and ether. By treatment with zinc and acetic acid it is converted into an oily base.

The green colouration of antipyrine with nitrous acid is delicate and, to a certain extent, characteristic, but is common to all pyrazolones. A. C. Stark recommends that the test should be applied by dissolving potassium nitrite in a test-tube in a little water, adding excess of strong sulphuric acid, and then filling the tube with the liquid to be tested.

Antipyrine dissolves without colour in pure anhydrous ethyl nitrite, but a green colour is immediately developed on addition of water. When antipyrine is added to spirit of nitrous ether containing free acid, the mixture rapidly acquires a dark-green tint, and green needles of iso-nitroso-antipyrine separate. The action (which does not occur if any free acid be neutralised by potassium hydrogen carbonate) derives practical importance from the fact that spirit of nitrous ether and antipyrine are not infrequently dispensed in conjunction. A mixture of the kind is alleged to have been fatal to the patient, but it is very doubtful if the nitroso-derivative of antipyrine was the cause of death; for direct exhibition of the compound to a small rabbit, both hypodermically and by the stomach, in doses commencing at 1/2 grain, and gradually increased to 4 grains, produced no perceptible toxic effect

[1] The liquid filtered from the crystals gradually changes colour from green to brown, and after standing for some hours is found to smell of hydrocyanic acid, but the quantity of this substance formed appears to be very minute (Wood and Marshall, *Pharm. Jour.* [*ui*], 19, 806).

(*Pharm. Jour.* [*iii*], **18**, 1085). Similar experiments have been made on dogs (*Pharm. Jour.* [*iii*], **19**, 807).

Antipyrine gives a very delicate and characteristic reaction with ferric chloride, which, in a 1% solution, produces a blood-red colouration. The reaction is still very distinct in a solution of 1 in 2,000, and perceptible at a dilution of 1 in 50,000. The red colouration is destroyed by excess of mineral acids. The reaction is at once given by urine containing antipyrine.

On mixing cold aqueous solutions of antipyrine and mercuric chloride, a white precipitate is formed. On boiling the liquid this disappears, but on continued boiling a brown resinous substance is deposited, which, when separated, is found to be soluble in hot alcohol and in nitric acid, and is coloured scarlet by concentrated sulphuric acid.

Antipyrine behaves in the general manner of alkaloids. Thus, in acid solutions it gives a yellowish-white precipitate with Mayer's reagent, and the same with Marmé's test (potassio-cadmium iodide); a green precipitate changing to orange-red with bismuth potassio-iodide; an abundant reddish-yellow precipitate with Nessler's reagent; a white with sodium phosphomolybdate and an abundant white precipitate with tannin.[1]

Pharmacopœia Requirements.

The requirements of the *United State Pharmacopœia*, Eighth Revision, are as follows:

M. p. 113°. Must not leave a "weighable residue" on ignition. If to an aqueous solution, tannic acid (T.S.) is added an abundant white ppt. is formed. If 0.1 grm. of sodium nitrite and 12 c.c. of an aqueous solution of antipyrine (1 in 100) be mixed, a nearly colourless liquid is obtained which upon the addition of 1 c.c. of dilute sulphuric acid develops a deep green colour (formation of isonitroso-antipyrine).

If to 2 c.c. of a dilute aqueous solution of antipyrine (1 in 1,000) 1 drop of ferric chloride T.S. be added, a deep red colour is produced which upon the addition of 10 drops of sulphuric acid is changed to light yellow.

Two c.c. of an aqueous solution of antipyrine (1 in 100) mixed with

[1] The reactions described in the text sufficiently indicate the pharmaceutical preparations with which antipyrine is incompatible. Thus it should not be dispensed in a mixture with nitric acid, nitrites, chloral hydrate, solid sodium salicylate, carbolic acid, tannin, iodine, mercuric chloride, salts of iron, permanganates, or tinctures of infusions of catechu, cinchona, roses, galls, rhubard, etc. (see Millard and Stark, *Pharm. Jour.* [*iii*], **20**, 860).

an equal volume of nitric acid assumes a yellowish colour, passing to crimson on warming (distinction from acetanilide and acetphenetidine).

On warming 0.1 grm. of antipyrine with sodium hydroxide T. S. and again warming after the addition of chloroform, the disagreeable odour of phenyl isocyanide should not be developed (absence of acetanilide).

According to the *German Pharmacopœia*, the solution of antipyrine in 2 parts of water should be neutral, free from acrid taste, and not changed by hydrogen sulphide water. A 2% solution should give a white precipitate with tannin; and on addition of 2 drops of fuming nitric acid to 2 c.c. of the solution, a green colouration should occur, changed to red on boiling and adding another drop of nitric acid.[1] 2 c.c. of a 0.2% solution gives a deep red colour with a drop of ferric chloride solution, changed to bright yellow on adding 10 drops of sulphuric acid.

The *Japanese Pharmacopœia* is similar to the German in its tests.

Antipyrine has now an established position and wide application in medicine. Although originally introduced as a febrifuge, it is taking a still higher place as an anodyne. Given in 10 to 20 grain doses in cases of bilious and nervous headache, it often effects a remarkably rapid and perfect cure. It has been usefully injected hypodermically in 8-grain doses as a substitute for morphia; and for the relief of pain in acute and chronic gout, neuralgia, sciatica, etc. The subcutaneous injection of antipyrine is said not to be followed by drowsiness, vomiting, or excitement. It is stated to be almost a specific in puerperal fever. It has been found valuable as a hemostatic, and has proved successful in some cases of sea-sickness, but by no means invariably. Antipyrine causes an almost immediate reduction in the temperature of the body (apparently from its influence on the brain-centres regulating the temperature), the effect continuing from 4 to 6 hours. It induces sweating and feeble pulse, and in excessive doses, or even small doses in certain cases, an eruption resembling nettle-rash, occasionally with vomiting and collapse.[2] Atropine has been found to act promptly as an antidote.

[1] This red colouration is said by Sperling (*Chem Centr.*, 1906, I, 1118) to be due to nitroantipyrine. It is not always distinctly apparent and sometimes a brown colouration is obtained. Antipyrine and all its derivatives except aminoantipyrine give the following reaction: 2 drops of fuming nitric acid are added to 2–3 c.c. of a 1 % solution in water and then 5 % of conc sulphuric acid are cautiously added. A cherry-red ring is formed at the surface of contact and when the layers are mixed the colour permeates the mixture

[2] The exhibition of antipyrine is unsafe when the heart is weak. A case where severe symptoms were produced by a dose of 1 grm. has been recorded by Schwabe (*Pharm. Jour. [iii]*, 20, 1059).

Antipyrine may be detected in the urine for 18 to 24 hours after it is taken by the stomach, but can be detected only for a few hours in the different organs. It has been detected, after putrefaction for a fortnight, in animals killed within 2 hours after its administration, either by the stomach or hypodermically.

Antipyrine is readily extracted from animal matters, by rendering the liquid ammoniacal and agitating it with chloroform or amyl alcohol.

Steensma (*Pharm. Weekblad.*, 1907, **44**, 1066) recommends *p*-dimethylaminobenzaldehyde as a means of detecting antipyrine. The reagent is prepared by diluting a solution of 1 grm. of the aldehyde in 5 c.c. of 25% hydrochloric acid to 100 c c. with absolute alcohol. When a small portion of this solution in presence of a trace of antipyrine is evaporated in a porcelain dish to dryness on the water-bath, a light red stain is left. The test serves to detect 0.001 mgrm. of antipyrine. Aqueous solutions should be extracted with chloroform, the solvent evaporated and the residue dissolved in the reagent.

Estimation of Antipyrine.

1. **Kippenberger's Iodometric Method.**—On adding a solution of iodine or an iodide to aqueous solutions of antipyrine, acidified or not, a brown tarry, non-crystallisable mass of the composition $C_{11}H_{12}ON_2$, HI, I_2, separates. Advantage may be taken of this fact to separate antipyrine from phenacetin, sulphonal, acetanilide and aniline salts if acid be present, hydrochloric acid being most suitable. The process is carried out as follows:

To the solution of antipyrine (as concentrated as possible) contained in a stoppered flask, a solution of iodine is added, made by mixing 100 c c. of an N/20 iodine solution, containing 10 or 20 grm. of potassium iodide per litre, with about 4 c.c. of hydriodic acid of sp. gr. 1.7 (52% HI). *Only a small excess of iodine solution is added* and the flask then well shaken until the liquid becomes clear, the precipitate adhering to the walls of the flask. The liquid is filtered through a small asbestos filter into a dry burette and in an aliquot proportion of the filtrate the iodine is estimated by N/20 thiosulphate. 21.3 c.c. of N/20 iodine = 0.1 grm. of antipyrine. The error due to the solubility of the periodide is generally negligible, but may be corrected

for by standardising the iodine solution against a solution of antipyrine of known strength.

Salipyrine and other antipyrine salts may be estimated in the same way.

2. **Picric Acid Method** (Lemaire, *Pharm. Jour.*, 1905, **74**, 13).—A known volume of the solution containing antipyrine is treated with a definite excess of an N/20 solution of picric acid and the sparingly soluble picrate filtered off after standing. The free picric acid is estimated in an aliquot portion of the filtrate by titration with N/10 sodium hydroxide, using phenolphthalein as indicator. 1 mol. of antipyrine (188) combines with 1 mol. of picric acid (229).

Detection of Adulterants in Antipyrine.

The following methods are given by Raikow and Schtarbonow (*Oester. Chem. Zeit.*, 1900, **3**, 125).

Acetanilide (antifebrin) and phenacetin (*p*-acetamino-phenetole) can be detected by boiling the antipyrine with concentrated phosphoric acid, the 2 anilides yielding acetic acid under these conditions, which can be recognised by its smell. Antipyrine gives a yellow colour with phosphoric acid which gradually changes to brownish-yellow, acetanilide gives a faint yellow colour which becomes brown on boiling. With phenacetin the solution is first rose coloured, then brownish red, changing through reddish-violet to violet, bluish-green to a dirty green. The appearance of a violet colouration is specially characteristic of phenacetin.

Acetanilide and phenacetin are distinguished by their different behaviour on hydrolysis with potassium hydroxide. A few grm. of the substance are heated with 2–4 c.c. of conc. aqueous potassium hydroxide in a test-tube, fitted with a rubber stopper through which passes a glass tube connected with a second test-tube containing 1–3 c.c. of a clear solution of calcium hypochlorite (bleaching powder). If acetanilide is present in the antipyrine the first drops of the distillate produce the well-known violet colouration characteristic of aniline. In the absence of acetanilide and presence of phenacetin the first drops give no colouration but subsequently a brick-red turbidity due to phenetidine is produced. Finally an amorphous red substance separates on the surface of the liquid, which becomes clear yellow in colour. If the receiving test-tube be changed when both acetanilide and phe-

nacetin are present, the two indications may be observed successively. On boiling a mixture of phenacetin and antipyrine with potassium hydroxide the distillate does not give the above described red colouration characteristic of phenacetin but the solution becomes yellowish-green and then yellowish-grey. With antipyrine alone the bleaching powder solution remains colourless.

Exalgin (methylacetanilide) when boiled with phosphoric acid is readily hydrolysed, giving acetic acid and the phosphoric acid becomes coloured intensely golden yellow. On boiling with potassium hydroxide, methylaniline distils over and collects in oily drops on the surface of the calcium hypochlorite: a green colour is produced which becomes greyish-green and finally a dirty green.

Salipyrine (Antipyrine Salicylate), $C_{11}H_{12}ON_2,C_7H_6O_3$.—If salicylic acid be gradually added to a dilute boiling solution of antipyrine, antipyrine salicylate separates as a yellowish oil. The compound can be more conveniently prepared by heating equivalent proportions of antipyrine and salicylic acid with a little water to 90°, or by shaking together an aqueous solution of antipyrine with an ethereal solution of salicylic acid, when the salt separates in fine crystals. Antipyrine salicylate melts at 91–92°, and decomposes at a somewhat higher temperature, dissolves in 250 parts of cold water and in 25 parts hot, and readily in alcohol, ether, chloroform, and carbon disulphide. The aqueous solution is faintly acid in reaction, and has a sweet taste and bitter after-taste. It gives a violet-red colouration with ferric chloride, and green with nitrous acid. Antipyrine salicylate has been employed with favourable results in medicine under the name of "salipyrine." A mixture of antipyrine and sodium salicylate gradually changes to an oily liquid on exposure to air. The change, which does not occur in a closed bottle, appears to be simply due to absorption of moisture by the salicylate and the solution of the antipyrine in the water thus absorbed. Antipyrine salicylate is official in the *Japanese Pharmacopœia.*

Resalgin (Resopyrine).—Antipyrine becomes pasty when mixed with β-naphthol, and appears to form a compound with phenol. Under the name of "resopyrin," Portes has described a compound obtained by mixing solutions of molecular proportions of resorcinol and antipyrine. It crystallises in oblique rhombic prisms, insoluble in water but soluble in alcohol.

Hypnal (Chloral-antipyrine), $C_{11}H_{11}(C_2H_2Cl_3O)ON_2$.—When dilute solutions of chloral hydrate and antipyrine are mixed no per-

ceptible reaction occurs, but on concentrating the liquid, or on mixing strong solutions of the 2 substances, a separation of oily globules takes place, and these immediately or gradually change to a mass of crystals of chloral-antipyrine. The same substance may be obtained by heating molecular proportions of chloral hydrate (165.5 parts) and antipyrine (188 parts) to 110–115°. The action consists in elimination of water and substitution of the group $CCl_3.CH(OH)$ for one of the hydrogen atoms of the antipyrine; but whether the replaced atom is one of those of the methyl groups, or the hydrogen atom of the CH group, is not definitely decided (compare *Pharm. Jour.* [*iii*], **20,** page 862 with page 889).

Chloral-antipyrine, also called hypnal, crystallises from alcohol in hard scales and from water in transparent rhombs. It melts at 67–68°, is almost odourless, and has a saline taste with an after-taste suggestive of chloral. It is only slightly soluble in cold alcohol, ether, and chloroform, but somewhat more soluble in boiling alcohol, and is dissolved by about 8 parts of warm water. The solution reduces Fehling's solution on warming, gives the blood-red reaction of antipyrine with ferric chloride, and yields chloroform when heated with dilute alkali hydroxide When chloral-antipyrine is kept in a melted state for some time, it deposits crystals of a *dehydration compound*, which is insoluble in water, melts at 186–187°, and gives no colour-change with ferric chloride. According to Reuter (*Pharm. Jour.* [*iii*], **20,** 602) chloral-antipyrine is physiologically inert, but Bardet found doses of 1 grm. to induce sleep as readily as chloral hydrate, while in cases of insomnia caused by pain it seemed to have the same anodyne effect as antipyrine. Schmidt found the monochloral-derivative to have more decided soporific effect and a less deleterious influence on the circulation than antipyrine.

Bichloral-antipyrine is obtained by heating antipyrine with excess of a strong solution of chloral hydrate, when an oily layer is formed, which solidifies to prismatic crystals melting at 67–68°, soluble with some dissociation in 10 parts of cold water, and giving the reactions of chloral-antipyrine.

Butylhypnal, a compound of antipyrine with butyl chloral hydrate, forms colourless needles, m. p. 70°.

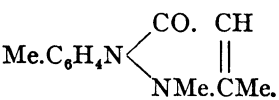
Tussol, antipyrine mandelate, $C_{11}H_{12}ON_2,C_6H_5.CHOH.CO_2H$, obtained by fusing together antipyrine and mandelic acid, forms colourless crystals, m. p. 52–53°; it is sparingly soluble in water (1 in 15),

easily so in alcohol. It may be recognised by its m. p., by giving a red colouration in aqueous solution (1 in 20) on adding ferric chloride, and a smell of benzaldehyde when warmed with potassium permanganate.

Migrainine (migranin) is a mixture of 90 9 parts of antipyrine with 6.6 parts of citric acid and 8.5 parts of caffein.

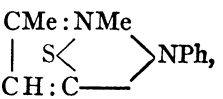
To estimate antipyrine in migrainine, 1.1 grm. is dissolved in 100 c c. of water, 20 c.c. of the solution is mixed with 20 c.c. of an alcoholic solution of mercuric chloride (2 5 grm. $HgCl_2$ to 100 c.c. of 95% alcohol) and an alcoholic solution of iodine is added which contains 1 351 grm of iodine per 100 c c. The iodine solution is standardised in the same manner against 20 c c. of a 1% solution of pure antipyrine. 20 c c. of the migrainine should use as much iodine as 0.2 grm. of antipyrine.

Tolypyrine is 1-*o*-Tolyl-2·3-dimethylpyrazolone,



For tests see table on following page.

Thiopyrine (Thioantipyrine)



(1-phenyl-2:3-dimethyl-2.5-thiopyrazole) is a less energetic antipyretic than antipyrine, but is said to have no injurious after-effects. It melts at 166°. It gives a transient green colouration with ferric chloride but not with nitrous acid. The crystalline *hydrochloride* has m. p. 128°, the platinichloride is brownish-red and melts and decomposes at 215.

Selenopyrine (selenoantipyrine), (Michaelis), forms lustrous pale yellow crystals, m. p. 168°, does not develop a colouration with ferric chloride, and only a faint green colouration with nitrous acid. The hydrochloride and sulphate do not crystallise.

Pyramidone (Dimethylaminoantipyrine, 1-Dimethylamino-2:3-dimethylpyrazolone). German Patents, 71261; 90959; 111724. M. p. 108°.

The following table, according to Hofmann (*Zeit. Unt. Nahr. u. Genussm.*, 1900, **6**, 419), shows differences in behaviour of antipyrine,

tolypyrine, amino-antipyrine and pyramidone. (Compare, however, Monferrino, *infra.*)

	Antipyrine	Tolypyrine	Amino-antipyrine	Pyramidone
Ferric chloride.	Red-brown colour, (1 in 2,000)	Red-brown colour.	Violet colour (1 in 20,000)	Blue by reflected, violet by transmitted light
Silver nitrate	No change	No change	Reddish to red-violet colour	Colour first blue then silver then separates
Nitrous acid	Bright green	Green	Fugitive red	Blue inclining to violet
Nitric acid	Warm, deep red	Cherry red		No red colouration
Wagner's reagent	Brown red ppt (1 in 2,000), which disappears on heating	Brown-red ppt (1 in 2,000) which disappears on heating	Yellowish-brown turbidity (1 in 2,000), disappears on heating giving red-violet solution	Violet colouration Excess of the reagent gives a turbidity, which dissolves on warming
Bromine water.	White pp	White ppt	Yellowish-white ppt (1 in 2,000) In 1% solution the ppt is brilliant violet-red Ammonia destroys the colour, sulphuric acid restores it	Concentrated solutions give a black or gray colouration
2% solution of blood mixed with 4 times its volume of hydrogen peroxide	Brown colour due to meta-hæmoglobin	Brown colour due to meta-hæmoglobin	When H_2O_2 added a faint red colour, which on adding blood turns dark red with tendency to blue	In very dilute solution gives violet colouration

Distinctive Tests for Antipyrine, Pyramidone and Nevralteine.

Monferrino (*Boll. Chim. Farm.*, 1909, **48**, 515) states that when present together in aqueous solution the 3 compounds may be detected by the following tests (Note: Nevralteine is sodium *p*-phenetidinemethanesulphonate).

Reagent	Antipyrine	Pyramidone
Potassium nitrite and concentrated sulphuric acid.	Green colouration changing to bluish-green.	Transient amethyst-violet colouration when present in greater quantity than antipyrine.

A little of the violet liquid obtained by the addition of ferric chloride when added to concentrated sulphuric acid gives a green colouration if nevralteine is present.

Detection of Pyramidone in Urine.

According to Jolles (*Zeit. Anal. Chem.*, 1898, **37**, 441) when a weak solution of iodine (a 10% alcoholic solution diluted with 10 vols. of alcohol) is poured on to the surface of, but not mixed with, urine containing pyramidone, a well marked violet-red ring forms at the surface of separation and gradually changes to red-brown. The test is said to be characteristic.

Estimation of Pyramidone.

Astruc and Pégurier (*Ann. Chim. Anal.*, 1905, **10**, 302) apply Lemaire's picric acid method of estimating antipyrine to the estimation of pyramidone. 0.231 grm of the sample is dissolved in 10 c.c of water and 40 c.c. of N/20 picric acid solution are added. After shaking during some minutes, the mixture is filtered and in 25 c.c. of the filtrate the excess of picric acid is titrated with N/10 alkali hydroxide using phenolphthalein as indicator. If n c.c. of alkali be used the per cent. of pyramidone $= 5(40-4n)$.

Estimation of Pyramidone in Presence of Antipyrine.

Pégurier (*Ann. Chim. Anal.*, 1905, **10**, 391) utilises the fact that while antipyrine is neutral to methyl orange, pyramidone behaves as a monacid base with this indicator. The 2 bases are estimated together according to the method of Astruc and Pégurier just given. 0 231 grm. of the sample is then dissolved in 10 c.c. water, the solution exactly neutralised with N/10 acid in presence of methyl orange and the antipyrine estimated by means of picric acid. For this purpose, 40 c.c. of N/20 picric acid are added and the solution after filtration titrated with N/10 KOH after adding phenolphthalein (see page 44).

Detection of Antipyrine in Pyramidone.

Steensma states that it is possible to detect 0.005 mgrm. of antipyrine in 100 mgrm. of pyramidone by dissolving the latter in *p*-dimethylaminobenzaldehyde reagent (see page 43) and evaporating the solution as already described.

For tests for halagen salts in pyramidone, see Kollo, *Pharm. Post.*, 1911, 173. *Pharm. J.*, 1911, **86**, 711.

ANILINE AND ITS ALLIES.

By S. S. SADTLER.

Aniline, $C_6H_5.NH_2$, or $\left.\begin{matrix}(C_6H_5)\\H\\H\end{matrix}\right\}N$

Aniline exists in minute quantity in coal tar, but is ordinarily produced by nitrating benzene and reducing the resulting nitrobenzene, $C_6H_5NO_2$, by suitable means.

If the treatment with nitric acid be carried further, dinitrobenzene is produced, and this by reduction is converted into meta-phenylene-diamine or metadiamino-benzene, $C_6H_4(NH_2)_2$.

If the reduction of nitrobenzene be effected by alkaline reagents, 2 molecules unite, and azobenzene, $C_6H_5.N{\cdot}N.C_6H_5$, is produced. On further treatment of this (especially in alcoholic solution) it is converted into hydrazobenzene, $C_6H_5\,NH.NH.C_6H_5$, which by intramolecular change is transformed into benzidine or di-para-amino-diphenyl, $NH_2.C_6H_4.C_6H_4.NH_2$. The relationship of aniline to the allied bases is shown below:

Aniline (aminobenzene)	Aniline.	Aniline (Phenylamine)
$NH_2.C_6H_4.H$	$C_6H_5.NH.H$	$C_6H_5\,NH.H$
Phenylene-diamine.	Phenylhydrazine	Diphenylamine
$NH_2.C_6H_4.NH_2$	$C_6H_5.NH.NH_2$	$C_6H_5.NH.C_6H_5$
Benzidine	Hydrazobenzene [1]	Hydrazobenzene [1]
$NH_2.C_6H_4.C_6H_4,NH_2$	$C_6H_5.NH.NH.C_6H_5$	$C_6H_5.NH.NH.C_6H_5$

Aniline forms two classes of homologues. The true homologues (Class A) coexist with aniline in coal tar, and are derived from aniline by the substitution of one or more methyl groups for a corresponding number of the hydrogen atoms of the benzene nucleus. They are ordinarily obtained by nitrating the corresponding hydrocarbons prepared from coal-tar naphtha, and reducing the resulting nitro-derivatives. Thus:

[1] Hydrazobenzene does not have basic properties.

Hydrocarbon.	Nitro-derivative	Amino-derivative.
Benzene—	Nitrobenzene—	Aniline—
$C_6H_5.H$	$C_6H_5.NO_2$	$C_6H_5.NH_2$
Toluene—	Nitrotoluene—	Toluidine—
$C_6H_4(CH_3).H$	$C_6H_4(CH_3).NO_2$	$C_6H_4(CH_3).NH_2$
Xylene—	Nitroxylene—	Xylidine—
$C_6H_3(CH_3)_2.H$	$C_6H_3(CH_3)_2.NO_2$	$C_6H_3(CH_3)_2.NH_2$
Cumene—	Nitrocumene—	Cumidine—
$C_6H_2(CH_3)_3.H$	$C_6H_2(CH_3)_3.NO_2$	$C_6H_2(CH_3)_3.NH_2$

Isomeric modifications are known of all the members of the series except those in the first line (page 77, *et seq.*).

The pseudo-homologues of aniline (Class B) are derived from aniline by the replacement of one or both of the hydrogen atoms of the amino-group by methyl or other alkyl radical. Similar substitutions can be effected in the amino-groups of toluidine, xylidine, etc.

These alkyl substituted anilines (Class B) are obtained by the action of methyl chloride or other alkyl salt on aniline, or of the corresponding alcohol on the hydrochloride or other salt of aniline. *p*-Toluidine has also been obtained in a very interesting manner by heating the hydrochloride of methyl-aniline[1] to 350° in a sealed tube, when change of position of the atoms within the molecule takes place thus:

$$\left.\begin{matrix} C_6H_5 \\ CH_3 \\ H \end{matrix}\right\} N \quad = \quad \left.\begin{matrix} C_6H_4(CH_3) \\ H \\ H \end{matrix}\right\} N$$

Methyl-aniline. *p*-Toluidine

By the same process methyl-toluidine may be converted into xylidine, and this by consecutive steps into a pseudo-cumidine, isoduridine, and amino-pentamethylbenzene. By treating aniline hydrochloride with aniline, diphenylamine or phenylaniline, $C_6H_5.NH\text{-}(C_6H_5)$, is obtained[2] (page 95).

Substitution of the hydrogen atoms of aniline and its homologues can also be effected by acid groups or chlorine, both in the benzene-nucleus and in the amino-group. In the latter case the derivatives are

[1] If the hydriodide of methyl-aniline be similarly treated, ortho- or meta- toluidine is obtained.

[2] Diphenylamine and aniline hydrochloride cannot be caused to react with formation of triphenylamine, $(C_6H_5)_3N$, but this substance can be obtained by the action of mono-brom-benzene on di-potassium aniline —

$$C_6H_5Br + C_6H_5.NK_2 = (C_6H_5)_3N + 2KBr.$$

called anilides (page 82), and are quite different from the substances resulting from the substitution of chlorine for the hydrogen of the benzene nucleus. In the compounds of the latter class, the basic character is either much weakened or entirely destroyed. Most of the derivatives exist in several isomeric modifications, according to the position of the substituting radicals in the benzene-nucleus. Examples of the substances of this class are:

Aniline-sulphonic acid or sulphanilic acid, $C_6H_4(SO_3H).NH_2$.
Nitraniline, $C_6H_4(NO_2).NH_2$.
Bromaniline, $C_6H_4Br.NH_2$.
Trichloraniline, $C_6H_2Cl_3.NH_2$.

Mixed substitution-products, belonging at once to 2 or more of the foregoing classes, are obtained by suitable means. As examples may be mentioned:

Paranitracetanilide,	$C_6H_4(NO_2).NH(C_2H_3O)$
Paranitroso-dimethylaniline,	$C_6H_4(NO).N(CH_3)_2$
Paranitroso-dimethyl-*p*-toluidine,	$C_6H_3(CH_3)(NO).N(CH_3)_2$

The more important of the allies and derivatives of aniline formulated on this and the preceding pages are described in greater detail in the sequel.

On treating aniline, and also many of the above-mentioned homologues and derivatives, with oxidising agents, a series of brilliant colouring matters are obtained, which form the well-known "aniline dyes" (Vol. 5).

By the action of nitrous acid on a cold solution of a salt of aniline a salt of diazobenzene is obtained. This and the allied products obtained by similar means from the homologues and analogues of aniline form the starting-point of the numerous and important colouring matters known as the "azo-dyes" (Vol. 5).

By the action of reducing agents on the salts of diazobenzene, phenylhydrazine, $C_6H_5NH(NH_2)$, is obtained. This substance has already been fully described.

Aniline. Amino-benzene. Phenylamine.

$$C_6H_7N = C_6H_5.NH_2 = \left.\begin{matrix} C_6H_5 \\ H \\ H \end{matrix}\right\} N$$

Aniline was first obtained in 1826 by Unverdorben by the dry distilla-

tion of indigo, and received the name *crystalline*. Runge in 1834 obtained it from coal tar, and termed is *kyanol*. The name *aniline* is due to Fritsche, who in 1841 obtained it by distilling indigo with caustic alkali. The name *benzidam* was given it in 1842 by Zinin, who prepared it by reducing nitrobenzene by sulphuretted hydrogen. The name *phenamine* has also been proposed for it. Aniline was first accurately described in 1843 by A. W. Hofmann.

Aniline occurs to a very limited extent ready-formed in the products of the distillation of coal, bone, and peat. Almost the whole of it, however, is obtained indirectly from coal-tar by the action of a reducing agent on nitrobenzene ("Aniline Oils," page 74). Aniline may also be obtained by passing ammonia and benzene vapour through a red-hot tube; $C_6H_6+NH_3=H_2+C_6H_7N$. It is also formed together with diphenylamine by the reaction of phenol and ammonia. The best yield is obtained by heating phenol to about 330° for 20 hours with ammonium chloride and magnesia or oxide of zinc (or ammonio-zinc chloride, $Zn(NH_3)_2Cl_2$). Aniline is also obtained by numerous other reactions.

Aniline may be purified by fractional distillation and conversion into the acetyl-derivative. This is recrystallised from water, and on saponification yields pure aniline.

Pure aniline is a colourless, oily liquid, of faintly vinous odour and aromatic, burning taste. It refracts light strongly, but has no rotatory action. Aniline, when very pure, freezes at 6°, but a slight impurity greatly reduces its solidifying point. It boils at 184°, and distils unchanged.

The sp. gr. of aniline is 1.0379 at 0° and 1.0342 at 4°, compared with water at 4°; and 1.0254 at 15°, compared with water at the same temperature. The coefficient of expansion is .000818.

Aniline becomes yellow or brown on exposure to air and light, especially at elevated temperatures, a resinous substance being ultimately formed. The change is due to oxidation, and does not occur *in vacuo* or in the dark. According to A. Bidet (*Comp. Rend.*, 1889, **108**, 520), aniline and toluidine prepared by the reduction of pure nitro-derivatives are colourless after distillation, and though they become yellowish in a few days, light has no further effect on them, and even this change Bidet attributes to the presence of amino-thiophene, $C_4H_3S.NH_2$.

Aniline is only slightly soluble in water, requiring 31 parts at the

ordinary temperature, but being more soluble in hot water. Water also dissolves in aniline, 5 parts being taken up by 100 of aniline at the ordinary temperature, and somewhat more at higher temperatures. The greater part can be separated by distillation, the water passing over first, but the last traces can only be removed by prolonged digestion over alkali hydroxides.

Aniline is soluble in all proportions in a 50% aqueous solution of its hydrochloride, and in smaller proportions in more dilute solutions.

Aniline dissolves readily in ethyl and methyl alcohols, ether, acetone, chloroform, carbon disulphide, and volatile hydrocarbons.

Aniline is itself a solvent for sulphur, phosphorus, indigotin, camphor and colophony, but does not dissolve caoutchouc or copal. It is employed sometimes as a solvent for aniline-blue.

Aniline is a powerful poison, coagulating albumin and producing symptoms similar to those caused by nitrobenzene (Vol. 3, page 213).

According to Letheby and Turnbull the action of aniline is chiefly on the nervous system. According to Grandhomme, the first symptom in slight cases of poisoning by aniline, caused by inhaling the vapour, is a blue colour on the edge of the lips, while the gait becomes unsteady, the speech thick, the head affected, and the face pale, while the appetite fails completely. Alcohol aggravates the symptoms. In more severe cases, such as may arise from the saturation of the clothes with aniline, the lips become dark blue or black, and the vertigo is so violent that standing becomes impossible. According to Wohler and Frerichs, aniline does not exert any poisonous action on dogs. Runge found the aqueous solution to kill leeches and the parts of plants immersed in it.

Aniline has marked basic properties, a long series of well-defined and crystallisable salts being obtained from it. It has, however, no action on phenol-phthalein, litmus or turmeric, though it affects a few of the more delicate vegetable colours such as hematoxylin. It expels ammonia from its salts at a boiling temperature, but is itself displaced in the cold. Aniline decomposes the solutions of many metallic salts, with precipitation of the corresponding hydroxides. When heated with strong sulphuric acid, aniline is converted into *p*-amino-benzene-sulphonic acid (sulphanilic acid). With hot fuming sulphuric acid, di-sulphonic acids are produced.

In presence of an excess of acid, aniline imparts a deep yellow colour to pine-wood and alder-pith.

According to Friswell, on adding cupric sulphate to an aqueous solution of aniline an apple-green crystalline precipitate is formed; or in extremely diluted solutions a green colouration.

Cold aqueous solutions of aniline salts are converted by treatment with nitrous acid (or a nitrite and mineral acid) into salts of diazobenzene. On boiling the solution phenol is formed, with evolution of nitrogen.

If aniline, or one of its salts in the solid state, be treated with a drop of chloroform, and then solid potassium hydroxide or a strong solution of potassium hydroxide in alcohol be added, and the whole gently heated by immersing the tube in hot water, a peculiar and highly unpleasant odour will be produced, due to the formation of phenylcarbamine, $C_6H_5.NC$. The reaction, which is known as "Hofmann's isonitrile test," is produced by other aromatic monamines, and by acetanilide.

Under the influence of oxidising agents aniline gives products and reactions which vary considerably according to the oxidising agent employed, thus:

a. When aniline is treated with excess of nitric acid, and the mixture evaporated at 100°, the base is decomposed with formation of a brown substance. With smaller proportions of nitric acid various coloured products are formed, including picric acid.

b. When treated with dilute sulphuric acid and manganese dioxide, aniline yields ammonia and quinone, $C_6H_4O_2$, but the greater part of the product undergoes still further change.

c. If aniline be dissolved in strong sulphuric acid, and a few drops of a solution of potassium dichromate is added, a red colour is produced, which rapidly changes to deep blue.

d. On treating aniline, or one of its salts in a solid state, with strong sulphuric acid, and then adding a minute fragment of manganese dioxide or other oxidising agent (in the manner described under "strychnine," page 449), a fine purple colouration is produced. A better result is obtainable by employing electrolytic oxygen; in this form the test is the most delicate and satisfactory which can be applied.

e. Chlorine acts on dry aniline with great violence, producing a black mass containing trichloraniline, $C_6H_4Cl_3N$. Bromine behaves simi-

larly; and, on adding bromine-water to the aqueous solution of an aniline salt, a precipitate of tribromaniline is formed. On the other hand, Mills and Muter (*J. Soc. Chem. Ind.*, 1885, **4**, 96) state that aniline in solution in carbon disulphide reacts with bromine, probably forming an additive compound.

f. When a solution of aniline is treated with a dilute solution of bleaching powder, avoiding excess, a fine purple colouration results, which gradually changes to brown. When carefully applied, the reaction is delicate and characteristic. The colour is destroyed by ether.

g. It a minute quantity of aniline be treated with an aqueous solution of phenol, and a solution of bleaching powder be then gradually added, the reagent produces yellow striæ, which change to a greenish-blue. The test, which is due to Jacquemin, is said to be very delicate.

The following work on the reduction of aniline has been done by E. Bromstein (*Ber.*, 1901, **34**, 1268–74).

The oxidation of aniline hitherto has resulted in products in which the amino group has been attacked, yielding for example, azo, azoxy-, nitroso- and nitrobenzene, phenylhydroxylamine, amino-phenol, and finally quinone. Bromstein oxidises aniline salts in neutral solution at a low temperature, and a certain concentration preferably with lead peroxide. A 20–25% yield of amino-diphenylquinonediimine, $H_2N.C_6H_3.(NC_6H_5)_2$ is obtained which on more energetic oxidation is converted into Azophenine, $C_6H_2(NHC_6H_5)_2(NC_6H_5)_2$. The conversion of the amino-quinone-imine into Azophenine can also be shown by dissolving the former in an excess of aniline and warming the solution for a short time with some aniline hydrochloride or zinc chloride. For the preparation of amino-diphenylquinonediimine aniline hydrochloride or sulphate is dissolved in 20–25 times its weight of water and oxidised with one and a half to twice its weight of lead peroxide (PbO_2) in the form of paste in the cold. The magenta coloured filtrate contains a dyestuff of no particular value, and the residue after drying is extracted with benzene and treated with light petroleum spirit which precipitates a tarry residue. The solution on standing deposits crystals, which recrystallise from alcohol separates in blackish-red nodules. The residue insoluble in alcohol consists of azophenine, melting at 246°. Amino-diphenylquinonediimine melts at 167°, and is very unstable towards acids. Reduction with ammonium sulphate yields a colourless subtsance melting as 83°, which gives an acetyl compound corresponding to the formula: $C_6H_3(NHC_2H_3O)(NHC_6H_5)_2$, m. p. at 171°.

Detection and Separation of Aniline.

The foregoing colour-reactions are amply sufficient for the recognition of aniline, provided that a proper process of separation be previously applied.

Aniline may be liberated from the aqueous solutions of its salts by addition of sodium hydroxide, and may then be extracted by agitating the liquid with ether. On separating the ethereal layer, and agitating it with dilute hydrochloric acid, the aniline passes into the aqueous liquid, which may then be concentrated or evaporated to dryness, and examined by the colour-reactions already described. From strychnine, which is the only substance with which aniline is at all likely to be confounded, if may be separated by adding sodium hydroxide to the concentrated solution, and distilling over the aniline by means of a current of steam. The strychnine remains in the flask, while the aniline will be found in the distillate if it be acidified with hyrdochloric acid and concentrated to a small bulk at 100°. The same plan may be employed for detecting aniline in toxicological cases, or the process used for isolating strychnine may be used, but instead of evaporating the ether-chloroform it should be separated and agitated with dilute hydrochloric acid in the manner above described.

F. Muller (*Trans.*, 1888, **52**, 514) found unchanged aniline in the urine of a person poisoned with it. The urine was optically inactive, but reduced Fehling's solution. A portion of the concentrated urine, when boiled with strong hydrochloric acid, neutralised with sodium hydroxide, and extracted with ether, gave an ethereal solution which showed the blue indophenol reaction. The ethereal extract of the unboiled urine did not give this reaction, a fact which Muller believes was due to the secretion of the aniline as *p*-aminophenylsulphate (compare "Phenyl-sulphuric Acid," Vol. 3, page 399); a substance which is split up by boiling with hydrochloric acid. In support of this, the original urine contained sulphates (estimated by barium chloride) equivalent to only 0.0475 grm. of sulphuric acid per litre; but after boiling with hydrochloric acid, 0.8085 grm. A direct test for the presence of *p*-aminophenylsulphates in urine consists in boiling the liquid with one-fourth of its volume of strong hydrochloric acid, adding a few c.c. of a 3% solution of phenol, and then some drops of a chromic acid solution. If *p*-amino-phenol be present, the liquid becomes red, and turns blue on adding ammonia.

The *estimation* of aniline may be effected by evaporating its ethereal solution, or preferably by extracting the base therefrom by agitation with dilute hydrochloric acid, evaporating the acid liquid, and weighing the residual hydrochloride. Under favourable circumstances it may be measured after liberation from a strong solution of the hydrochloride by addition of alkali hydroxide.

Instead of weighing the aniline hydrochloride, the salt may be redissolved in water, and the solution titrated with standard silver nitrate. Or it may be titrated with standard alkali hydroxide and phenolphthalein or litmus, as aniline hydrochloride acts on these indicators exactly like an equivalent quantity of free hydrochloric acid, and the end-point is sharply marked. The process allows of the titration of aniline in presence of neutral ammoniacal salts. On the other hand, with helianthin (methyl-orange), the basic character of free aniline is distinctly marked, but the end-point is not sufficiently definite to render the indicator available for accurately titrating aniline.

According to Julius (*J. Soc. Dyers, etc.*, **21**, 79), free aniline in aqueous solution can be satisfactorily titrated with standard sulphuric or hydrochloric acid, if congo-red be employed as an indicator and the neutral point be regarded as that at which a bluish-violet colour is obtained, not changed by further small additions of acid; but a much larger excess is required to produce a pure blue. Results are said to be obtainable agreeing within 0.2% with the theoretical.

Salts of Aniline.

Aniline combines readily with acids forming a series of salts which crystallise well. The following are the most important.

Aniline Hydrochlorate.—Hydrochlorate of Aniline. C_6H_7N,HCl. This salt crystallises with great facility in colourless needles or large plates, which are very soluble in water and alcohol. M. p. 198°, and boils at 245° unchanged. It yields double salts with stannic, mercuric, antimonious, platinic and auric chlorides; *aniline chloroplatinate*, $(C_6H_7N,HCl)_2PtCl_4$, crystallises from hot water in yellow needles. *Aniline salt* is the ordinary commercial name for aniline hydrochloride. It is manufactured by mixing the calculated weights of aniline and hydrochloric acid in stone-tanks, freeing the crystals formed from the mother-liquor by a centrifugal machine, and drying them. According to another process, aniline is dissolved in petroleum spirit of 0.720 sp. gr.,

and hydrochloric acid gas passed in till the solution is saturated. The aniline salt is deposited as a white powder, which is separated from the adhering petroleum spirit by hydraulic pressure, and ground to powder.

Aniline salt is employed largely in calico-printing, its chief use being for the production of *aniline-black* (Vol. 5). It is important that the salt intended for this purpose should be made from pure aniline, and should be dry and neutral. The presence of free acid in the aniline salts is liable to cause the cloth dyed black to rot in the steaming process. It must be free from sand or grit, which would injure the printing rollers, and will produce streaks on the printed cloth. *Grit* remains undissolved when the sample is treated with hot water, and may be filtered off, dried or ignited, and weighed. *Free acid* is best determined by titration with decinormal sodium hydroxide, using methyl-orange as an indicator, but the results are not very satisfactory. A useful method of examination consists in titrating the aqeuous solution of 2 grm. of the sample with normal sodium hydroxide, using litmus or phenolphthalein as an indicator. The amount neutralised corresponds to the total acid, both free and combined with aniline. Theoretically, 2 grm. or pure aniline hydrochlorate would require 15.4 c.c. of normal sodium hydroxide, but owing to the presence of toluidine and moisture commercial samples of good quality require between 14 and 15 c.c.[1] The process will indicate the presence of ammonium chloride, which will not neutralise alkali, and hence a sample containing it will require a less volume of the standard solution. *Ammonium chloride* is occasionally met with in considerable proportion as an adulterant of aniline salts. For its accurate determination the sample should be dissolved in water, excess of sodium hydroxide added, the liberated aniline separated, and the aqueous solution distilled in the usual way. On titrating the distillate with standard acid and methyl-orange, only the ammonia will be indicated. *Fixed impurities* will be detected on igniting the sample; only a mere trace should be present. An idea of the proportion of *toluidine* present in the sample can be obtained by liberating the mixed bases from the solution of the salts by sodium hydroxide, and heating a few centimetres of the aniline with an equal quantity of strong arsenic acid solution to 180° for some time. On boiling the product with water, the intensity of the crimson colouration will

[1] This method of examining aniline salts is due to R. Williams (*Chem. News*, 50, 299) but he appears to attribute the reaction to the presence of free acid.

increase with the proportion of toluidine in the sample. A more accurate result can be obtained in the manner indicated on page 78.

Aniline Sulphate, $(C_6H_7N)_2H_2SO_4$.—This salt forms a crystallien powder, which is readily soluble in water and slightly so in alcohol. It is insoluble in ether, a fact which distinguishes it from the sulphate of methylamine.

Aniline Phosphate, $(C_6H_7N)_2.H_3PO_4$, crystallises in plates. Easily soluble in water, ether and hot alcohol, slightly soluble in cold alcohol.

Aniline Oxalate, $(C_6H_7N)_2H_2C_2O_4$, is easily soluble in water, soluble with difficulty in absolute alcohol, and insoluble in ether.

Aniline Acetate, $C_6H_7N,HC_2H_3O_2$, does not appear to have been obtained in a definite crystalline form. When heated it loses the elements of water and forms acetanilide.

Aniline-sulphonic Acids. Amino-benzene-sulphonic Acids.

When aniline is treated with an equivalent amount of dilute or concentrated sulphuric acid it is converted into aniline sulphate. If an excess of acid be used, a high temperature employed, or sulphuric anhydride be present, aniline-sulphonic acid is produced:

$$C_6H_5.NH_2 + SO_2(OH)_2 = C_6H_4\begin{cases} SO_2.OH \\ NH_2 \end{cases} + H.OH$$

Three modifications of this substance exist, which differ according to the relative positions of the NH_2 and SO_3H groups in the benzene-nucleus. The ortho-sulphonic acid (1:2) has no practical interest, but the meta- and para-acids are manufactured on a large scale for the production of aniline- and azo-dyes.

M-amino-benzene-sulphonic acid, $C_6H_4(NH_2)^{(1)}.SO_3H^{(3)}$, is employed for the manufacture of *Metanil-yellow* (Vol. 5). It is prepared by warming nitrobenzene with fuming sulphuric acid, or by treating a solution of benzene in strong sulphuric acid with fuming nitric acid, when a mixture of nitro-benzene-sulphonic acids, $C_6H_4(NO_2)SO_3H$, is obtained, in which the meta-acid predominates, and may be roughly separated from its isomers by conversion into the barium or calcium salt. The meta-nitro-sulphonic acid yields, on reduction, the corresponding amino-sulphonic acid.

P-amino-benzene-sulphonic acid, $C_6H_4(NH_2)^{(1)}.SO_3H^{(4)}$, likewise called *Sulphanilic Acid,* is prepared on a large scale by heating 1 part of aniline and 3 of concentrated sulphuric acid to 195°. With

fuming acid, the reaction occurs more rapidly and at a lower temperature. On pouring the cooled product into water, the acid separates as a crystalline mass, which can be recrystallised from hot water.

Sulphanilic acid crystallises in rhombic tables containing 1 H_2O, which effloresce in the air, and are only slightly soluble in cold, but readily in hot, water. Treatment with potassium dichromate and sulphuric acid oxidises it to quinone, $C_6H_4O_2$. The solution of the sodium salt, on treatment with sodium nitrite, yields sodium diazobenzene-sulphonate (Vol. 5). Aniline sulphanilate loses all its asbic properties at 100°.

Nitranilines.—When aniline is treated with dilute nitric acid it yields nitraniline. With the concentrated acid it reacts far more violently than benzene, and is converted into quinone and other products. To obtain a nitro-derivative by such means, the aniline must be protected by employing its acetyl-derivative, or by nitrating in presence of an excess of strong sulphuric acid. In the latter case a mixture of the 3 isomeric nitranilines is obtained, but chiefly the *meta*-compound; in the former case *para*-nitracetanilide, $C_6H_4(NO_2).NH(C_2H_3O)$, is formed, together with some of the *ortho*-compound, both of which readily yield the corresponding nitraniline, $C_6H_4(NO_2).NH_2$, on boiling with concentrated hydrochloric acid or alkali hydroxide

Another method of preparing the nitranilines, especially the *meta*-modifications, is the reduction of the corresponding dinitrobenzenes in alkaline alcoholic solution. Under these circumstances only one of the NO_2 groups is reduced to NH_2, whereas in acid solution diaminobenzene, $C_6H_4:(NH_2)_2$, is obtained (page 105).

	Nitranilines, $C_6H_4(NO_2)\ NH_2$		
Appearance and Crystalline form,	*Ortho* $NO_2\ NH_2 = 1\ 2$	*Meta* $NO_2\ NH_2 = 1\ 3$	*Para* $NO_2\ .\ NH_2 = 1\cdot 4$
	Orange-yellow needles	Long yellow needles	Long yellow needles.
Taste,		Sweet, burning	Nearly tasteless
M. p.,	71·5°	110°	147°
Volatility, ...	Distils in a current of steam	Sublimes at 100° Distils in a current of steam	Not volatile with steam.
Salts, ...	Very unstable	Fairly stable	Unstable
Behaviour when boiled with strong sodium hydroxide	....	Unchanged.	Forms *p*-nitrophenol—$C_6H_4(NO_2).OH$

The nitranilines are yellow crystalline substances, readily soluble in alcohol but only slightly so in water. They are weak bases forming yellow salts, and yield the corresponding diamino-benzenes on reduction. The preceding table shows their chief differences.

There are 6 *dinitranilines*, $C_6H_3(NO_2)_2.NH_2$, melting as follows: 2–6, 138°; 2–4, 182°; 2–3, 127°; 2–5, 137°; 3–4, 154°; 3–5, 159°. Also a *trinitraniline*, $C_6N_2(NO_2)_3.NH_2$, or picramide, m. p. 188°, and is converted into picric acid, $C_6H_2(NO_2)_3.OH$, and ammonia when boiled with alkali hydroxide.

Homologues of Aniline.

As already stated, the true homologues of aniline are substances in which 1 or more atoms of the hydrogen of the benzene-nucleus are replaced by a corresponding number of atoms of methyl or other alkyl radical. The compounds in question may be prepared, and are produced commercially, by processes exactly similar to those which result in the formation of aniline. That is, the hydrocarbons toluene, xylene, etc., are treated with nitric acid, and the resulting nitro-derivatives are reduced to the bases by nascent hydrogen (usually produced by iron and hydrochloric acid).

In their general chemical relationships the homologues present the closest resemblance to aniline, and yield substitution-products of a strictly parallel character. They are also diazotised similarly.

The only homologues of aniline which require separate descriptions are the toluidines, C_7H_9N, and the xylidines, $C_8H_{11}N$. Their consideration will be followed by a section describing "aniline oils," under which term is included commercially pure aniline and toluidine, and various mixtures of these bases.

Toluidines. Amino-toluenes. Amino-methylbenzenes. Tolylamines.

$$C_7H_9N = C_7H_7.HN_2 = C_6H_4 \left\{ \begin{matrix} (CH_3) \\ H \\ H \end{matrix} \right\} N$$

The toluidines exist in small quantity together with aniline in coal-tar. They are produced commercially from toluene by processes exactly analogous to those by which aniline is prepared from benzene, and together with aniline constitute nearly the whole of the "aniline

oils" of commerce (page 74). An interesting method of producing toluidine is mentioned on page 52.

Three isomeric modifications of toluidine are known. The chief physical differences between them are shown in the following table, in which they are also contrasted with aniline and their meta-isomeride benzylamine, $C_6H_5.CH_2NH_2$.[1]

	Aniline	*o-Toluidine* $CH_3 NH_2 = 1\ 2$	*m-Toluidine* $CH_3 NH_2 = 1\ 3$	*p-Toluidine* $CH_3 NH_2 = 1\ 4$	*Benzyl-amine*
Sp. gr. at 15°,...	1.0268	1 0037	0 998 (at 25°)	Solid	0 990
M p.,. . ..	Solidifies at −8°	Does not solidify at −20°	Does not solidify at −13°	+45°	Liquid
B. p , .. .	184°	199 5°	197°	198°	185°
Characters of the acetyl-derivative. M. p , ...	114°	107°	65–66°	147°	57–61°
B p, ...	295°	296°	302–304°	307°	300°
1,000 pts of water dissolve,	3 4 at 14°	8 6 pts at 19°	4 4 pts at 13°	0 89 at 22°	Soluble
Solubility of the acid oxalate· In 1,000 pts of water at 15°,	.....	23 8	26 5	8 7	
In 1,000 pts of ether at 15°,	... .	0 50	Very slight	0 016	

Ortho-toluidine is formed by the reduction of *o*-nitrotoluene. It is a colourless liquid, turning brown on exposure to air or light, and otherwise closely resembling aniline. It differs from its isomerides by giving a green colouration when treated with ferric chloride and a little para-diamino-benzene. A solution of 1 in 10,000 gives a fairly deep colouration, and one of 1 in 100,000 assumes a distinct greenish tint. All commercial aniline gives this colouration, and even that prepared by the distillation of indigo with alkali hydroxide.

Meta-toluidine is produced by the reduction of *m*-nitrotoluene, preferably by an acid solution of stannous chloride. It is only present in small proportion in commercial toluidine. For its detection and approximate estimation the mixed bases are converted into hydro-

[1] BENZYLAMINE is a colourless liquid of faint aromatic odour, and is not affected by light. It is miscible in all proportions with water, alcohol and ether, but is separated from its aqueous solutions by alkali hydroxides (compare "Pyridine") It has a strongly alkaline reaction, fumes with hydrochloric acid, and absorbs carbon dioxide from the air, with conversion into silky needles of the *carbonate*.

chlorides, and the greater part of the isomeric salts removed by fractional crystallisation. The mother-liquor is evaporated to dryness, and the residue heated with methyl alcohol to 200°, under pressure, for a considerable time. This produces a mixture of the three isomeric dimethyl-toluidines, but only the meta-modification yields a nitroso-derivatives, $C_6H_3(NO)(CH_3).N(CH_3)_2$, on adding sodium nitrite to an ice-cold solution of its hydrochloride. The hydrochloride of nitroso-dimethyl-*m*-toluidine thus prepared, crystallises from a hot acidified solution in greenish-yellow needles only slightly soluble in cold water. On treatment with sodium carbonate the free base is obtained, melting at 92°, crystallising from water or ether in small green plates or long needles, and precipitated in moss-green needles on adding petroleum ether to its chloroformic solution. All its solutions have a deep green colour. Nitroso-dimethyl-*m*-toluidine forms steel-blue compounds with aniline and *o*-toluidine.

A detailed study of the oxidation products of *p*-toluidine has been made by E. Bromstein (*Ber.*, 1901, **34**, 1274–84), and resumé of the work is here given.

According to Rosenstiehl, the 3 modifications of toluidine may be distinguished by the following reactions:

	o-Toluidine	*m-Toluidine*	*p-Toluidine*
1 To a solution of the base in sulphuric acid, of 1 75 sp gr, add a solution of chromic acid in sulphuric acid of the same strength	Blue colouration changing on dilution to a permanent red-violet	Yellow-brown colouration, becoming greenish-yellow on slight dilution, and colourless on further addition of water	Yellow colouration
2 To a solution of the base in sulphuric acid of 1 75 sp gr, add nitric acid	Orange colouration, or in very concentrated solutions, brown, becoming yellow on dilution	At first red, rapidly changing to intense blood-red, and then dirty red, on dilution, orange	Blue streaks which soon tinge the whole liquid; (in presence of aniline or *o*-toluidine, blood red). The colour quickly becomes violet, then red, and, after some hours, brown
3 Dissolve the base in ether, and add an equal volume of water. Then add a few drops of clear solution of bleaching powder.	The aqueous layer becomes first yellow and then brown The ethereal layer, after separation, gives a permanent reddish-violet colouration with dilute sulphuric acid.	The aqueous layer becomes a thick brownish-yellow. The ethereal layer becomes reddish, and after separation and addition of dilute sulphuric acid is coloured violet at the under-surface.	No reaction. In presence of aniline the ether becomes blue on agitation

p-Toluidine is produced by the reduction of the nitrotoluene derived from the toluene produced by the dry distillation of Tolu balsam; also by heating *p*-cresol to 300° with ammonia and chloride of zinc; and by molecular transposition from methylaniline hydrochloride (page 52). It crystallises from hot dilute alcohol in colourless plates melting at 45°, and has a peculiar odour recalling that of aniline.

In 1880 W. H. Perkin, by the action of potassium dichromate on *p*-toluidine sulphate, obtained 2 oxidation products, having the formulas $C_{21}H_{21}N_3$ and $C_{28}H_7N_3$, respectively. The latter compound was investigated by later workers, its constitution being determined by A. G. Green (*J. Soc. Chem. Ind.*, 1894, **13**, 143), who showed it to be an amino-ditolyl-*p*-toluquinonediimine. Perkin applied the oxidation with lead peroxide in similar manner to that employed in the case of aniline (see page 57), and obtained according to the conditions one or other of Perkin's compounds.

By oxidising *p*-toluidine hydrochlorate or sulphate with lead peroxide (2 1/2–3 times the weight of the hydrochloride) in a dilution of 10–60 times, in the former case Perkin's[1] and in the latter Barsilowsky's[2] base is obtained. A 6% yield of the latter is produced by dissolving 100 grm. of *p*-toluidine in 1 litre of water, together with just the necessary quantity of hydrochloric acid, and oxidising with 587 grm. of manganese peroxide paste (15%). There were recovered 4 grm. of *p*-toluidine and 9.6 grm. of peroxide, so that 96 grm. of toluidine or 128 grm. of the hydrochloride required 78 grm. of manganese peroxide or in the proportion of 10:6. No Perkin's base was obtained but 6 grm. of Barsilowsky's base and a considerable amount of azotoluene. An 18% yield of Perkin's base was obtained by dissolving 218 grm. of *p*-toluidine and 98 grm. of sulphuric acid, in 6.5 litres of water and adding 294 grm. of potassium dichromate in 35 litres of water. After 24 hours the dark brown precipitate was filtered off, dried, and extracted with benzene, which, after evaporation yielded the mixture of bases. On boiling with 20 times the weight of absolute alcohol, 39 grm. of Perkin's base remained behind, while only 0.2 grm. of Barsilowsky's base was formed. In another preparation, 267.5 grm. of *p*-toluidine gave 54 grm. of 20% of Perkin's base, and 3 grm. of Barsilowsky's base. The latter melts at 235° and dissolves in concentrated sulphuric acid with a pure blue colour. As already stated on boiling this base

[1] Perkin's base (*p*-tolylamino-ditolyl-*p*-toluquinonediimine).
[2] Barsilowsky's base (amino-ditolyl-*p*-toluquinonediimine).

with *p*-toluidine and its hydrochloride in alcoholic solution it is converted not into Perkin's base, but into the hydrochloride of the product $C_{35}H_{35}N_5$, which crystallises in brassy yellow plates, melting at 282°. The base separates from dilute alcohol in orange-red plates melting at 251°. Perkin's base, or tolylaminoditolyltoluquinonediimine, melts at 183°, and dissolves with a violet colour in concentrated sulphuric acid, which turns green, blue, and then violet-red on dilution with water. The compound is basic and dissolves with a violet-red colour in hydrochloric acid, but the salts are easily decomposed. On treatment with 20 times its weight of 5% alcoholic sulphuric acid and standing 24 hours, the purple red solution passes through violet to pure blue. On saturation with ammonia and recrystallisation from ethyl or methyl alcohol, brownish-red shining needles melting at 181°, are obtained, the yield being 75% of the theoretical. The solution in concentrated sulphuric acid is green, becoming more and more reddish on standing and turning deep orange-yellow on dilution with water.

Commercial toluidine consists chiefly of a mixture of the ortho- and para- modifications. According to Friswell, the sp. gr. of the *o-toluidine* of commerce should be about 1.0037, and its b. p. from 197 to 198°. It ought not to solidify on cooling to —4°, though the majority of samples contain sufficient *p*-toluidine to cause them to commence crystallising at this temperature. The *p-toluidine* of commerce occurs in white dry crystals, m. p. 43–45°, and distils between 196 and 198°. *Liquid* commercial toluidine should boil at 197–198°, have a sp. gr. of about 1.000, and contain from 30 to 40% of *p*-toluidine and 60 to 70% of *o*-toluidine.

A portion of the *p*-modification separates from the commercial mixture of the isomers when the liquid is cooled by a freezing mixture. A further separation is effected in practice by fractionally saturating the mixture of the bases with sulphuric acid, and then distilling in a current of steam. *o*-Toluidine being a weaker base than the para-compound, the former will alone pass into the distillate if the quantity of sulphuric acid employed be somewhat in excess of that requisite to neutralise the *p*-toluidine.

L. Lewy (*Trans.*, 1887, **50**, 872) has proposed to separate *o*- and *p*-toluidine by converting the bases into phosphates. It appears that when *p*-toluidine and orthophosphoric acid are brought together, *di*-toluidine orthophosphate, $(C_7H_9N)_2H_3PO_4$, is produced as a salt crystallising in scales and very sparingly soluble in cold water, but

more readily, with partial dissociation, in boiling water. Aniline acts similarly, forming a sparingly soluble *di*-aniline orthophosphate, $(C_6H_7N)_2H_3PO_4$. On the other hand, *o*-toluidine forms a *mono*-toluidine orthophosphate, $(C_7H_9N)H_3PO_4$, and never a di- or tri- salt. Hence in the phosphates obtained from a mixture of the 2 toluidines the proportions of the bases might be deduced from the percentage of phosphoric acid. The mono-orthotoluidine phosphate is more readily soluble in water than diparatoluidine or dianiline phosphate. Further, when its solution is shaken with free aniline or *p*-toluidine, the *o*-toluidine is set free. Hence pure *o*-toluidine can be obtained from commercial toluidine[1] by adding rather more of a 21% aqueous solution of phosphoric acid than will suffice to form diphosphates with the aniline and *p*-toluidine present. On warming the liquid, the free *o*-toluidine forms a layer at the surface, which may be separated and distilled. The process may be modified by adding a further quantity of phosphate to convert the *o*-toluidine into monophosphate, and then cooling the liquid and allowing it to stand to secure the complete deposition of the *p*-toluidine phosphate.

Wölfing (*Ber.*, 1886, **19**, 2132) states that *o*-toluidine prepared by Lewy himself by the above process, both on the small and large scale, still contained as much as 4% of *p*-toluidine. For the preparation of pure *p*-toluidine he recommends (*Dingl. Polyt. J.*, **263**, 260) that the hydrochlorates of the bases should be treated with an amount of sodium nitrite only sufficient to convert the *o*-toluidine present into amino-azotoluene. Only when this change is complete does the *p*-toluidine react with the nitrite to form a diazo-amino-compound.

A method of determining the proportions of the ortho- and para-modifications of toluidine in the commercial product has been based by Rosenstiehl on the different solubilities of the acid oxalates of the two bases. The acid oxalate of *p*-toluidine requires 6,660 pts. of ether for solution, while the corresponding salt of *o*-toluidine dissolves in 200 pts. of ether. The method, somewhat modified, is as follows: 0.2 grm. of the sample is dissolved in 80 c.c. of anhydrous ether free from alcohol; 1.059 grm. of anhydrous oxalic acid, or 1.177 grm. of the crystallised, acid is dissolved in 250 c.c. of anhydrous, alcohol-free ether. Each c.c. of this solution will precipitate 0.005 grm. of toluidine. An excess is added to the ethereal solution of the sample, the liquid allowed to stand in a stoppered bottle for 12 hours, then filtered through

[1] The xylidines and cumidines behave like *o*-toluidine, and form only monophosphates.

paper and the precipitate washed with ether. The precipitate is the washed into the bottle with water, and the solution titrated with N/10 alkali hydroxide and phenolphthalein. 1 c.c. of N/10 alkali represents 0.00535 grm. of *p*-toluidine. Miniati, Booth, and Cohen (*J. Soc. Chem. Ind.*, 1889, **6**, 419) find that if too long a time be allowed for the precipitation, the product is liable to contain the *o*-toluidine oxalate, and hence the result will be above the truth. They recommend that a repetition of the experiment should be made, in which the amount of oxalic acid solution used is only that requisite to combine with the *p*-toluidine found by the first test, so reducing the error to a minimum.

G. Lunge (*Chemische Ind.*, **8**, 74) estimates the proportion of *p*- and *o*-toluidine in a mixture of the two by a careful observation of the sp. gr. The determination is made by the bottle, and referred to water at 15°. If the sample does not contain more than 50% of *p*-toluidine it is liquid at 15°, and consequently the observation is made at that temperature. With 50 to 60% of *p*-toluidine the method is still available if the bottle be filled at 20°; but with still larger proportions the results are unreliable, as the correction for temperature loses in accuracy, and the differences in sp. gr. become very small for considerable alterations in the composition of the mixture. It is very desirable to adhere rigidly to the prescribed temperature, as an error of 1° causes an error of 7% in the estimation. The correction is ±0.0008 for 1°, when the sp. gr. is above 1.0008, and ±0.0007 when below that point. All water must be removed by treating the sample with powdered potassium hydroxide and redistilling. The distillation also serves to show the presence of aniline or xylidine, in presence of notable quantities of which the method is inapplicable.

Lunge gives the following table of densities of mixtures of *p*- and *o*-toluidine, water at 15° being taken as unity:

A method of separating *o*-toluidine from *p*-toluidine has been based by P. Schoop (*Chem. Zeit.*, 1885, **9**, 1785) on the observation of Weith and Merz, that the acetyl-derivatives of *o*-toluidine is far less soluble in water than that of the isomer and of aniline. Schoop's method has been found unsatisfactory by several chemists, and need not be further described.

A method of estimating *p*-toluidine in admixture with *o*-toluidine has been based by G. A. Schoen (*Chem. Zeit.*, **12**, 494; *J. Soc. Chem. Ind.*, **7**, 594) on the intensity of the red colour produced with potassium

Sp gr at 15°	*o*-Toluidine, %	Sp gr at 15°	*o*-Toluidine, %	Sp gr at 20°	*o*-Toluidine, %	Sp gr at 20°	*o*-Toluidine, %
1 0037	100	1 0016	82½	0 9995	65½	0 9939	50
36	99	15	82	94	65	38	49½
35	98	14	81	93	64	37	48½
34	97	13	80	92	63	36	48
33	96	12	79½	91	62	35	47½
32	95	11	78½	90	61½	34	46½
31	94	10	77½	89	61	33	46
30	93½	09	77	88	60	32	45
29	92½	08	76	87	59	31	44½
28	91½	07	75	86	58½	30	44
27	91	06	74	85	58	29	43
26	90	05	73	84	57½	28	42
25	89½	04	72½	83	56½	27	41
24	88½	03	72	82	56	0 9926	40
23	88	02	71	81	55		
22	87	01	70	80	54½		
21	86½	1 0000	69	79	54		
20	86	0 9999	68½	78	53		
19	85	98	68	77	52½		
18	84½	97	67	76	51½		
1 0017	83½	0 9996	66½	0 9975	51		

dichromate. If the sp. gr. indicates the presence of more than 8% of *p*-toluidine it is reduced below that proportion by adding *o*-toluidine. 1 c.c. of the oil is then dissolved in 2 c c. of hydrochloric acid and 30 of water, and 1 c c. of a cold saturated solution of dichromate of potassium added. The mixture is allowed to stand for an hour, with occasional stirring, and is then filtered. *o*-Toluidine gives a black lake and a colourless liquid, but in presence of *p*-toluidine the precipitate is light brown, and the filtrate has a red colour, intense in proportion to the *p*-toluidine present. Pure aniline behaves like *o*-toluidine, but in presence of the latter a red filtrate is produced. Hence aniline must be absent, or its amount must be deduced from the b. p. and sp. gr. of the sample, and a corresponding amount added to the standard mixture with which the sample is compared.

A method of estimating small quantities of impurities in *o*-toluidine and *o*-nitrotoluene has been proposed by A. F. Holleman. (*Rec. trav. chim. Pays-Bas*, 1908, **27**, 458–642). The impurity usually encountered in *o*-toluidine is the *p*-compound and the amount of this is estimated by converting the sample into the acetyl-derivative and observing the solidifying point. The solidifying points of known mixtures of the acetyl compounds of *o*-and *p*-toluidine are given in the following table:

p-Compound, %.	Solidifying point.
0	109.15°
1.12	108 45°
2 42	107 75°
9.58	103.2°
13.6	100.8°

42.8 grm. of the toluidine to be tested are added slowly to a solution of 25.2 grm. of oxalic acid discovered in 1 litre of hot water. After cooling to 0°, the crystals are collected on a filter and washed once with a litte water. The toluidine is then regenerated from both crystals and filtrate by adding sodium hydroxide and distilling in steam. After separating the oil, the aqueous liquor must be extracted twice with ether to avoid loss. Both portions of toluidine are now converted into the acetyl compound by using for 1 grm. of toluidine, 2 c c. of glacial acetic acid and 1 c.c. of acetic anhydride, evaporating on a water-bath and distilling in a vacuum. The solidifying point of the 2 portions is determined and the amount of *p*-toluidine deducted from above table. When the amount of *p*-compound exceeds 1–2% the toluidine can be directly converted into the acetyl derivative without first preparing the oxalate. The presence of *p*-nitrotoluene in *o*-nitrotoluene is detected by first reducing with iron and hydrochloric acid and treating the resulting toluidine in the above matter.

Xylidines.—Amino-dimethylbenzenes. $C_6H_3(CH)_2.NH_2$.

Six isomeric substances of the above formula are theoretically possible, and all of them are known. Thus:[1]

	v-Orthoxylidine	*as*-Orthoxylidine	*v*-Metaxylidine	Wroblewsky's so-called Orthoxylidine
Hydrochloride	$+1\ H_2O$	$+1\ H_2O$	$+\frac{1}{2}H_2O$, needles	$+\frac{1}{2}\ H_2O$
Solubility in 100 of water at 18°	11 2	Very soluble	9 2	Very soluble
Nitrate	Anhydrous	Anhydrous	Anhydrous	Anhydrous
Solubility in 100 of water at 18°	6 6	0 4	2 2	2 7
Normal sulphate	Anhydrous	Anhydrous	Anhydrous	Anhydrous
Solubility in 100 of water at 18°	1 4	5 6	Very soluble	
Acid sulphate	Is not formed under ordinary conditions		$+2\frac{1}{2}\ H_2O$	$+2\frac{1}{2}\ H_2O$
Solubility in 100 of water at 18°			6 2	Very soluble

[1] The table is chiefly drawn up from the descriptions of the isomeric xylidines given by Roscoe and Schorlemmer. The characters differ considerably from those attributed to the isomers by Wroblewsky (*Annalen*, 207, 91). Nölting and Pick (*Ber*, 1888, 21, 3150), however, consider that Wroblewsky's *v*-oxylidine was simply impure *v*-metaxylidine, and give the following table of characters of xylidine salts:

Base	Positions of Groups CH_3 CH_3 NH_2	B. p.	Acetyl-derivative		Characters of Hydrochloride
			M. p.	Appearance, etc	
v-Orthoxylidine	1 2 3	223°	134°	White needles	Moderately soluble white needles containing 1 H_2O
a-Orthoxylidine	1 2 4	226° (melts at 49°)	99°	Long vitreous prisms	Long, very thin prisms, containing 1 H_2O
v-Metaxylidine	1 3 2	216°	176·8°	White needles, not saponified by boiling alkali or acid.	Thin anhydrous plates, readily soluble
a-Metaxylidine	1 3 4	212°	129°	White needles	Anhydrous rhombic tablets, slightly soluble in cold water
s-Metaxylidine	1 3 5	220°	140·5°	Large flat needles	Large anhydrous needles
Paraxylidine	1 4 2	213·5°	139°	Long lustrous needles	Flat needles or large tablets

The modifications of xylidine produced by nitrating the xylenes of coal-tar naphtha and reducing the nitro-derivatives are chiefly *a-o*-xylidine, *a-m*-xylidine, and *p*-xylidine, but 2 of the other isomers are also said to be produced. Only the *a*-meta-modification is of any value for the manufacture of azo-colouring matters, and of the cumidines, $C_6H_2(CH_3)_3.NH_2$, which are prepared by heating xylidine hydrochlorides with wood spirit, only pseudo-cumidine is of value. On this account, the useless isomers are removed as far as possible from the metaxylene before nitrating (Vol. 2, page 219), and in fact the presence of even a few units per cent. of *o*-xylene will occasion considerable practical inconvenience by the formation of tarry matters during its conversion into xylidine. On the other hand, commercial xylidine often contains as much as 25% of *p*-xylidine. *v-m*-xylidine (1:3:2) is prepared by converting commercial xylidine into the sulphate, which is allowed to crystallise, and the base liberated from the mother-liquor by alkali. The fraction distilling between 212° and 216° is heated with acetic anhydride. The *v-m*-acetxylidide formed is not acted on by boiling for several hours with 4 times its weight of dilute sulphuric acid containing 25% of H_2SO_4, but its isomers are decomposed. On cooling, the unchanged acetyl-compound separates, and after recrystallisation from hot water melts at 176°.8. On heating it

for some time to 200°, with three parts of sulphuric acid containing 70% of H_2SO_4, the sulphate of *v-m*-xylidine is formed. This salt differs from the sulphate of the isomeric xylidines in its very ready solubility in water.

a-o-**Xylidine** (1:2:4) is the only modification of xylidine which is solid at ordinary temperatures. By gradually evaporating its solution in petroleum ether, it is obtained in thick monoclinic prisms, but when rapidly deposited, or caused to solidify quickly, it forms transparent vitreous tablets. It melts at 49°, and is sparingly soluble in cold water, but readily in hot water, and also in alcohol and ether. Its aqueous solutions are not coloured by bleaching powder solution. The hydrochloride is readily soluble in water, but only slightly in strong hydrochloric acid; its aqueous solution imparts an intense yellow colour to fir-wood.

a-m-**Xylidine** (1:3·4). or ordinary xylidine, is best obtained by converting commercial xylidine into the hydrochloride and crystallising the product from water. Both the hydrobromide and hydrochloride are only slightly soluble in cold water. The last traces of impurity can be removed from *m*-xylidine by converting it into the acetyl-derivative, and recrystallising this substance from benzene till it has a m. p. of 129°. It is then decomposed by sulphuric acid.

p-**Xylidine** (1:4:2) has a sp. gr. of 0 980. It is prepared by treating commercial xylidine with fuming sulphuric acid containing sufficient sulphuric anhydride to convert the bases into sulphonic acids. The mixture is heated to 100° for some time, allowed to cool, and the solid mass pressed under water to separate *m*-xylidine-sulphonic acid in the crystalline state; or the hot liquid is poured upon ice, when the *m*-sulphonic acid, being with difficulty soluble in dilute sulphuric acid, crystallises out. The mother-liquor is neutralised with chalk, filtered, precipitated with sodium carbonate, and again filtered. On concentrating the filtrate, the sodium salt of *p*-xylidine-sulphonic acid separates in nacreous plates, which are washed with a little cold water to free them from traces of the readily soluble meta-sulphonate. The salt yields *p*-xylidine on dry distillation with ammonium chloride, while the sodium salt of *m*-xylidine-sulphonic acid chars under the same treatment. *p*-Xylidine may also be obtained by nitrating and reducing *p*-xylene, which may readily be prepared from commercial xylene (Vol. 3, page 219).

Cumidines.—Amino-trimethylbenzenes. $C_6H_2(CH_3)_3.NH_2$.

Various isomerides of this formula are known. The solid variety of commercial cumidine is made by heating xylidine hydrochloride and methyl alcohol together under pressure, to about 300°. The bases are liberated and converted into nitrates, and the difficultly soluble nitrate of pseudocumidine separated from the mother-liquor. The base is again liberated and distilled. The fraction passing over between 230 and 240° crystallises on cooling, and consists of amino-pseudocumene: (CH_3:CH_3:CH_3:NH_2 = 1:2:4:5). It crystallises from hot water in long needles, and from alcohol in large prisms, melts at 68°, and boils at 234–236°. When converted into diazocumene it can be used for the preparation of azo-colours by reaction with naphthol-mono- and di-sulphonic acids.

Isoduridine. Amino-tetramethylbenzene. $C_6H(CH_3)_4.NH_2$.

When the hydrochloride of pseudocumidine is heated with methyl alcohol to 300°, the hydrochloride of isoduridine is formed. The free base, which also occurs among the bye-products of the manufacture of pseudocumidine, is an oily liquid which boils at 250–253°, and solidifies on cooling to crystals, m. p. at 14°.

Amino-pentamethylbenzene. $C_6(CH_3)_5.NH_2$.

This base is obtained by heating dimethyl-*a*-pseudocumidine with methyl iodide. It forms large white needles, m. p. 151° and b. p. 277°.

Aniline Oils.

The term "aniline oils" is applied commercially to all the different varieties of aniline manufactured on a large scale, equally whether the product in question consists of nearly pure aniline, of toluidine, or of a mixture of the two. The method of manufacturing the different varieties of aniline oil is substantially the same, the composition of the product depending on that of the hydrocarbon employed. The details of the method of manufacture are, of course, subject to variation, but the following is an outline of the method pursued in a well-known aniline works: Crude coal-tar naphtha is redistilled to a temperature of 170°. The product of the distillation, called "once-run naphtha," is treated with strong sulphuric acid (sp. gr. 1.845) which removes the bases, hydrocarbons of the olefine and acetylene series, and some of the higher homologues of benzene. A subsequent treatment with milk of lime or sodium hydroxide eliminates the phenols and other substances of an acid character. The purified oil is washed with water and redistilled

to obtain "50/90 benzol," and this when fractionated with the aid of a dephlegmating column at once yields 99% benzol, toluol, and solvent naphtha (compare Vol. 3, page 233). Solvent naphtha is now generally further treated for the isolation of xylene, but the benzols and toluol are directly converted into the nitro-compounds by placing them in a vessel surrounded with cold water, and gradually running in a cold, previously made mixture, of 150% by weight of nitric acid of 1.4 sp. gr. with 200% of concentrated sulphuric acid. When the reaction is complete the mixture is allowed to stand, and the lower layer of acid is tapped off and concentrated again in glass for repeated use. The nitrobenzol is washed several times with sodium hydroxide, and then treated with open steam to drive off unchanged benzol and "light stuff." The nitrobenzol (or nitrotoluol obtained in a precisely similar manner) is then placed in a still with hydrochloric acid, and borings or filings of grey cast iron added gradually. High-pressure steam is blown in, and the nitrobenzol which distils over is separated from the condensed water, and returned to the still until the complete solubility of the distilled oil in hydrochloric acid shows that the reaction is complete. Milk of lime is then introduced, and the liberated aniline distilled off by the aid of steam. Aniline sinks to the bottom of the condensed water, but when toluidine is being made the oil floats on the surface. The condensed water contains from 2 to 3% of dissolved bases, and is converted into steam for the aniline stills. The iron is converted into a black paste, consisting chiefly of Fe_3O_4, which is sold for purifying gas. The aniline oil is distilled to separate water, etc. The addition of lime to liberate the aniline is not strictly necessary, and in many works it is omitted. The first reaction seems to be:

$$C_6H_5.NO_2 + Fe_3 + 6HCl = 3FeCl_2 + C_6H_5.NH_2 + 2H_2O.$$

The ferrous chloride formed also acts as a reducing agent, being converted into ferric chloride, which in presence of water gives ferric oxide and aniline hydrochlorate. The end-products are chiefly aniline, ferroso-ferric oxide, and a weak solution of ferrous chloride. The hydrochloric acid seems to act chiefly as a carrier, so that the general reaction may be represented by the equation: $4C_6H_5.NO_2 + 9Fe + 4H_2O = 3Fe_3O_4 + 4C_6H_5.NH_2$. Acetic acid was formerly employed in place of hydrochloric acid, but its use is now almost, if not entirely, obsolete. Its use in too large a proportion tended to the formation of acetanilide. Too large an excess of iron, or its too rapid

addition, may cause loss from a reproduction of benzene, which deficiency of both iron and acid favours the production of azo-benzene.

Composition and Assay of Aniline Oils.

There are 3 leading kinds of aniline oil now recognised in the market, namely: (1) Pure aniline oil; (2) aniline oil for red; and (3) toluidine. The demand for xylidine for the manufacture of azo-reds has considerably influenced the character of commercial aniline; since the 50/90 benzol, which was commonly used for the manufacture of "aniline for red," formerly contained a notable quantity of xylene, which is now removed and converted separately. Since the employment of dephlegmating columns has become usual, benzene and toluene of almost constant b. p. have been manufactured. From the pure hydrocarbons the corresponding bases are prepared, while from the intermediate oil, containing about 25% of benzene and 75 of toluene, an aniline oil for red is manufactured, which contains about 25% of aniline, from 20 to 25 of *p*-toluidine, and 45 to 50% of *o*-toluidine.[1]

In addition to the foregoing leading qualities of aniline oil, products of very varying composition and degrees of purity have to be dealt with by the dye-manufacturer. Thus in making magenta by the arsenic acid process, fully one-fourth of the aniline distils off and is condensed. But this recovered aniline is found on rectification to have a considerably higher density than the original oil (1.015 to 1.009 against 1.0075), and to consist almost entirely of aniline and *o*-toluidine, whereas the original oil contained from 15 to 25% of *p*-toluidine. This is either employed for the maufacture of safranine or very red shades of blue, or crude *p*-toluidine is added to it in such proportion as to bring it approximately to the original composition. Similarly, in the manufacture of magenta by the nitrobenzene process, the recovered aniline contains notable quantities of nitrobenzene, while from other processes methylated and ethylated anilines are obtained. *Recovered anilines* are deeper in colour and of greater body than unused oils, and often have a strong and somewhat characteristic odour. They are rarely met with outside the colour-works in which they have their origin.

On next page is a tabulated list of the more important or frequently-occurring constituents of aniline oils.[2] With the exception of aniline

[1] The composition of aniline oil for red is often judged of by the consumer solely from the sp. gr., and he or the aniline-maker adjusts it accordingly by adding aniline or toluidine to the crude oil as the gravity may indicate.

[2] Hell and Rockenbach (*Ber.*, 1889, 22, 505) have investigated some other non-basic constituents of aniline and toluidine tailings.

and its homologues, and the substituted anilines, very little is known respecting the effect of the substances formulated in the table on the colouring matters produced. For the most part the objectionable impurities are got rid by fractionating the crude aniline oil.

Name	Formula	M p	B p	Remarks
Aniline	$C_6H_5NH_2$	−8°	183 7°	
Toluidine (*o*-, 1 2; *m*-, 1 3; *p*-, 1 4)	$C_6H_4(CH_3)NH_2$	below −20° below −13° 45°	199° 197° 198°	
Xylidine (several isomers)	$C_6H_3(CH_3)_2NH_2$		212–226°	
Cumidine (several isomers, chiefly pseudocumidine)	$C_6H_2(CH_3)_3NH_2$	63°	235°	
Methyl-aniline	$C_6H_5NH(CH_3)$		192°	
Dimethyl-aniline	$C_6H_5N(CH_3)_2$	0 5°	192°	
Ethyl-aniline	$C_6H_5NH(C_2H_5)$		204°	
Diphenylamine	$C_6H_5NH(C_6H_5)$	54°	302°	
Acetanilide	$C_6H_5NH(C_2H_3O)$	112°	295°	
Acetotoluidide (*o*-, *p*-)	$C_6H_4(CH_3)NH(C_2H_3O)$	65–66° 147°	302–304° 300–307°	Produced by action of heat on toluidine acetate
Nitranilines	$C_6H_4(NO_2)NH_2$			From imperfect reduction of dinitrobenzene.
Paraniline	$C_{12}H_{14}N_2$	192°	330°	
Xenylamine	$C_{12}H_9NH_2$	45°	322°	
Phenylene-diamine (*para*-)	$C_6H_4(NH_2)_2$	63°	287°	Reduction of dinitrobenzene.
Toluylene-diamine (*para*-)	$C_6H_3(CH_3)(NH_2)_2$	99°	283–285°	See page 107
Azobenzene	$C_6H_5N_2C_6H_5$	65°	293°	Imperfect reduction of nitrobenzene
Nitrobenzene	$C_6H_5(NO_2)$	3°	210°	Vol 3, page 211
Dinitro-benzenes (*o*-, *m*-, *p*-)	$C_6H_4(NO_2)_2$	118° 90° 172°		Monoclinic tables Long needles or thin rhombic tables Monoclinic needles
Nitrotoluenes (*o*-, *m*, *p*-)	$C_6H_4(CH_3)(NO_2)$	below −20° 16° 54°	223° 230° 238°	Sp. gr. 1.163 at 23 5° Sp. gr. 1 168 at 22°
Benzene ...	C_6H_6	5 5°	80 5°	Vol 3, page 199
Toluene .. .	$C_6H_5(CH_3)$	below −20°	111°	Vol 3, page 215
Aminothiophene	$C_4H_3SNH_2$	.		
Paraffins. . .	C_2H_{2n+2}	.		Especially in aniline oils derived from cannel-tar benzols

The *assay* of aniline oils is usually limited to observations of the colour, odour, and sp. gr., supplemented by a careful fractional distillation and tests for water, nitrobenzene, hydrocarbons, etc

The *sp gr.* of aniline oil is a valuable indication of its composition. The observation must be made by the plummet or sp. gr. bottle at exactly 15°, and the result referred to water at the same temperature taken as unity.[1]

The following figures represent the densities as thus observed:

	Sp gr. at 15°.
Pure aniline,	1.0268.
Aniline oil for red,	1 0075 to 1.0012.
o-Toluidine,	1.0037.
Mixture of equal parts of *o*- and *p*-toluidine,	0 9975.
p-Toluidine (solid),	1.046.

The odour of pure aniline is very different from that of the toluidines. The presence of toluidine in aniline is indicated by the density of the sample, its diminished solubility in dilute alcohol (page 79), and by the results of the fractional distillation (page 79). In addition to these characters, the following tests are sometimes of service:

Pure aniline affords no rosaniline on treatment with oxidising agents, but if toluidine be present magenta is readily formed. The test is best made by mixing 5 c c. of the sample of anilme with an equal measure of a concentrated solution of arsenic acid, containing about 75% of As_2O_5 and having a density of 2.04. The mixture, contained in a small flask or long test-tube, is immersed in a paraffin-bath heated to 180°. The mixture rapidly changes in colour, and swells considerably. When the action is complete, the contents of the tube acquire a metallic bronze appearance and no longer intumesce. The product is treated with boiling water, when, if the sample contained toluidine, arseniate of rosaniline dissolves and communicates an intense crimson colour to the liquid. Neither pure aniline nor toluidine alone gives this reaction.

If a sample of commercial aniline be mixed with some solid magenta and a few drops of glacial acetic acid, and the whole heated to 180°, as described above, ammonia is abundantly evolved, and in a short time the mixture becomes intensely blue from the formation of tri-

[1] P. Schoop (*Chem Zeit*, 1885, 9, 178) gives the sp gr of pure aniline as 1 0377 at 1°; *o*-toluidine as 1 0143, and *p*-toluidine as 1 0045 at the same temperature, the coefficient of expansion being in each case 0 00081 for 1°.

phenyl-rosaniline. With pure aniline the blue is very pure in shade, but when toluidine or xylidine is treated in a similar manner the product is intensely purple, and a mixture of the bases gives proportionate intermediate shades of colour. If a little of the "melt" be withdrawn from the tube, diluted considerably with alcohol, a few drops of acetic acid added, and then streaked on white filter-paper by means of a glass rod, the purple tint is readily observed, especially if the paper be held up before a gas-flame.

A valuable indication of the general composition of an aniline oil is obtained by submitting the sample to fractional distillation, and noting the proportions of distillate obtained at various temperatures. The distillate may be measured after each rise of 5° in the b. p. of the sample, or the temperature may be observed when each consecutive 5 or 10% fraction has passed over. The latter is the plan now commonly adopted, 100 c.c. of the sample being employed, and the arrangement of the apparatus being exactly the same as in the fractional distillation of benzols (Vol. 3, page 233).

The heat is applied cautiously at first, in order to dissipate any water. When this is effected, which will be known by the rapid rise of the thermometer, the heat is so regulated that the distillate shall fall in distinct drops, about 60 per minute. With each increase of 10 c c. in the volume of the distillate the temperature indicated by the thermometer is observed and recorded, the process being continued till 90 or 95 c c. have passed over.

A very simple test for aniline oils was devised and communicated to Allen by the late B. Nickels, who found it to give useful results, and to indicate differences between samples not readily distinguishable by the ordinary fractional distillation process. The test is based on the greater solubility in dilute alcohol of aniline as compared with toluidine and xylidine, and is thus performed: 5 c.c. measure of the sample is taken with a pipette and diluted to 40 c.c. with methylated spirit. Distilled water is then gradually added from a burette, with constant shaking, till a permanent turbidity is produced, when the volume of water employed is noted. Operating in this way, a sample of very pure aniline required 126 c.c. of water to produce permanent turbidity. The following figures, obtained by Nickels in 1881, show the results yielded by three typical specimens of commercial aniline as then manufactured:

	A Pure aniline	B Heavy aniline	C Toluidine
Colour	Pale amber	Amber	Deep brown
Sp gr. at 15 5°	1 025	1 011	1 002
Water required for precipitation	106 4 c c	73 7 c c	63 2 c c
10% distilled over at	183½°	189°	195°
20% distilled over at	183½	189¾	195¾
30% distilled over at	183¾	190	196
40% distilled over at	184	191	196½
50% distilled over at	184¼	191½	197
60% distilled over at	184	192¼	197½
70% distilled over at	184	193	198
80% distilled over at	184	194¼	198½
90% distilled over at	184½	197	199¼
95% distilled over at	184¾	201	

Sample A was a fair commercial specimen of the quality known as "pure aniline," and actually contained some 95% of real aniline. An article of this high purity is required for the manufacture of aniline blue, triphenyl-rosaniline (see page 76), any notable admixture of toluidine resulting in a product dyeing with reddish tinge.[1]

The quality known as "heavy aniline," exemplified by B, is a fair sample of aniline oil for red (see page 76). This class of aniline is produced from benzols containing a considerable proportion of toluene, and the aniline oil itself is a mixture of aniline and toluidines. Good samples of aniline oil for red contain from 35 to 42% of real aniline, 35 to 50% of *o*-toluidine, and 14 to 24% of *p*-toluidine.

R. J. Friswell thinks 100 c.c. an undesirably small quantity for fractional distillation. He prefers to operate on 250 c.c. which he distils in a flask with a side-tubulure, and he recommends an observation of the temperature at which the last drop disappears from the bottom of the flask. A naked flame is used, and a few fragments of platinum wire or fire-brick added to the contents of the flask. The following figures were obtained by Friswell (Thorpe's *Dict. Applied Chem.*, **1**, 165) by the examination of commercially pure aniline.

	No 1	No 2	No. 3
Sp. gr. at 15°	1.02710	1 02684	1 02690
10% over at	184 7°	184 6°	184 6°
20% over at	184 7	184 8	184 6
30% over at	184.7	184 8	184.7
40% over at	184 7	184 8	184.7
50% over at	184 8	184.8	184.8
60% over at	184.9	184 8	184.8
70% over at	185 0	184.8	184.9
80% over at	185 1	184 8	184.9
90% over at	185.1	184.8	185.0
Dry at	186 7	186 8	

[1] In good samples the b p. holds closely together, differing by 1 or 2 only. Inequalities or jumps in the b p., especially at the beginning and end of the distillation, indicate badly-made samples or mixtures.

Any *water* present in aniline oil will be found in the very first portions (first fraction of 10%) whenever the sample is submitted to distillation. It takes the form of globules, which are not miscible with the next fraction of the distillate nor with petroleum spirit. Water may exist in aniline in any proportion from a trace up to 3 or 4%, but a good commercial rectified specimen should no contain more than 0.5%. Aniline is readily soluble in a strong aqueous solution of aniline hydrochloride. A solution of the kind, of 1.08 sp. gr. is stated by Watson Smith to be sometimes sold as aniline oil, which in colour and taste it closely resembles. Such a fraud would be at once detected on distillation.

Benzene, toluene, and other **hydrocarbons** will separate when the first fraction of 10% (10 c.c.) is treated with an equal volume or slight excess of hydrochloric acid, and water added to 100 or 150 c.c. They assume the form of oily globules which float even on diluting the liquid. The best samples of pure aniline show only a slight opalescence when thus treated, but the smell of the "light stuff" is always perceptible. In recovered anilines these impurities exist to a notable extent, since they survive the reactions by which the bases are consumed. Aniline for red usually contains somewhat more hydrocarbons than pure aniline.

Nitrobenzene and **nitrotoluene** may be recognised, even when mere traces are present, by the milky appearance of the liquid produced by saturating 10 c.c. of the original sample of oil with hydrochloric acid. On diluting the liquid with water, and leaving it at rest for some hours, any considerable quantity of nitrobenzene will collect at the bottom in the form of oily globules, which, after separating the acid liquid, may be identified by the smell and other characters. Still smaller quantities of nitrobenzene may be recognized if the "tailings" be operated upon instead of the original sample. Nitrobenzene occurs more frequently in magenta-aniline and toluidine than in the oils of lower b. p.

Nitrobenzene is also indicated by the yellow colour of the froth produced when the sample is violently agitated.

Acetanilide and **acetotoluide** were impurities characteristic of aniline prepared by the reduction of nitrobenzene with acetic acid and iron, but are now rarely met with in aniline oils. In any case they would become concentrated in the "tailings," together with phenylenediamine, azobenzene, paraniline, xylylamine, etc.

Aniline tailings is the name applied to the least volatile portion of

aniline oils. They contain little or no aniline; some toluidine, xylidine and cumidine; nitrobenzene and its homologues; and some or all of the bi-products tabulated on page 77 which boil above 200°.

The composition and special methods of examination of commercial *toluidine* are described on page 63 *et seq.*

Anilides.

The anilides are derivatives of aniline in which one or both of the hydrogen-atoms of the amino-group are replaced by acid-radicals. The homologues of aniline yield similar derivatives (*e. g.*, aceto-toluide. The most important and typical member of the class is acetanilide or phenylacetamide:

$$C_6H_5.NH(C_2H_3O); \text{ or } C_2H_3O.NH(C_6H_5).$$

A number of derivatives of acetanilide have been prepared, and certain of them have found some employment as analgesics and antipyretics, as for instance:

Acetanilide. Phenylacetamide. Antifebrin. $C_6H_5\ NH(C_2H_3O)$.

Bromacetanilide. Antiseptin. Brominated antifebrin. } $C_6HBr.N_4H(C_2H_3O)$.

Methylacetanilide. Exalgin. Methylated antifebrin. } $C_6H_5.N(CH_3)(C_2H_3O)$.

Aceto-amidophenol. Hydroxy-antifebrin. $C_6H_4(OH).NH(C_2H_3O)$.

Aceto-anisidine. Methacetin. Methoxy-antifebrin. } $C_6H_4(O.CH_3).NH(C_2H_3O)$.

Acet-phenethidine. Phenacetin. Ethoxy-antifebrin. } $C_6H_4(O.C_2H_5).NH(C_2H_3O)$.

Amido-phenacetin. Phenocoll. $C_6H_4(O.C_2H_5).NH(C_2H_2O.NH_2)$.

Most of these substances are described in the following pages. The relationship of antifebrin to hypnone, hydracetin (pyrodine), and phenyl-urethane, is shown by the following formulæ:

Acetophenone. Hypnone. $C_6H_5.(CO.CH_3)$.

Acetanilide. Antifebrin (see below). $C_6H_5.NH.(CO.CH_3)$.

Acet-phenylhydrazine. Hydracetin. $C_6H_5.NH.NH.(CO.CH_3)$.

Lævulinyl-phenylhydrazine. Antithermin. } $C_6H_5.NH.NH.(C_5H_7O_2)$.

Phenyl-urethane. Euphorin. $C_6H_5.NH.(CO.O.C_2H_5)$.

Acetanilide. Phenylacetamide. $C_6H_5.NH(C_2H_3O)$.

This substance was originally obtained by the action of acetyl chloride on aniline. It is more conveniently prepared by boiling aniline with glacial acetic acid for many hours under an inverted condenser, until the product solidifies on cooling. The mass is then melted and poured into water, to remove unconverted aniline and acetic acid. It may be purified by distillation and crystallisation from alcohol, benzene, or hot water, from which it separates in colourless unctuous laminæ, resembling boric acid, soluble in about 190 parts of cold or 18 of boiling water. Acetanilide is odourless, but produces a slight burning sensation on the tongue. It occurs commercially as a crystalline powder or scales. It melts at 113°, and distils unchanged at 295°. Acetanilide dissolves in 3 5 parts of alcohol, and is very soluble in ether, chloroform, and benzene, yielding neutral solutions.

Acetanilide is a weak base. The *hydrochloride* is obtained by passing hydrochloric acid gas through a solution of acetanilide in acetone. It forms needles which are decomposed into their constituents by water, and gradually converted into acetic acid and aniline hydrochloride on exposure to moist air.

Acetanilide dissolves in strong sulphuric acid without change of colour. On treating the solution with nitric acid, the acetanilide is converted chiefly into *para*-nitroacetanilide, some of the *ortho*-compound and, in presence of a large excess of sulphuric acid, a little of the *meta*-compound being also formed. Nitrous acid, passed into its acetic acid solution, converts acetanilide into an unstable nitrosamine, $C_6H_5.N(C_3H_3O)(NO)$. When heated with zinc chloride to about 250°, acetanilide yields *flavaniline*, $C_{16}H_{14}N_2,HCl$ (Vol. 5). Treated in alcoholic solution with sodium ethylate, acetanilide yields a sodium derivative, $C_6H_5.NNaC_2H_3O$, but when this is boiled with water it splits into aniline and sodium acetate. Acetanilide behaves like aniline on treatment with alkali hydroxide and chloroform (page 56), and the formation of the disagreeably smelling isonitrile is a delicate reaction for its presence.

Acetanilide behaves like aniline when treated with phenol and a solution of bleaching powder (page 57).

When treated with a solution of potassium chlorate in strong sulphuric acid, acetanilide gives a red colouration, changed to yellow

on dilution. With a crystal of a nitrite and a drop of concentrated hydrochloric acid it produces a yellow colour, changing on heating to green and blue; and, on evaporating the liquid to dryness, an orange residue is obtained, changed to red on adding ammonia (Vitali).

When acetanilide is heated gently with mercurous nitrate, a substance is produced which dissolves in alcohol with green colour (Yvon). If a few centigrams of acetanilide be gently heated with 2 or 3 drops of a solution of mercurous nitrate, and when solution has been effected 2 or 3 drops of sulphuric acid added, a blood-red colouration will be produced (Cella and Arzeno). The same reaction is produced by phenol, resorcinol, thymol, and salicylic, gallic, and tannic acids, but not by benzoic acid.

Acetanilide gives no colour-reactions with ferric chloride, nitrites in very dilute solutions, or potassium dichromate in aqueous solution. These reactions distinguish it from antipyrine and kairine.

Various other colour-reactions of acetanilide have been described. The tests given in the *United States Pharmacopœia* are included in those given herewith. As a rule, the most satisfactory method for its positive identification is to heat the substance with alcoholic potassium hydroxide, dilute with water, and shake with ether. The ethereal layer is examined for aniline, while the aqueous liquid is tested for an acetate.

To detect acetanilide in urine, Vulpius boils the liquid with hydrochloric acid, cools, extracts with ether, and tests the ethereal solution with phenol and bleaching powder solution.

E. Ritsert (*Pharm. Zeit.*, 1890, **35**, 306) gives the following tests for the purity of commercial acetanilide: The sample should leave no ash on ignition, and after drying for 2 hours at 105°, should melt at 114°. A higher or lower m. p. indicates the presence of aceto-toluides. 0.1 grm. dissolves in 1 c.c. of strong hydrochloric acid to a clear solution, which, after a few minutes, precipitates acetanilide hydrochloride (methyl-acetanilide does not yield a similar reaction). No change should be produced on adding a drop of nitric acid, which, after a time, produces a yellow or brown colouration if phenacetin or methacetin be present. If 0.1 grm. be boiled in portions in 2 c.c. of strong hydrochloric acid, the solution cooled, and a drop or two of chlorine water added, a fine blue colouration is produced. The aqueous solution of acetanilide should be free from acid reaction (indicating acetic acid). On boiling it and adding ferric chloride, a deep reddish-brown colour

should be produced, destroyed by a mineral acid. If a drop of dilute solution of potassium permanganate (1:1000) be added to a boiling aqueous solution of 1 grm. of acetanilide in 30 c.c. of water, the pink colouration at first produced should persist at least 5 minutes, and should not change to yellow on again boiling. Precipitation at this stage indicates the presence of free aniline, resinous products, aceto-toluides, or other impurities.

In the *additions* (1890) to the *British Pharmacopœia*, acetanilide is described as melting at 112.8°, and dissolving in sulphuric acid without colouration. The solution in 18 parts of boiling water should be clear, neutral, and odourless; and *after cooling* should not be coloured on adding ferric chloride. This is directly opposed to the experience of Ritsert above quoted. In the *German Pharmacopœia* the direction is to add ferric chloride to a cold saturated solution, thus avoiding the dissociation and formation of acetic acid liable to occur on boiling. According to the *German Pharmacopœia*, on heating with alkali hydroxide solution, acetanilide gives off an aromatic vapour, which, after addition of a drop of chloroform and renewed application of heat, is changed to the disagreeable smell of the isonitrile. Further, 0.1 grm. of acetanilide should yield a clear solution when boiled with 1 c.c. of hydrochloric acid for 1 minute; and, after adding to the liquid 2 c.c. of carbolic acid, a cloudy red colouration should be produced by solution of bleaching powder, changed to a permanent indigo blue (indophenol) on adding excess of ammonia.

Acetanilide has powerful antipyretic properties, and has received an extensive application in medicine under the name of "antifebrin,"[1] though dangerous symptoms are sometimes produced by it (*Pharm. Jour.*, [iii], **20**, 1059). The dose is from 3 to 10 grains.

According to Salzer, commercial antifebrin is liable to certain unchanged aniline, which may be detected by dissolving the sample in cold hydrochloric acid, and pouring on the liquid a solution of bleach-

[1] When administered to rabbits, acetanilide is oxidised to para-aminophenol, $C_6H_4(OH).NH_2$, with complete elimination of the acetyl-group. In dogs there is a small formation of para-aminophenol, but the chief change consists in a simultaneous oxidation of the aniline-residue to ortho-aminophenol, of the acetyl-group to carboxyl, and in the formation of carbonyl-ortho-hydroxyamidophenol, $C_6H_3(OH)\begin{Bmatrix} NH \\ O \end{Bmatrix}CO$, the anhydride of which is excreted in the urine as a sulphate In both the rabbit and the dog the amido-phenols are also eliminated as sulphates. In man, the acetyl-group is not wholly oxidised, the urine containing the sulphate of aceto-paraminophenol. In all cases there is an oxidation of one of the hydrogen atoms of the benzene-nucleus to hydroxyl, while the proportion of ethereal sulphates is increased, the urine is red from excess of bilirubin, reduces alkaline cupric solution, and is strongly levorotatory; the optically active substance probably being the above-mentioned sulphate (Gressly and Nencki, *Monatsh*, 1890, 11, 253).

ing powder. Pure acetanilide yields a white precipitate, which dissolves on shaking the liquid, but after a time colourless silky needles separate. In presence of aniline the well-known violet colouration is produced.

Acetanilide has been used as an adulterant of antipyrine. The m. p. of the pure substances are nearly identical, but a mixture of equal proportions of the two melts at 45°.

Of the 3 isomeric *aceto-toluides*, only the metacompound possesses antipyretic properties.

Diacetanilide, $C_6H_5N:(CH_3CO)_2$, is a crystalline substance melting at 111°. It is purified by crystallising out of benzine.

Formanilide, phenyl formamid, $C_6H_5:NH(HCO)$. Colourless crystals, m. p. 46°, soluble in water, alcohol and glycerin. This substance is prepared by heating aniline and formic acid in a way similar to the preparation of acetanilide. It is an antipyretic analgesin and local anesthetic.

Gallanilid, gallanol, $C_6H_3NH.CO.C_6H_2(OH)_3$, colourless crystals of bitter taste, m. p. 205°. Hot water, alcohol and ether are solvents. It is used as an astringent for wounds.

Para-brom-acetanilide, $C_6H_4Br.NH(CO.CH_3)$, has been introduced as a remedy under the name of "antisepsin." It forms small pearly prisms, melting at 164.5°, and devoid of taste or smell. It is soluble with difficulty in cold, but readily in hot water, as also in alcohol and ether.

Acet-methylanilide or methylacetanilide, $C_6H_5.N(CH_3)(C_2H_3O)$, is prepared by warming together methylaniline and acetyl chloride. The product is boiled with water, when the new substance crystallises on cooling. Methylacetanilide has been introduced as an anti-rheumatic and analgesic under the name of "exalgin." In doses of 0.5 to 4 grains its effects are said to be very satisfactory. Exalgin forms fine needles or large white tablets (compare "Acetanilide"). It melts at 100–101°, boils without decomposition between 240 and 250°, and is slightly soluble in cold water, but more so in boiling, and very soluble in water containing a little alcohol. It is saponified with difficulty by alkali hydroxide, but completely by concentrated hydrochloric acid, with formation of acetic acid and methylaniline.

Acetanilide and methylacetanilide may, according to V. Zotier (*L'Union Pharm.*, 1910, **51**, 255), be distinguished by the following reactions: About 0.05 grm. is treated with 10 drops of hydrochloric

acid and boiled for 2 minutes. The liquid is then cooled, 5 more drops of hydrochloric acid are added, then 1 drop of 1% sodium nitrite solution; after allowing reaction to take place for 10 minutes, 1 c c. of phenol is added and then, gradually, enough strong sulphuric acid to give a homogeneous mixture. Of this, 0.5 c.c. is treated with sufficient sodium hydroxide solution, sp. gr. 1.332, to give a clear solution. In the case of exalgin a blue colour will be obtained and with acetanilide a yellow tint. Mixtures will naturally vary from yellowish to green. The various details of the test must be rigorously observed.

Hirschsohn states that methylacetanilide may be distinguished from acetanilide and phenacetin by treating 1 grm. with 2 c c. of chloroform, which dissolves the exalgin only. A chloroform solution of exalgin remains clear on adding 10 volumes of petroleum ether, whereas the solutions of antifebrin and phenacetin become turbid. 20% of acetanilide, or 10 of phenacetin, may be detected in exalgin by these reactions. An aqueous solution of antifebrin gives a brom-derivative on adding bromine-water, thus differing from exalgin and phenacetin.[1]

Benzanilide, $C_6H_5.NH(CO.C_6H_5)$, is obtained by the action of benzoyl chloride on aniline, or by boiling together equivalent quantities of benzoic acid and aniline. It forms a white, crystalline powder, melting at 160–161° and volatile without decomposition. It is almost insoluble in water, but dissolves in 58 parts of cold, or 7 of boiling, alcohol, crystallising on cooling in nacreous plates. It is difficultly soluble in ether. Benzanilide is not attacked by aqueous alkalies or acids, but is saponified by fusion with potassium hydroxide. It has been found valuable as an antipyretic for children, in doses of 2 to 8 grains, and is said not to produce objectionable secondary effects.

Phenyl-urethane, $C_6H_5.NH(CO.OC_2H_5)$.—This compound has recently acquired a practical interest owing to its introduction as a synthetic remedy under the name of "euphorin." It is produced by the reaction of aniline on ethyl-chlorocarbonate, and occurs as a white crystalline powder, of a faintly aromatic odour and scarcely perceptible taste, which subsequently becomes acrid and clove-like. It melts at 49 to 51°, boils at 237°, and is only slightly soluble in cold water, but very freely soluble in alcohol, and sufficiently soluble in sherry and

[1] Exalgin may also be distinguished from antifebrin, methacetin, and phenacetin by treating 0 1 grm. with 1 c c. of concentrated hydrochloric acid Phenacetin remains insoluble. Antifebrin dissolves, but separates again in crystals of the hydrochloride Methacetin also dissolves, but is recognised by the reddish-brown colouration produced on adding 1 drop of nitric acid.

other alcoholic liquids to be conveniently given in solution in such menstra. According to Sansoni, after administration of phenyl-urethane, the urine shows the para-aminophenol reaction either directly or after distillation with potassium carbonate. The proportion of urea is increased, but the urine is free from phenol, aniline, albumin, and sugar.

Substituted or Alkylated Anilines.

These bases result from the replacement of one or both of the hydrogen atoms of the amino-group of aniline by alkyl or similar radicals.

The bases of this class are obtained by heating the hydrochloride or other salt of aniline (or its homologues) with the alcohol with which it is intended to react, or the halogen salt of this alcohol with free aniline.

The only substituted anilines which require special description are the following:

	Formula	Sp gr	B p.
Methyl-aniline . .	$C_6H_5NH(CH_3)$	0.976 at 15°	192
Dimethyl-aniline . . .	$C_6H_5.N(CH_3)_2$	0 9553 at 15°	192
Ethyl-aniline . .	$C_6H_5.NH(C_2H_5)$	0 954 at 18°	204
Diethyl-aniline ...	$C_6H_5N(C_2H_5)_2$	0 937 at 13°	213 5
Phenyl aniline (Diphenylamine)	$C_6H_5NH(C_6H_5)$	1 161	302
Diphenyl-aniline (Triphenylamine)	$C_6H_5N(C_6H_5)_2$		

Diphenylamine is a very weak base, and in triphenylamine the basic character is entirely lost.

Methyl-aniline. $C_6H_5.NH(CH_3)$.

This base is obtained by the action of iodide, nitrate, or chloride of methyl on aniline, or by heating methyl alcohol with aniline hydrochloride.[1] In all cases dimethyl-aniline is formed simultaneously, and hence in the production of mono-methyl-aniline a portion of the aniline remains, in practice, unattacked.[2]

[1] Pure methylaniline may be obtained by the reaction of methyl iodide on sodium acetanilide, $C_6H_5.NNa(C_2H_3O)$, and saponification of the resultant compound by alkali hydroxide.

[2] To separate this from its mono- and di-methyl-derivatives, dilute sulphuric acid is added as long as aniline sulphate continues to separate The sulphuric acid solution is separated from the solid aniline sulphate by pressure in a linen cloth, and the expressed liquid treated with sodium hydroxide. The substance which separates is dried and treated with acetyl chloride until no further rise of temperature is observed, when the product is poured into cold water. On cooling, methyl-acetanilide, $C_6H_5.N(CH_3)(C_2H_3O)$, separates in long needles, while dimethylaniline hydrochloride remains in solution. The former product is saponified by boiling with dilute hydrochloric acid, which converts it into acetic acid and methyl-aniline hydrochloride. Another method of separating aniline from its mono- and di-methyl-derivatives is referred to in the footnote on p 92 Methyl-aniline can be re-formed by treating its nitroso-derivatives with tin and hydrochloric acid

Methylaniline is a liquid boiling at 192°. It resembles aniline, but is lighter than water, and its odour is stronger and more aromatic. The *sulphate* is soluble in ether and uncrystallisable. A solution of bleaching powder first colours it violet and then brown. The conversion of methylaniline into toluidine is referred to on page 52.

Methylaniline-nitrosamine, $C_6H_5.N(CH_3)(NO)$, separates as a yellow oil on treating a cold solution of methylaniline hydrochloride with sodium nitrite, while any aniline and dimethylaniline are converted into soluble products. If the nitrosamine be extracted by ether, and treated with tin and hydrochloric acid, it is reduced to methylaniline, which may thus be obtained in a pure state. The nitrosamine is destitute of basic properties. It has an aromatic odour, and may be distilled in a current of steam, but not alone. When methylaniline-nitrosamine is warmed with phenol and sulphuric acid, the mixture diluted with water and saturated with alkali hydroxide, it yields the intense green-blue colouration produced by all nitrosamines (Liebermann's reaction). When heated with alcoholic hydrochloric acid it undergoes molecular transformation into paranitroso-methylaniline, $C_6H_4(NO).NH(CH_3)$, a substance crystallising in green-plates or steel-blue prisms, and otherwise resembling paranitroso-dimethylaniline (page 90).

Dimethyl-aniline. $C_6H_5.N(CH_3)_2$.

This important base is obtained by the action of excess of methyl iodide on aniline. On the large scale, methyl iodide was formerly employed, but was afterward replaced by the nitrate, and this again (owing to its explosive properties) was superseded by the very volatile methyl chloride. The product obtained in this way contained about 5% of monomethyl-aniline, but no other admixtures. Dimethylaniline is now always manufactured by heating together a mixture of aniline hydrochloride, aniline, and methyl alcohol.[1] The methyl alcohol employed must be quite free from ethyl alcohol and acetone, the latter of which not only reduces the yield, but gives a product unsuitable

[1] The aniline must be free from toluidine and impurities insoluble in hydrochloric acid; and the methyl alcohol employed must be quite free from ethyl alcohol and acetone, the latter of which not only reduces the yield, but gives a product unsuitable for the preparation either of methyl-violet or malachite green, owing to the formation of a base of the formula $CH_2(C_6H_4.N(CH_3)_2)_2$. 93 parts of aniline are used, of which 18 are saturated with hydrochloric acid and 75 parts of methyl alcohol The excess of methyl alcohol, and comparatively small quantity of hydrochloric acid, tend to produce a purer oil With more hydrochloric acid, the reaction takes place at a lower temperature, but there is a danger of forming toluidine. The mixture is heated at first to a temperature of 270°, at a pressure not exceeding 27 atmospheres. When the reaction is complete, in about 15 hours, the pressure decreases without the temperature being reduced (Schoop, *Chem. Zeit*, 1887, 11, 253).

for the preparation either of Methyl-violet or Malachitegreen, owing to the formation of a base of the formula· $CH_2(C_6H_4.N(CH_3)_2)_2$.

Dimethylaniline is a colourless oily liquid, solidifying at 0.5° and boiling at 192°. It has a sharp basic odour, and forms uncrystallisable salts. It unites with methyl iodide, with energy at the ordinary temperature, to form the iodide of trimethyl-phenylammonium, which breaks up again into its constituents on distillation, but by reaction with argentic oxide yields trimethyl-phenylammonium hydroxide, $Me_3PhN.OH$, a crystalline, very deliquescent, corrosive, and very bitter base.

With bleaching-powder solution, dimethylaniline merely gives a pale yellow colouration, a reaction by which any contamination by aniline or mono-methylaniline can be detected, as these bases give a violet colour with the same reagent (page 57). Mild oxidising agents, such as chloranile, carbon oxychloride, and cupric chloride, convert the methylaniline into Methyl-violet (Vol. 5). With acid chlorides and aldehydes, it yields complex compounds. Thus with benzaldehyde it gives tetra-methyl-paradiamino-triphenylmethane, and the corresponding hydroxide or carbinol, $C_6H_5.[N(CH_3)_2]_2.OH$, obtained from this by oxidation, is the base of Malachite or Benzaldehyde Green (Vol. 5). By reaction with diazobenzene chloride, dimethylaniline is converted into dimethyl-amino-azobenzene, $C_6H_5.N_2.C_6H_4.N(CH_3)_2$, or Butter Yellow; while with diazobenzenesulphonic acid it yields Helianthin or Methyl-orange (Vol. 4).

Paranitroso-dimethylaniline, $C_6H_4(NO).N(CH_3)_2$, is produced by the action of nitrite of sodium or nitrite of amyl on dimethylaniline.[1] It is manufactured on a large scale for the production of Methylene-blue, Indophenol, and Toluylene-red (Vol. 5). It crystallises in large green plates or tables, soluble in ether. By oxidation with potassium permanganate or ferricyanide, it is converted into *p*-nitro-dimethylaniline, $C_6H_4(NO_2).N(CH_3)_2$, which forms long, sulphur-yellow needles, melting at 162–163°. When boiled with alkali hydroxide, nitroso-dimethylaniline is completely split up into dimethylamine, $H.N(CH_3)_2$ (which may, by this reaction, readily be obtained pure), and nitrosophenol or quinonoxime, $C_6H_4O(NOH)$ (Vol. 5).

[1] Ten parts of dimethyl-aniline are dissolved in 50 of strong hydrochloric acid and 200 of water, and to the cold solution is gradually added a solution of 5.7 parts of sodium nitrite in 200 of water, when the hydrochloride of the nitroso-compound is obtained as a substance crystallising in yellow needles, from which the free base is obtained by treatment with topassium carbonate and solution in ether.

Commercial dimethylaniline usually contains more or less aniline and methylaniline. By the entrance of methyl into the benzene-nucleus, more or less dimethyl-toluidine, $C_6H_4(CH_3).N(CH_3)_2$, and higher homologues are usually present in addition. Hence the dimethylaniline of commerce usually boils between 198° and 205°. The smaller the range in the b. p. the better the sample.

O. de Coninck (*Comptes Rend.*, 1900, **131**, 945–946) gives a summary of the reactions of substituted anilines.

The following reactions are given by alcoholic solutions of the bases with solutions of salts of copper, nickel, and cobalt:

	Methylaniline	Dimethylaniline	Ethylaniline	Diethylaniline
Dilute copper chloride.	Bluish opalescence, darkening gradually In time, slight bluish-white precipitate Fleeting white fluorescence	Bluish opalescence in time, slight green precipitate	Bluish opalescence, bluish-white fluorescence, In time, slight green precipitate	Bluish-white opalescence then slight green precipitate
Concentrated copper chloride.	Turbidity, then greenish precipitate Bluish-white fluorescence Gradual change through grey, violet-grey, dark violet to (after 12 days) carmine	Bright green precipitate The liquid gradually becomes turbid	Abundant greenish-white precipitate	Abundant green precipitate
Dilute copper sulphate	No opalescence or fluorescence Bluish-white precipitate	Thick, flocculent, bluish-white precipitate.	Abundant bluish-white precipitate, flocculent	Bluish opalescence Then slight blue precipitate
Concentrated copper sulphate	Immediate green colouration Then abundant green precipitate	Immediate precipitate abundant, bright blue.	Immediate greenish-white precipitate Liquid slowly turns greenish-yellow	Bright blue precipitate fairly abundant.
Dilute copper acetate.	Colouration rose, dark rose, amber, dark brown Then dark brown precipitate	Bluish tint turning to lilac.	Brown colouration after a time	Bluish opalescence. Then slight bluish precipitate.
Concentrated copper acetate	Immediate emerald-green colouration, rapidly darkening to black Then slight black precipitate.	Turbidity Then green precipitate, which dissolves in strong alcohol to a bright blue liquid, becoming bright green, afterward dark green.	Emerald-green coloration Then bright green precipitate, soluble in strong alcohol. The solution after 4 hrs turns dark green, and deposits the same precipitate	Turbidity Afterward slight green precipitate.

	Methylaniline	Dimethylaniline	Ethylaniline	Diethylaniline
Dilute cobalt chloride.	No colouration In time precipitate of cobalt hydroxide.			
Concentrated cobalt chloride.	Violet-carmine colouration. After 4½ hrs, turbidity and deepening of the tint.			
Dilute nickel chloride.	Gradual turbidity but no precipitate even after a long time			
Concentrated nickel chloride	After 6 hrs extremely slight green precipitate Afterward colour changes to dirty green, then to very dark brown			

The presence of aniline and methyl-aniline is indicated by the rise of temperature produced on treating 5 c.c. of the dry oil with an equal measure of acetic anhydride. This is stated to be 0.815° for each unit per cent. of methylamine present. For small percentages this appears to be fairly correct, but with a product actually containing 30%, an excess of over 7% is said to be indicated. A serious objection to the method is that it wholly fails in presence of aniline. But the presence of aniline can be recognized by mixing a few drops of the oil with a few drops of ether, and adding 1 drop of conc. sulphuric acid, when, if aniline be present, its sulphate will separate as a white precipitate.

A more plausible method is that of Nolting and Boasson (*Ber.*, **10**, 795), based on the different behaviour of the bases with nitrous acid,[1] but the results yielded in practice have been found unreliable by Reverdin and de la Harpe. These chemists recommend (*Chem. Zeit.*, 1889, **13**, 387, 407) for the estimation of the aniline and methyl-aniline

[1] When aniline hydrochloride is treated in cold solution with sodium nitrite, it yields diazobenzene chloride, while dimethylaniline is converted into the hydrochloride of its nitroso-derivative. Both these substances are freely soluble in water, while methyl-aniline is converted by the same treatment into the non-basic methylaniline-nitrosamine, which can be extracted by agitating the liquid with ether. If this reaction occurred in its simplicity, the monomethyl-aniline could be estimated from the weight of the nitrosamine left on evaporating the ethereal solution But when this is distilled in a current of steam, in which the nitrosamine is volatile, a considerable quantity of nitrophenyl-methyl-nitrosamine, $C_6H_4(NO_2)N(NO)(CH_3)$, remains as a residue This substance is clearly produced by the oxidation of the nitrosamine, and direct experiment shows that pure methyl-aniline, on treatment with excess of nitrous acid, is converted into it, to the exclusion of the simple nitrosamine. As the molecular weights of the two bodies are materially different (181:136), the indefinite character of the reaction prevents the accurate estimation of the methylamine (Reverdin and de la Harpe, *Chem. Zeit.*, 1889, 13, 387, 407).

conjointly, acetylisation of the bases, and estimation of the excess of acetic anhydride by titration with alkali; and for the estimation of the aniline, diazotising and treating the product with beta-naphthol disulphonic acid.

Methylaniline and dimethylaniline may be distinguished in mixtures with Recognition of- in the presence each other according to H. Emde (*Arch. Pharm.*, 1909, **247**, 77–79).

The platinum salts can be used to distinguish between methylaniline and dimethylaniline in the presence of each other. Methylaniline platinic chloride, $(BHCL)_2PtCL_4$, forms short orange-coloured crystals, which decompose at 199° and can be crytallised from hot water containing hydrochloric acid. Dimethylaniline platinic chloride, $(BHCL)_2PtCL_4$, is produced by dissolving the base in strong hydrochloric acid and adding the solution to 10% platinum chloride solution. The orange-yellow precipitate is filtered off in the cold and recrystallised from alcohol containing hydrogen chloride. It then forms orange-red needles, decomposing at 173°. If a mixture of the bases is suspected, a small portion dissolved in acid is added to a platinum chloride solution, and the platinum double salts separated by crystallising from alcohol. If methylaniline only is to be looked for, the mixture can be crystallised from water. The presence of 5% of methylaniline in dimethylaniline can be recognized in this way.

At ordinary temperatures acetic anhydride has no action on dimethylaniline, but on prolonged heating tetramethyl-diamino-phenylmethane is formed in considerable quantity, if the reagent be in excess. Methylaniline is converted into methyl-acetanilide, $C_6H_5.N(CH_3)(C_2H_3O)$, and aniline in the cold yields acetanilide, $C_6H_5.NHC_2H_3O$, but on heating more or less diacetanilide, $C_6H_5.N(C_2H_3O)_2$, is produced. To avoid the formation of these secondary products the following method of working is recommended: From 1 to 2 grm. weight of the sample is mixed as rapidly as possible with an accurately known quantity (about twice its weight) of acetic anhydride, in a small flask fitted with a reflux condenser. After standing for half an hour at the ordinary temperature, 50 c.c. of water should be added, and the flask heated on the water-bath for 50 minutes to effect the conversion of the excess of acetic anhydride into acetic acid. The liquid is then cooled, diluted to a known volume, and an aliquod part titrated with standard caustic alkali, using phenolphthalein as an

indicator.[1] By this means the excess of acetic anhydride, $C_4H_6O_3$, is ascertained, and the difference between the amount so found and that employed is the weight which has reacted with the *aniline* and *methyl-aniline* contained in the sample. 51 parts of acetic anhydride consumed in the reaction correspond to 107 of base in terms of *methyl-aniline*, and the percentage of base thus found (*a*) is calculated and recorded.

The *aniline* itself is determined as follows: From 7 to 8 grm. of the sample in dissolved is hydrochloric acid (28 to 30 c.c.), and diluted with water to 100 c.c. 10 c.c. of this solution is further diluted with water and cooled by ice. The solution is then diazotised by adding a solution of sodium nitrite in quantity sufficient to react with the whole of the sample if it consisted of aniline solely A solution of the sodium salt of β-naphthol-disulphonic acid known as "R Salt" (Vol 5), is meanwhile prepared of a strength approximately corresponding to 10 grm. of naphthol per litre, and its precipitating power is calculated from its known strength, or exactly ascertained by experiment with pure aniline.

A measured quantity of this solution is then treated with excess of sodium carbonate, and to it the ice-cold solution of the diazotised sample is slowly added. Common salt is then added till a precipitate ceases to form, when the liquid is filtered, and portions of the filtrate are tested with R salt and the diazo-solution respectively, to ascertain which of these two is present in excess. Another experiment is then made with suitably varied volumes, until after a few trials exact precipitation of the colouring matter is attained without sensible excess of either the naphthol or diazo-solution. The reactions which occur are as follows:

$$C_6H_5.NH_2,HCl + HNO_2 = C_6H_5N:N.Cl + 2H_2O;$$ and $$C_6H_5.N_2.Cl + C_{10}H_5(OH)(SO_3Na)_2 = HCl + C_6H_5.N_2.C_{10}H_4(OH)(SO_3Na)_2.$$

From these formulæ, and the volumes of the 2 solutions required for exact reaction, the weight of aniline present can be calculated. 1 grm. of R salt will react with 0·2672 grm. of *aniline*. The percentage of aniline thus found (*b*) is multiplied by 1.15 ($=\frac{107}{93}$), which gives its equivalent in methyl-aniline, and this (*c*) subtracted from the sum of aniline and methyl-aniline in terms of methyl-aniline found by the acetylisation process (*a*) gives the percentage of real *methyl-aniline* (*d*) present. The *dimethyl-aniline* is estimated by difference.

[1] H. Giraud (*Bull Soc Chim*, 1889, **2**, 142) modifies this process by employing the acetic anhydride dissolved in 10 times its volume of dimethylaniline 10 c c of this solution is added to 1 grm of the sample After standing for 1 hour in a corked flask, water is added, and the liquid (boiled for some time and) titrated with standard barium hydroxide in the presence of phenolphthalein.

In the case of a sample of known composition, Reverdin and de la Harpe obtained the following satisfactory results by the foregoing process:

	Present.	*Found.*
Aniline,	10.42%	10.30%
Methylaniline,	10 97	11.16
Dimethylaniline (by difference),	78.61	78 54
	100.00	100.00

The presence of methylaniline is more objectionable in dimethylaniline intended for the manufacture of green than in that to be used for violet. Schoop (*Chem. Zeit.*, 1887, **11**, 254) states that the proportion seldom exceeds 2%, and that the best qualities of dimethylaniline are nearly or quite free from it. When present, monomethylaniline can be removed by shaking the oil with a small quantity of dilute sulphuric acid, or by boiling with acetic acid for 2 hours.

Diethylaniline. $C_6H_5.N(C_2H_5)_2$.

This base is best prepared by heating 1 molecule of aniline hydrobromide with 10% in excess of one molecule of ethyl alcohol to 145° for 8 or 10 hours. Nearly the theoretical yield is obtained. The base boils at 213.5°. Diethyl-*o*-toluidine and diethyl-*p*-toluidine may be obtained by exactly similar means.

Diphenylamine. Phenylaniline. $C_6H_5.NH.C_6H_5$.

This base is obtained by heating aniline with the hydrochloride or other salt of aniline. 6 parts of aniline and 7 of aniline hydrochloride are heated to 250° under a pressure of 4 or 5 atmospheres for 24 hours. The ammonia formed is allowed to escape at intervals to prevent reconversion of the diphenylamine into aniline. The product is treated with warm hydrochloric acid and a large quantity of water, which dissolves any unchanged aniline hydrochloride, and decomposes the hydrochloride of diphenylamine, which latter base separates out and is purified by distillation. Diphenylamine crystallises in small white plates, having an agreeable flowery odour and burning taste. It melts at 54° and boils at 302° (Graebe). It is almost insoluble in water, but readily in alcohol, ether, benzene, and aniline. Diphenylamine has very feeble basic properties. The *hydrochloride* is a white crystalline powder, which turns blue in the air, and is decomposed by water. The most characteristic test of diphenylamine is the deep blue colour produced by adding a trace of nitric acid to its solution in

strong sulphuric acid. The reaction, which is very delicate, is employed as a test for nitric acid.

Diphenylamine; Reaction of—— with nitric acid.

The deep colouration produced by the action of nitric acid on diphenylamine sulphate, which has been looked upon as a characteristic reaction for nitric acid, is also, according to I. Bay (*Comptes Rend.*, 1905, **140**, 796–797), observed with other oxidising agents, and also by long exposure to the atmosphere. It is pointed out that, in general, all aromatic amines give rise to more or less highly coloured oxidation products.

Commercial diphenylamine should be pale yellow, melt not much below 54°, be free from unpleasant odour and oily matters, and give no violet colouration with bleaching powder. It is used for making Diphenylamine Blue, Aurantia, and Orange IV.

Diphenylamine in alcoholic solution reacts with bromine to form tetrabromodiphenylamine (W. Dreger, *Z. ges Schiess- und Sprengstoffn.*, 1909, **4**, 123) $(C_6H_5)_2NH8Br = (C_6H_3Br_2)_2NH4HBr$. This bromo-derivative is insoluble in water, sparingly soluble in alcohol, readily soluble in benzene, xylene, chloroform, and ethylacetate, especially on warming; it melts at 102°, and crystallises in reddish needles having a silky lustre. For the determination of diphenylamine in the commercial product, the sample is dissolved in alcohol or, if already in ethereal solution, the ether may be driven off by adding alcohol and warming. Excess of bromine is then added, drop by drop, with constant stirring. The solution is then mixed with twice its volume of water and the whole boiled until the alcohol and excess of bromine are driven off and the bulk is reduced to one-half, constant stirring is needful. The precipitate of tetrabromdiphenylamine is transferred to a funnel, or Gooch crucible, connected to a water pump, and washed with warm water to remove the last traces of alcohol and bromine, and lastly dried at 98–100° until of constant weight. Smaller quantities may be estimated by evaporating to dryness in a previously weighed glass vessel. If it be desired to estimate the diphenylamine contained in gelatinised nitro-cotton, the nitro-compound is gradually decomposed by means of soda lye in a capacious flask. The flask is closed by a doubly perforated stopper fitted with a stoppered funnel, and a bent tube attached to a condenser, which communicates with a suitable receiver. The free diphenylamine and any camphor that may be present are carried into the receiver which

contains ether. The distillate is well shaken with common salt; the ether completely dissolves the diphenylamine and any camphor, and the estimation is then completed as before indicated.—J. W. G.

Methyl-diphenylamine, $C_6H_5.N(CH_3)C_6H_5$, boils at 282°, and gives various colour-reactions with oxidising agents. In dilute sulphuric acid it dissolves to form a liquid of the colour of solution of potassium permanganate.

Warm nitric acid converts diphenylamine and its methyl-derivative into $C_6H_2(NO_2)_3.NH.C_6H_2(NO_2)_3$, hexanitro-diphenylamine, the ammonium salt of which constitutes the colouring matter known as *Aurantia* (Vol. 5).

p-Amino-diphenylamine results from the reduction of phenyl-amino-azobenzene, nitro-phenylamine, or Tropæolin OO (Vol. 5).

Triphenylamine. Diphenylaniline. $(C_6H_5)_3N$.

This substance is formed by the action of brombenzene on dipotassium aniline. It is a neutral substance, melting at 127°, and crystallising from ether in monoclinic pyramids. It forms no isonitrile, picrate, nor acetyl-compound, but yields iodide of triphenyl-methyl-ammonium on treatment with methyl iodide. Its solution in glacial acetic acid is coloured green on adding a little nitric acid, but with sulphuric acid it gives a violet colouration changing to blue.

Aminophenols.

By the reduction of the nitrophenols, corresponding amino-compounds are obtained. These substances may also be prepared by heating either of the 3 isomeric amino-hydroxybenzoic acids, $C_6H_3(NH_2)OH.COOH$, with barium hydroxide.

In the aminophenols the acid character of the phenols is neutralised by the presence of the amino-groups, so that they only yield salts with acids; but as phenols they are still capable of yielding alkyl-derivatives (*e. g.*, anisidine), while the hydrogen of their amino-groups may be replaced for acetyl, etc., as in phenacetin.

The aminophenols form colourless crystalline scales or plates, which are very readily oxidisable on exposure to air, with blackening and formation of resinous products, especially if impure. On the other hand, their hydrochlorides are relatively stable, and often capable of sublimation. The solution of *p-minophenol* hydrochloride is coloured first violet and then green by solution of bleaching powder, quinone

chlorimine, $C_6H_4O(NCl)$, being formed; while with chromic acid mixture, and other oxidising agents, it yields quinone, $C_6H_4O_2$. Treatment with hydrogen sulphide and ferric chloride converts it into compounds of the *methylene-blue* group (Vol. 5).

The formyl- and acetyl-derivatives of the aminophenols are converted with great facility into anhydro-bases. Thus ethenyl-aminophenol, a basic liquid boiling at 200 to 201°, is obtained by boiling ortho-aminophenol with acetic anhydride.

$$C_6H_4(OH).NHC_2H_3O = C_6H_4\begin{cases} N \\ \diagup\!\diagdown \\ O \end{cases} C\,CH_3 + H_2O.$$

When this substance is heated with dilute acids, the reverse action occurs, acetyl-*o*-aminophenol being formed.

The methyl esters of the aminophenols (anisidines or amino-anisols), and the corresponding ethyl esters (phenethidins or aminophenatols), are bases resembling aniline, and are employed for producing certain azo-dyes (*e. g.*, Anisol Red, Phenatol Red; Vol. 5). The acetyl-derivatives of these esters are used in medicine under the names of *metacetin* and *phenacetin* (see below), the latter being widely used.

The following table shows the characters of the isomeric aminophenols and their derivatives·

	o-1 2		*m*-1 3		*p*-1 4	
	M p	B p	M p	B p	M p	B p
Aminophenol (page 97) . $C_6H_4\{^{OH}_{NH_2}$	° 170	° sub-limes.	°	°	° 184	°
Acetyl-derivative . . . . . $C_6H_4\{^{OH}_{NH(COCH_3)}$	201	.	. .		179	
Methyl-ester (anisidine) . $C_6H_4\{^{O(CH_3)}_{NH_2}$		228		251	56	246
Ethyl-ester (phenethidine) $C_6H_4\{^{O(C_2H_5)}_{NH_2}$		229		180–205 (at 100 mm)	.	253
Methacetin (page 104). . . . . $C_6H_4\{^{O(CH_3)}_{NH(COCH_3)}$	84	204	.	. . .	127	
Phenacetin (page 99) . . . . . $C_6H_4\{^{O(C_2H_5)}_{NH(COCH_3)}$	70	. .	97	. . . . .	135	
Aminophenacetin. Phenocoll . . . . . $C_6H_4\{^{O(C_2H_5)}_{NH(CO\,CH_2\,NH_2)}$	. . .	. . .	. . . .	. . . . .	100.5	

Phenacetins. Acet-phenetidines.

$$C_6H_4 \begin{cases} O(C_2H_5) \\ NH(CO.CH_3) \end{cases}$$

The substances of this formula have acquired value as antipyretics and analgesics.

The phenacetins are prepared by ethylating the corresponding mononitrophenols, thus obtaining the isomers of the formula $C_6H_4(NO_2).OC_2H_5$. On treatment with zinc or iron and hydrochloric acid, these are reduced to the corresponding phenethidines, $C_6H_4(NH_2).OC_2H_5$, which are purified and acetylised by heating with glacial acetic acid for some hours, the products being recrystallised from water.

Of the 3 isomeric phenacetins, the *m*-compound is unimportant. It forms tasteless and odourless scales, melting at 96°.

Para-acetphenetidin is the official variety in the *German*, *British*, and *United States Pharmacopœias*. It forms white, odourless, tasteless, glistening scaly crystals. It requires 1400 parts of cold, or 70 parts of boiling, water for solution, and is soluble to a notable extent in chloroform. Its solution in 16 parts of alcohol is precipitated by the smallest addition of water. The crystals melt at 135°.

Ortho-acetphenetidin forms brilliant white, very light spangles, without taste or odour, and melting at 70°. It is very slightly soluble in cold, but more readily in hot, water, separating again on cooling. It dissolves in about 3 parts of rectified spirit, and abundantly in chloroform.

Besides the differences in their m. p. and solubilities, *p*- and *o*-phenacetin are distinguished by their behaviour when boiled for several hours with dilute sulphuric acid (sp. gr. 1.26). When thus treated, the para-compound yields acetic acid and sparingly soluble sulphate of phenetidin. Orthophenacetin, on the other hand, is not decomposed by the same treatment, requiring the action of acid of 1.575 sp. gr. for 2 hours at 90° to effect its hydrolysis.[1] If in either case the acid liquid be diazotised, and then treated with an ammoniacal solution of naphthol-disulphonic acid, a fine red-yellow colour will be obtained if *p*-phenacetin was employed, while with the *o*-compound a cherry-red colouration is produced. In either case the colouring matter may be precipitated by brine.

[1] S. Lüttke detects *o*-phenacetin by boiling 15 grm of the sample with 25 grm. of dilute hydrochloric acid, when *o*-phenetidin hydrochloride is formed, from which the free base may be separated by sodium hydroxide, and its b p. (given by Lüttke as 242 5°) determined. The hydrochloride gives a blood-red colouration with ferric chloride.

This formation of an azo-colouring matter may be employed to detect the phenacetins in urine and other organic liquids. The urine is evaporated to dryness, and the residue treated with hot alcohol. The solution is filtered, evaporated, and the residue boiled for 2 hours with dilute sulphuric acid (sp. gr. 1.26) under a reflux condenser. The resultant solution is cooled to 5 or 6°, treated with a 1% solution of sodium nitrite for 5 minutes, and then poured into a solution of naphthol-disulphonic acid in excess of ammonia, taking care that the mixture remains alkaline. If either modification of phenacetin be present in the urine a characteristic colouration will be produced, from the intensity of which the amount of phenacetin may be estimated.

For medicinal use, phenacetin is said to present considerable advantages over antipyrine, and especially over antifebrin (acetanilide), for while the latter body is decomposed in the system with formation of aniline, which has marked toxic properties, phenacetin yields phenetidin, $C_6H_4(OC_2H_5).NH_2$, and amidophenol, $C_6H_4(OH).NH_2$, which are said to be harmless. Paraphenacetin, in doses ranging from 8 to 20 grains for adults, and from 2 to 3 grains for children, is said to be a valuable antipyretic and anti-neuralgic, without producing nausea, vomiting, cyanosis, or disagreeable after-effects Being nearly insoluble, it is best given in the form of powders. The dose of *o*-phenacetin required to produce the same effect is larger than that of the *p*-compound.

According to Reuter (*Pharm. Zeit.*, 1891,) phenacetin is liable to contain unconverted *p-phenetidin*, which appears to be poisonous in very small doses, if taken for some time, producing nephritis and albuminuria. To detect the impurity, Reuter melts 2.5 grm. of chloral hydrate at 100°, and adds 0.5 grm. of the sample. On agitation the phenacetin dissolves, and, if pure, the solution will remain colourless when heated on the water-bath for 5 minutes, though after longer heating it will assume a rose tint. In presence of para-phenetidin, an intense colouration, ranging from red-violet to blue-violet, is produced in 2 or 3 minutes at most.

S. Lüttke detects *diaminophenols* or *diaminophenatols* in phenacetin by grinding 0.5 grm. of bleaching powder to a fine paste with hydrochloric acid, and adding about 0.03 of the sample, when a red colour will be produced.

G. M. Beringer (*Amer. Pharm. Assoc.*, 1903; *Chem. and Druggist*, 1903, **63**, 377) suggests the following test. 0.1 grm. of the phenacetin

is boiled with 3 c.c. of a 50% solution of sodium hydroxide for 1 minute, then cooled and shaken with 5 c.c. of sodium hypochlorite solution. If the sample be pure, a clear yellow liquid is obtained, but if acetanilide be present, a purple-red or brownish-red turbidity or precipitate is produced.

The lower price of *acetanilide*, and its close physical resemblance to phenacetin, have suggested the possibility of the partial or complete substitution of the former body for the latter, and a flagrant instance of such a practice is actually on record (*Pharm. Jour.*, [iii], **21**, 377). The presence of 5% of acetanilide lowers the m. p. of the sample to 127–128°.

H. Schwartz (*Pharm. Journ.*, [iii], **18**, 1085) recommends that 1 grm. of the suspected sample should be heated with 2 c c. of sodium hydroxide solution, a fragment of chloral hydrate or a few drops of chloroform added, and the mixture again gently heated. With phenacetin the odour is aromatic and not disagreeable, but in presence of acetanilide, the penetrating and repulsive smell of phenyl-carbamine, $C_6H_5.NC$, is produced. On boiling the sample with sodium hydroxide solution, oily drops of aniline separate if acetanilide be present in considerable quantity. If the cooled liquid, together with the separated globules, be shaken with ether, and the ether separated and evaporated, the residue when dissolved in water and treated with a drop of carbolic acid, and a clear solution of bleaching powder added, gives a blue-green colouration changed to onion-red by hydrochloric acid, and restored by ammonia. (See also *J. Soc. Chem. Ind.*, 1888, **7**, 772).

The following tests for distinguishing acetanide from phenacetin have been suggested by E. Barral (*J. Pharm. Chim.*, 1904, **19**, 237).

Phosphomolybdic acid reagent gives a bright yellow precipitate in an aqueous solution of acetanilide which is soluble on heating. Phenacetin gives a similar precipitate which is insoluble on heating.

Mandelin's reagent (1 grm. of ammonium vandate in 200 grm. of sulphuric acid) gives a red colour, changing rapidly to greenish-brown with acetanilide. With phenacetin the colour is olive-green while cold and reddish-brown after being warmed.

Barral has also suggested the following additional colour tests for phenacetin. *Sodium persulphate* produces a yellow colour when warmed with phenacetin, when boiled for some time the colour becomes orange.

Bromine water when heated in contact with a few crystals of phe-

nacetin, colours the latter rose, the liquid becomes orange-yellow, and a brown precipitate is gradually formed on cooling.

Million's reagent when heated in contact with phenacetin turns yellow and then to a red; nitrous fumes are finally formed.

For the detection of acetanilide in phenacetin, M. J. Schroder recommends that 0.5 grm. of the sample should be boiled with 8 c.c. of water, and the liquid filtered when cold from the recrystallised phenacetin. The filtrate is boiled with a little potassium nitrite and dilute nitric acid, a solution of mercurous nitrate containing a little nitrous acid added, and the whole again boiled. A red colour will be obtained if the proportion of acetanilide in the sample exceeds 2%.

TABLE OF TESTS FOR ACETANILIDE AND PHENACETIN

Comparative	Acetanilide	Phenacetin
M p	112°	135°
Boils	295°	
Solubilities at 25°	2 5 pts alcohol 0.4 pts boiling alcohol 5 pts chloroform 179 pts water 18 pts boiling water Solution conc H_2SO_4 without change of colour	12 pts alcohol 2 pts boiling alcohol. 925 pts water 70 pts boiling water. 63 pts ether 20 pts chloroform Solution conc H_2SO_4 without change of colour
Phenol and bleaching powder solution (See page 57)	Behaves like aniline	No reaction
Iso-nitrile test with alkali hydroxide and chloroform (See page 56)	Odour of *iso*-nitrile at once	Odour of *iso*-nitrile only on standing a few minutes
Boiling with a strong solution of sodium hydroxide	Oily drops (aniline separates)	No separation
Addition of sodium nitrite and dilute nitric acid boiled	Red colour	No colour
Then mercurous nitrate added and boiled	Bright yellow ppt soluble on heating	Bright yellow ppt insoluble on heating
Phosphomolybdic acid to aqueous solution.		
Mandelin's reagent in aqueous solution.	Red colour changing rapidly to greenish-brown	Olive-green colour while cold and reddish-brown after being warmed

If 1 grm. of a mixture of equal parts of phenacetin with acetanilide be shaken with 200 c.c. of water, the whole of the acetanilide goes into solution together with 0.130 grm. of phenacetin, while the remainder of the phenacetin remains insoluble. If this be separated, its weight,

when corrected by an addition of 0.130, will represent the phenacetin present in 1 grm. of the sample (*Pharm. Jour.*, [III], **21**, 377).

Phenacetin is distinguished from *exalgin* and *antifebrin* by boiling 0.1 grm. for a minute with 1 c.c. of hydrochloric acid, adding 10 c.c. of water, filtering, and adding to the filtrate 3 drops of a 3% solution of chromic acid, when a ruby-red colour will be gradually developed. (See *Pharm. Jour.*, [III], **21**, 978.) Strong sulphuric acid should dissolve phenacetin without becoming coloured, while a saturated solution, if free from phenol and acetanilide, will not become turbid on adding bromine-water.

No reliable method has been so far singly known for the estimation of acetanilide and phenacetin in complex mixtures. The following method of J. L. Turner and C. E. Vanderkleed (*Amer. Jour. of Pharm.*, 1907, **79**, p. 521), however, is said to give good results. It is based on the saponification of acetanilide by an alkali, distillation of acetic acid from the resulting acetate by means of a phosphoric acid solution, and titration of the distillate. It is carried out as follows: 1 grm. of acetanilide is saponified by heating to boiling under a reflux condenser, for 1 1/2 to 2 hours, with 3 grm. of sodium hydroxide, .020 grm. of alcohol and .010 grm. of water. The solution is transferred to an evaporating dish, and the alcohol driven off on the water-bath. The residue is transferred to a separator and shaken out once with ether in order to remove the aniline separated from the acetanilide. The ethereal solution is shaken out twice with water to remove traces of sodium acetate, which is somewhat soluble in ether, and the washings added to the orginal aqueous residue. The aqueous solution is then transferred to a flask of 1 litre capacity, acidified with 0.025 grm. of 85% phosphoric acid, and the acetic acid completely distilled off with steam, this being shown when no further reaction is given with litmus. The distillate is then titrated with N/1 sodium hydroxide, with the addition of 0.001 grm. of 1% solution of phenolphthalein. 0.001 grm. of N/1 sodium hydroxide = 0.13409 grm. of acetanilide. Phenacetin, being closely allied to acetanilide, is estimated in exactly the same way, 1 mgrm. of N/1 sodium hydroxide being equivalent to 0.17779 grm. of phenacetin. The two substances, however, cannot be estimated when present in the same preparation. Acetates, nitrates, and nitrites interfere with the method. These salts are removed by means of chloroform extraction; but when they are present in the phosphoric acid, or in the alkali used for saponification, they must

first be removed. Chlorides do not interfere, but if carbonate be present in the alkali used for saponification, the carbon dioxide formed on the addition of phosphoric acid would be distilled over with the acetic acid and vitiate the result of the titration; it is therefore advisable to heat the acidified solution to boiling under a reflux condenser before distillation, in order to drive off the carbon dioxide.

Methyl-phenacetin, $C_6H_4(O.C_2H_5).N(CH_3)(C_2H_3O)$. This substance is prepared by treating *p*-phenacetin in xylene solution with sodium, and causing the resultant sodium-derivative to react with methyl iodide (*Pharm. Jour.*, [III], **21**, 81). The new product distils at about 300° as an oil, which crystallises on standing. It may be purified by recrystallisation from alcohol or ether, when it forms colourless crystals, moderately soluble in water, and having marked narcotic as well as antipyretic characters.

Amino-p-phenacetin, $C_6H_4(O.C_2H_5).NH(CO.CH_2.NH_2)$.—The *hydrochloride* of this base is readily soluble in water and alcohol, and has been introduced, under the name of "*phenocollum hydrochloricum*," as an antipyretic and antirheumatic. Prolonged boiling with alkalies splits it into *p*-phenethidine and glycocine.

Para-phenetol carbamine, Dulcin, $CO(NH_2)NH.C_6H_4OC_2H_5$. Colourless crystals, m. p. 173°. Sparingly soluble in cold water, much more so in hot water, alcohol and ether. This substance is 200 times as sweet as sugar.

Formyl-p-phenetidin, $C_6H_4(O.C_2H_5)\,NH(CO.H)$, though having a constitution similar to acet-phenetidin, appears to have no antipyretic properties, but has been suggested as an antidote in cases of poisoning by strychnine.

Para-diethoxyethenyldiphenylamidin, $CH_3C:(N.C_6H_4OC_2H_5)NH.C_6H_4OC_2H_5$. It is a white crystalline powder, insoluble in water, m. p. 121°. The hydrochloride, m. p. 189° is more used as a local anesthetic.

Methacetin is the commercial name of *p*-acet-anisidine, $C_6H_4(O.CH_3).NH.C_2H_3O$. It is, consequently, the lower homologue of phenacetin. It forms a crystalline powder or small lustrous scales or plates, odourless, but of a faintly bitter taste. It melts at 127°, and at a higher temperature boils and distils unchanged. It dissolves in 526 parts of cold, or 12 of boiling, water, and is easily soluble in alcohol, acetone, chloroform, and dilute acid and alkaline liquids. It is less soluble in benzene, and only with difficulty in ether,

carbon disulphide, petroleum spirit, and oil of turpentine, but dissolves freely, on warming, in glycerin and fixed oils. In its general reactions and physiological effects, metacetin closely resembles phenacetin, though according to some authorities it has a less powerful, and according to others a more powerful, action. Its efficacy in cases of neuralgia and rheumatism is said to greatly exceed phenacetin, from which it may be distinguished by its physical characters, or by heating it with a quantity of water insufficient for its solution. When thus treated, methacetin melts and solidifies again on cooling, whereas phenacetin undergoes no apparent change. 1 c.c. of hydrochloric acid dissolves 0.1 grm. of methacetin very easily, whereas the same quantity of phenacetin is mainly undissolved.

Diaminophenols. $C_6H_3(OH)(NH_2)_2$.

These substances are weak bases, forming salts which crystallise well and give aqueous solutions which turn brown in the air; and are coloured an intense violet or dark red by potassium dichromate, ferric chloride, or bleaching powder.

Triaminophenol. $C_6H_2(OH)(NH_2)_3$.

This substance is an unstable base resulting, from the complete reduction of picric acid, $C_6H_2(OH)(NO_2)_3$, in acid solutions. If alkaline reducing agents be employed, the action does not proceed beyond the formation of dinitro-amino-phenol or picramic acid, $C_6H_2(OH)(NH_2)(NO_2)_2$ (see Vol 5) A dilute solution of triamino-phenol is coloured deep blue by ferric chloride.

Phenylene-diamines. Diaminobenzenes.

Three modifications of phenylene-diamine or diaminobenzene, $C_6H_4(NH_2)_2$, are known, differing from each other in properties according to the positions of the amino-groups, thus:

	Ortho-compound 1 2	Meta-compound 1 3	Para-compound 1 4
Appearance	Tablets or plates	Crystalline mass	Tablets or small plates
M p	102–103°	63°	140°
B p	252°	287°	267°
Characters of hydrochloride.	Groups of radiating needles, readily soluble	Concentrically arranged crystals.	Readily soluble tablets, very sparingly soluble in hydrochloric acid
Reaction in neutral solution with sodium nitrite.	Separation of amino-azo-phenylene as a colourless oily liquid	Yellow or brown colouration, or precipitate of triamino-azobenzene	No reaction

o-Phenylene-diamine is distinguished from its isomerides by its reaction with sodium nitrite, and by the separation of ruby-red needles on adding ferric chloride to the solution of its hydrochloride. On treating an alcoholic solution of the base with a drop of phenanthraquinone dissolved in glacial acetic acid, and boiling for a short time, a bright yellow precipitate of diphenylene-quinoxaline, $C_{20}H_{12}N_2$, is formed. It consists of small needles which are coloured a deep red by strong hydrochloric acid, and its production affords the most delicate reaction for ortho-phenylenediamine. Its isomerides do not give the reaction, but its homologue, *o*-toluylenediamine, behaves similarly.

Meta-phenylene-diamine may be prepared by the reduction of meta-dinitrobenzene (Vol. 5). It often remains in a state of superfusion for some time, but is instantly solidified by adding a crystal of the solid substance. *m*-Phenylene-diamine is sparingly soluble in water, the solution being alkaline in reaction. It is readily soluble in ether, and may be extracted by this solvent from alkaline aqueous liquids. It is a di-acid base, the *hydrochloride* being $C_6H_4(NH_2)_2,2HCl$. The reaction of *m*-phenylene-diamine with sodium nitrite is characteristic and extremely delicate. It is due to the formation of Bismarck or Phenylene Brown (Vol. 5), and by means of it 1 part per million of nitrous acid can be detected in water.

m-Phenylenediamine possesses marked poisonous properties, its physiological action resembling that of the leucomaines and ptomaines. Dubois and Vignon (*Compt. Rend.*, **107**, 533) experimented on dogs, and found that a dose of 0.1 grm. per kilogrm. of the animal produced salivation, vomiting, diarrhœa, abundant excretion of urine at intervals, and death by coma in 12 to 15 hours. Besides these severer symptoms, all those of intense influenza were produced, such as acute coryza and sneezing, coughing, and extreme depression.

p-Phenylene-diamine occurs in aniline tailings. It may be prepared by the reduction of *p*-nitracetanilide. It is but slightly soluble in water, but readily in alcohol and ether. When heated with dilute sulphuric acid and manganese dioxide it yields quinone, $C_6H_4O_2$, which reaction distinguishes it from its isomerides. On passing hydrogen sulphide through a solution of the hydrochloride, and then adding ferric chloride, Thionine or Lauth's Violet is formed (Vol. 5).

Para-phenylene-diamine possesses poisonous properties similar to

those of meta-phenylene-diamine, but death occurs more rapidly than with the latter base. It also exerts a special action on the eye, which is gradually forced out of its orbit by the swelling of the conjunctiva or intra-orbital cellular tissue; while the lachrymal glands are blackened by a deposit of pigment (compare "Toluylene-diamines").

Dimethyl-p-phenylene-diamine, $H_2N.C_6H_4.N(CH_3)_2$, may be obtained by the reduction of nitrosodimethyl-aniline or of Helanthin (Vol. 5). A neutral solution of the hydrochloride is coloured a beautiful purple by ferric chloride; and on treating it with a hydrochloric acid solution of hydrogen sulphide, and then adding ferric chloride till the smell of sulphuretted hydrogen has disappeared, a fine blue colouration is obtained, due to the formation of Methylene Blue (Vol. 5). This reaction is the most delicate test for

Toluylene-diamines. Diamiotoluenes. $C_6H_3(CH_3)(NH_2)_2$.

These bases closely resemble the phenylene-diamines. The *ortho-para*-modification ($CH_3:NH_2:NH_2 = 1:2:4$) is obtained by the reduction of ordinary dinitrotoluene. It melts at 88°, is used for the production of Toluylene Red and Toluylene Orange. The 1:3:4 (*meta-para*) modification is obtained by nitrating acet-*p*-toluide, saponifying, and reducing.[1] Janovsky (*J. Soc. Chem. Ind.*, 9, 383) gives the following table of reactions of neutral or slightly acid solutions of the two isomeric toluylene-diamines:

Reagent	α-Toluylene-diamine $CH_3\ NH_2\ NH_2 = 1\ 2\ 4$	β-Toluylene-diamine $CH_3\ NH_2\ NH_2 = 1\ 3\ 4$
Ferric chloride	No change at first, after standing for a long time an orange colouration	Wine-red colouration
Potassium dichromate	Yellowish-brown colouration	Reddish-brown precipitate
Potassium ferricyanide	Olive-green crystalline plates	Dark-red colouration
Bromine water	Yellowish-white precipitate	Brown flocks and magenta-red solution
Platinic chloride	Yellowish-brown colouration	Reddish-brown precipitate
Auric chloride	Brown precipitate	Red solution with blue reflex and metallic mirror in the cold
Potassium nitrite	In very dilute solutions a golden brown colouration, in concentrated a brown precipitate	No colouration, but a salmon-coloured precipitate.
Solution of bleaching powder.	Reddish-brown colouration and then a light brownish-yellow precipitate.	Dark-red colouration, then an olive-green precipitate.

[1] This modification appears to be identical with the *p*-toluylene-diamine isolated by Hell and Schoop from aniline tailings (*Berichte*, 1881, 12, 723).

The foregoing reactions are available, even in presence of other substances, for the detection and identification of the toluylene-diamines, which often result from the reduction of azo-dyes.

The toluylene-diamines are powerful poisons (compare "*m*-phenylenediamine," page 106).[1]

Benzidine. Di-*p*-amino-diphenyl.

$$C_{12}H_{12}N_2 = NH_2.C_6H_4.C_6H_4.NH_2 (1, 4:1, 4).$$

This substance is obtained by the reduction of diparanitro-diphenyl, $NO_2.C_6H_5.C_6H_4NO_2$, by nascent hydrogen (tin and hydrochloric acid). A readier method of preparation is the following: An alcoholic solution of 10 parts of azobenzene, $C_6H_5.N:N.C_6H_5$, is treated with a solution of 3.5 parts of tin in concentrated hydrochloric acid, and the liquid warmed for some time. Hydrazobenzene, $C_6H_5.NH.NH.C_6H_5$, is formed, which by intramolecular change is converted into benzidine (dihydrochloride). Some of the isomeric *o*-*p*-diamino-diphenyl is simultaneously formed, and a portion of the azobenzene is reduced to aniline, $C_6H_5.NH_2$. The alcohol is distilled off, the residue dissolved in water, and sulphuric acid added. The nearly insoluble benzidine sulphate is precipitated, while the sulphates of the isomeric base and of aniline remain in solution. The precipitate is washed with dilute hydrochloric acid (to remove tin salts) and treated with ammonia, the liberated benzidine being crystallised from dilute alcohol. Benzidine is also produced by treating azobenzene with sulphur dioxide. Benzidine is manufactured on a large scale by heating nitrobenzene with sodium hydroxide, a little alcohol, and the proportion of zinc-dust theoretically sufficient to reduce it to hydrazobenzene. The product is washed with cold dilute hydrochloric acid to remove oxide of zinc. On subsequently heating it with dilute hydrochloric acid, it is converted into benzidine dihydrochloride.

Benzidine forms large pearly plates, which are colourless when pure, but rapidly turn red on exposure to the air. It melts at 122°, and boils with partial decomposition above 360°. Benzidine is very sparingly soluble in cold, but readily in boiling, water, and is easily soluble in alcohol and ether.

[1] Engel and Kiener (*Compt Rend*, 1887, 105, 465) find the symptoms to vary considerably according to the time required to produce death, which ranges from a few hours in acute cases to several weeks in chronic cases. When death ensues in a few days, there is always icteria, and often hæmoglobinuria, and the urine is loaded with fat and yellow and brown pigment-granules, which sometimes contain iron. This ferruginous pigment accumulates in the spleen and marrow, and seems to be formed from the hæmoglobin in the protoplasm from the cellules, and not from the red corpuscles.

Benzidine is a well-defined di-acid base, forming crystallisable salts. The sulphate is very sparingly soluble in water, even when boiling.

On adding potassium dichromate to a concentrated solution of benzidine hydrochloride, a deep blue crystalline precipitate, containing $C_{12}H_8(NH_2)_2CrO_4$, is immediately formed. The same precipitate is formed on warming, even in very dilute solutions.

When chlorine-water is added in small quantity of a solution of benzidine hydrochloride, the liquid assumes a fine blue colour, which on further addition of chlorine-water changes to green; and ultimately, when the chlorine is in excess, a flocculent red precipitate is formed, apparently containing $C_{12}H_7Cl_3N_2O$, soluble in alcohol and ether, and forming a colourless compound on reduction. Bromine-water and a solution of bleaching powder act similarly; but in presence of a large quantity of free hydrochloric acid bromine forms tetrabrombenzidine, melting at 285°. With nitrous acid, solutions of benzidine salts react to form tetrazo-compounds which react with phenols, phenolsulphonic and carboxylic acids, amidosulphonic acids, etc., to form the important class of bodies known as "tetrazo-dyes," of which *Congo-Red* is the type (Vol. 5, page 206), and which are remarkable for dyeing cotton without a mordant.

Orthotoluidine. $NH_2.C_6H_3(CH_3).(CH_3)C_6H_3.NH_2$.—This base is homologous with benzidine, and is prepared from ortho-nitrotoluene by the same process by which benzidine is prepared from nitrobenzene. It melts at 128°, and presents a close resemblance to benzidine. The tetrazo-dyes prepared from it are less readily altered by acids than are the similar dyes prepared from benzidine.

According to Roesler and Glasmann, *Chem. Zeit.*, 1903, **27**, 986. Benzidine and Tolidine may be estimated iodometrically.

The method depends on the reaction of the bases with 1 molecule of iodine, according to the equations

$$C_{12}H_{12}N_2 + I_2 = C_{12}H_{11}IN_2 + HI$$
(Benzidine.)
$$C_{14}H_{16}N_2 + I_2 = C_{14}H_{15}IN_2 + HI$$
(Toluidine.)

About 5 grm. of the base are dissolved in 5 c.c. of hydrochloric acid, sp. gr. 1.19, and water, by the aid of heat, and when cold, diluted to 500 c.c. with water. 25 c.c. of this solution are neutralised with sodium bicarbonate solution until precipitation commences.

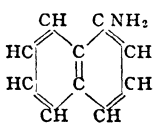
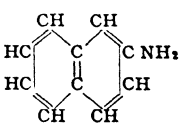
This precipitate is redissolved in a trace of very dilute hydrochloric acid, care being taken that the final solution is perfectly neutral, since the presence of free acid will vitiate the results. The neutral solution thus obtained is diluted to 500 c.c., and titrated in the usual manner with N/20 iodine solution, running drop by drop. The iododerivatives of the bases form voluminous dark blue precipitates, so the end reaction is best observed either with starch paper or by spotting out with starch solution on a white tile.—

NAPHTHYLAMINES, PYRIDINE, QUINOLINE AND ACRIDINE BASES.

By W. H. GLOVER, Ph.D.

NAPHTHYLAMINES AND THEIR ALLIES.

When naphthalene, $C_{10}H_8$, is treated cautiously with nitric acid, α-nitronaphthalene, $C_{10}H_7.O_2N$, is formed, and this is converted by reducing agents into α-aminonaphthalene or α-naphthylamine, $C_{10}H_7.NH_2$. By other reactions the isomeric β-naphthylamine may be obtained. These two substances differ from each other in a notable manner, as indicated in the following table:

	α-Naphthylamine	β-Naphthylamine
Structural formula		
M p	50°	112°
B p	300°	294°
Odour	Disagreeable, persistent	None
Appearance. .	Flat needles or prisms .	Pearly plates
Reactions of hydrochloride in solution With ferric chloride .	Blue precipitate	No reaction
With nitrous acid in alcoholic or acetic acid solution.	Yellow colour, turned crimson by hydrochloric acid.	No reaction.
With sulphanilic acid and sodium nitrite, followed by hydrochloric acid.	Red colouration.	

α-Naphthylamine. $C_{10}H_7.NH_2$.

This base is obtained by the reduction of nitronaphthalene, or by heating α-naphthol with the double compound of calcium chloride and ammonia.[1]

α-Naphthylamine has a most disgusting and persistent odour, resembling that of feces. It turns violet or brown in the air, but when purified by sublimation this change occurs very slowly, and only on exposure to air and light. It is slightly volatile with steam.

α-Naphthylamine is nearly insoluble in water, but very soluble in alcohol and ether. It forms a series of readily-crystallisable, easily-soluble salts; the *hydrochloride*, $C_{10}H_7.NH_2,HCl$, forms long needles and glistening scales, sublimes at 200°, and is soluble in water, ethyl alcohol and ether; the *sulphate* (tech. *naphthylamine* S), $(C_{10}H_7NH_2)_2,H_2SO_4$, crystallises with $2H_2O$ in white, silvery scales and is soluble with difficulty in cold water and ethyl alcohol, but readily soluble in hot ethyl alcohol; the *oxalate*, $(C_{10}H_7.NH_2)_2,H_2C_2O_4$, forms small, glistening leaflets; the *hydrogen oxalate*, $C_{10}H_7.NH_2,H_2C_2O_4$, crystallises in white nodules; the *picrate* has m. p. 165°. When ammonia is added to a solution of a salt, the free base is precipitated in white, silky needles.

On adding ferric chloride to a solution of α-naphthylamine, or of one of its salts, an azure blue precipitate of *naphthamein* is produced, which rapidly becomes purple, but is unchanged by treatment with sulphurous acid. Other oxidising agents (*e. g.*, chromic acid, bleaching powder) produce precipitates varying in colour from blue to violet or red.

On adding an alcoholic solution of nitrous acid to a solution of α-naphthylamine in alcohol or glacial acetic acid, a yellow colour is produced, which, on adding a little hydrochloric acid, changes to an intense violet or magenta colour; or, in presence of only traces of naphthylamine, to a reddish colour.

A red colouration is produced when hydrochloric acid is added to a cold solution of α-naphthylamine, sulphanilic acid and sodium nitrite,

[1] On a large scale, α-naphthylamine is prepared in a manner very similar to that employed for the production of aniline. Nitronaphthalene is reduced by iron and hydrochloric acid at a temperature of about 50° When the reduction is complete, milk of lime is added and the naphthylamine distilled off by the aid of superheated steam. The crude product is purified by redistillation, when it is obtained as a nearly colourless oil, which solidifies to crystalline cakes of a greyish colour. It appears to be wholly free from β-naphthylamine, but contains an impurity which is probably 1.8-naphthylenediamine, $C_{10}H_6(NH_2)_2$ (O. N. Witt, *Dingl. Polyt. Jour*, 1887, 265, 225).

owing to the formation of aminonaphthylazobenzenesulphonic acid, $NH_2.C_{10}H_6.N_2.C_6H_4.SO_3H$.

Commercial α-naphthylamine is met with in white, grey or brown crystalline cakes; it should be free from oily constituents and almost completely soluble in dilute hydrochloric acid. *Naphthalene*, the presence of which causes incomplete solubility, may be estimated by distilling the acidified solution in a current of steam, agitating the distillate with ether, separating the ethereal layer, evaporating at a low temperature and weighing the residue. The technical product may also contain some *β-naphthylamine;* this is isolated by the fractional crystallisation of the hydrochloride and, subsequently, of the sulphate.

α-Naphthylamine, when boiled with glacial acetic acid, yields *α-acetnaphthalide*, $C_{10}H_7$ NHAc, m. p. 159–160°, and when treated with benzoyl chloride and aqueous sodium hydroxide yields *benz-α-naphthalide* (benzoyl-α-naphthylamine), $C_{10}H_7.NHBz$, m. p. 156°. α-Naphthylamine is used in the preparation of α-naphthol, α-naphthylamine sulphonic acids, azo-dyes, Magdala Red (Vol. 5), and naphthalene fancy colours for cotton

β-Naphthylamine. $C_{10}H_7 NH_2$.

This modification of aminonaphthalene is most readily obtained by heating β-naphthol under pressure with ammonia at 160°, or with the double compound of zinc chloride and ammonia at 200–210°.

β-Naphthylamine is odourless and more stable than the α-modification. It volatilises in a current of steam, and is slightly soluble in cold, more readily in hot, water, the solution exhibiting a blue fluorescence, which, however, is not shown by β-naphthylamine salts. β-Naphthylamine gives no colouration with oxidising agents, nor with nitrous and hydrochloric acids in alcoholic solution.

The *hydrochloride*, $C_{10}H_7.NH_2$, HCl, crystallises in leaflets and is readily soluble in water and ethyl alcohol; the *sulphate*, $(C_{10}H_7NH_2)_2,H_2SO_4$, crystallises in leaflets and is less soluble in water than the α-salt; the *picrate* crystallises in long, yellow needles, m. p. 195° (decomp.), and is soluble in alcohol.

β-Naphthylamine, when heated with (1) formic acid (sp. gr. 1.2), yields *formyl-β-naphthalide*, $C_{10}H_7.NH.CHO$, m. p. 129°, and (2) glacial acetic acid yields *β-acetnaphthalide*, crystallising in white leaflets, m. p. 132°.

Commercial β-naphthylamine should have m. p. 112° and be almost completely soluble in dilute hydrochloric acid; the insoluble portion is probably *β-naphthol* and *β-dinaphthylamine;* the β-naphthol is isolated by extracting the product with dilute aqueous potassium hydroxide and acidifying the alkaline extract with a mineral acid.

β-Naphthylamine is used in the preparation of red azo-dyes.

Tetrahydro-β-naphthylamine. $C_{10}H_{11}.NH_2$.

This base has been introduced into medicine under the name of "Thermine." It is a colourless, slightly viscous liquid, of peculiar odour. It is a strong base, a drop soon becoming converted into a crystalline mass of the *carbonate* on exposure to air. The *hydrochloride* forms well-defined white crystals, m. p. 237°, and is readily soluble in water, alcohol, and amyl alcohol.

The physiological effects of thermine embrace the two strongly marked characteristics of mydriasis (accompanied by pain) and elevation of the temperature, which latter effect has been observed to the extent of 4 5°.

ALKYL- AND ACYL-NAPHTHYLAMINES.

Ethyl-α-naphthylamine, $C_{10}H_7.NHEt$, when freshly distilled is a colourless oil, but on exposure to the air it becomes brownish-red by transmitted light and steel-blue by reflected light; it has sp. gr. 18°/18° 1.073.

Ethyl-β-naphthylamine, when freshly distilled, is a colourless, viscid oil, b. p. 315–316°; 305°/716 mm., 191°/25 mm., sp. gr. at 18° 1.062, soluble with difficulty in hydrochloric acid. It comes on the market as *Developer* B and its use has been proposed for the preparation of black azo-dyes.

A method for estimating the above two substances is given by Vaubel (*Chem. Zeit.*, 1903, **27**, 278); reliable results are obtained, however, only by an experienced worker.

Phenyl-α-naphthylamine, $C_{10}H_7.NHPh$, is prepared by heating aniline with α-naphthylamine hydrochloride or α-naphthylamine with aniline hydrochloride at 240°; it crystallises in prisms, m. p. 62°, b. p. 226°/15 mm., 335°/258 mm., is insoluble in dilute acids, and easily soluble in most organic solvents. The *acetyl* derivative has m. p. 115°; the *benzoyl* derivative has m. p. 152°. The *commercial* product

is usually met with as pale brown cakes; it is used in the preparation of Victoria Blue.

Phenyl-β-naphthylamine is obtained by heating β-naphthol, aniline and aniline hydrochloride at 200–210°; it crystallises in needles, m. p. 108°, b. p. 395°, is slightly soluble in cold alcohol, ether, benzene and glacial acetic acid, and readily soluble in the hot solvents forming solutions with a blue fluorescence. The *hydrochloride*, formed by passing hydrogen chloride into a solution in benzene, is decomposed by water; the *acetyl* derivative has m. p. 93°; the *benzoyl* derivative has m. p. 136°.

o-Tolyl-α-naphthylamine, $C_{10}H_7.NH.C_6H_4.Me$, is prepared by heating α-naphthol and *o*-toluidine with calcium chloride at 280°; it crystallises in needles, m. p. 94–95°, is slightly soluble in light petroleum, readily soluble in the ordinary organic solvents. The addition of nitric acid to the greenish-yellow solution in sulphuric acid produces a dark greenish-blue colour changing to yellowish-brown while, potassium dichromate, in small quantity, gives a dirty dark-green, in larger quantity, a reddish-brown colouration.

o-Tolyl-β-naphthylamine, prepared from β-naphthol, *o*-toluidine and calcium chloride, crystallises in glistening leaflets, m. p. 95–96°; it is decomposed by concentrated hydrochloric acid yielding *o*-toluidine and β-naphthol. The pale yellow solution of the base in concentrated sulphuric acid is coloured dark reddish-yellow by nitric acid, and brownish-violet by potassium dichromate. The *picrate* has m. p. 110°; the *benzoyl* derivative has m. p. 117–118°.

p-Tolyl-α-naphthylamine is obtained by heating α-naphthylamine with *p*-toluidine hydrochloride or α-naphthol and *p*-toluidine with calcium chloride; it crystallises in short prisms, m. p. 79°, is slightly soluble in cold alcohol and light petroleum, readily soluble in boiling alcohol, ether and benzene. The pale yellow solution in concentrated sulphuric acid is coloured green by the addition of nitric acid or potassium dichromate. It is used in the preparation of Night Blue.

p-Tolyl-β-naphthylamine is prepared by heating β-naphthol, *p*-toluidine, and *p*-toluidine hydrochloride at 200–210°; it forms reddish leaflets, m. p. 102–103°, is soluble in hot organic solvents, and slightly soluble in cold alcohol and light petroleum. The yellow solution in concentrated sulphuric acid gives a brownish-red colour with nitric acid and a raspberry-red colour with potassium dichro-

mate. It is decomposed by concentrated hydrochloric acid yielding *p*-toluidine and *β*-naphthol. The *acetyl* derivative forms colourless needles, m. p. 85°; the *benzoyl* derivative crystallises in needles, m. p. 139°. The base finds application in the preparation of Wool Black.

Benzyl-α-naphthylamine, $C_{10}H_7.NH.CH_2Ph$, is prepared from α-naphthylamine and benzyl chloride; it has m. p. 66–67° and is used in the preparation of Nile Blue B.B.

Dimethyl-α-naphthylamine, $C_{10}H_7.NMe_2$, is prepared by heating α-naphthylamine with methyl iodide and methyl alcohol at 100° or α-naphthylamine hydrochloride with methyl alcohol at 180°; it is a liquid with a green fluorescence, b. p. 267°, and has an odour resembling petroleum.

Dimethyl-β-naphthylamine, is obtained by the action of trimethylamine on β-naphthol at 200°; it forms crystals, m. p. 46°, b. p. 305°.

Diethyl-α-naphthylamine, $C_{10}H_7.NEt_2$, is formed by heating α-naphthylamine with ethyl bromide and alcohol under pressure at 120°; it is a yellow oil, b. p. 290°.

Methylphenyl-α-naphthylamine, $C_{10}H_7.NMePh$, is prepared by acting on phenyl-α-naphthylamine and methyl alcohol with hydrogen chloride under pressure; it is a greenish oil with a blue fluorescence, and is employed in the preparation of Victoria Blue.

α-Dinaphthylamine, $(C_{10}H_7)_2NH$, is prepared by heating α-naphthylamine with its hydrochloride at 150°. It crystallises in large rectangular leaflets, m. p. 113°, and is insoluble in water, moderately soluble in alcohol, readily soluble in the other organic solvents. The solution in concentrated sulphuric acid is yellow at first, but quickly, especially when warmed, turns green. An alcoholic solution gives a pale green colouration with ferric chloride. The base is not altered by acetic anhydride, but with acetyl chloride it yields an *acetyl* derivative which crystallises in needles, m. p. 217°. The *picrate*, $(C_{10}H_7)_2NH,2C_6H_3O_7N_3$, forms needles, m. p. 168–169°.

αβ-Dinaphthylamine, $C_{10}H_7[\alpha]NH[\beta]C_{10}H_7$, is prepared by heating α-naphthylamine and β-naphthol with crystalline calcium chloride at 280°; it crystallises in long, stout, glistening prisms, m. p. 110–111°, and is readily soluble in warm alcohol, ether and benzene; the *picrate* crystallises in needles, m. p. 172–173°; the *acetyl* derivative forms stout needles, m. p. 125°.

β-Dinaphthylamine is formed by heating β-naphthol with zinc ammonium chloride under pressure at 280–300°; it crystallises in silvery leaflets, m. p. 170.5°, is slightly soluble in alcohol, readily in hot glacial acetic acid and benzene; the solutions exhibit a blue fluorescence. The *picrate* has m. p. 164–165°; the *acetyl* derivative crystallises in needles, m. p. 114–115.°

Naphthylene-Diamines. $C_{10}H_6(NH_2)_2$.

The naphthylenediamines or diaminonaphthalenes are prepared by heating the corresponding dihydroxynaphthalenes with ammonia, by the reduction of the dinitronaphthalenes, and in other ways.

The following table exhibits the leading properties of the most important members, which find application in the dyeing industry:

Naphthylaminesulphonic Acids.—It is not possible in a work of this kind to give a complete account of all the naphthylaminesulphonic acids known, but in the following pages the properties of the most important technical products are briefly summarised. The general methods of preparation are also mentioned as this gives an indication of the impurities to be looked for in testing the commercial material.

General Methods of Preparation.

(1) By sulphonation at different temperatures of the naphthylamines.

(2) By sulphonation of the nitronaphthalenes and reduction of the product.

(3) By nitration of the naphthalenesulphonic acids and reduction of the product.

(4) By the action of ammonia on the naphtholsulphonic acid, the OH group being replaced by the NH_2 group.

Monosulphonic Acids of α-Naphthylamine.

Most of the naphthylaminemonosulphonic acids are commercially important chiefly in connection with the manufacture of the dyes which are obtained by combining them with diazo-compounds.

1-Naphthylamine-2-sulphonic acid (*orthonaphthionic acid*) is prepared by heating sodium naphthionate with naphthalene at 200°, the SO_3H group changing from position 4 to 2; it crystallises in triclinic needles, 1 part dissolving in 225 parts of water at the ordinary temperature; reduces solutions of gold salts; gives a greyish-green precipitate with ferric chloride and yields a *diazo*-compound which forms diffi-

Position of the amino groups	1·2-	1·3-	1 4-	1 5-	1 8-	2 3-	2 7-
Mode of preparation.	By reducing azo-compounds of β-naphthylamine	From 1 3-dinitronaphthalene and from 3-amino-α-naphthol	From 4-nitro-α-naphthylamine and from azo-compounds of α-naphthylamine	From 1 5-dinitronaphthalene	From 1 8-dinitronaphthalene	From 2 3-dihydroxynaphthalene	From 2 7-dihydroxynaphthalene.
Form of crystals	Plates	Leaflets	Leaves	Prisms	Needles	Leaves	Leaflets
M p . . .	95°	96°	120°	189 5°	66 5°	191°	159°
Hydrochloride	Short prisms	Needles	Plates	Needles	Leaves, m p 280°		
Sulphate	Leaflets	Needles		Needles	Small crystals		
Reaction of the hydrochloride with ferric chloride.	Green, then yellow colouration, brown precipitate	Dark-brown colouration	Green colouration	Blue colouration then blue precipitate	Chestnut-brown precipitate	No colouration	
Action of nitrous acid	. . .	Deep yellow colouration	Soluble tetrazo-compound	Soluble tetrazo-compound	Vermilion precipitate	Yields naphthylene-azo-imide, $C_{10}H_6N_3H$, yellow needles, m p 187°	
Action of the azo-dyestuffs on unmordanted cotton	. .		Do not dye	Dye the fibre			
Diacetyl derivative	Needles, m p 234°.	Slender white needles, m p 363°.	Crystals, m p 303–304°.	.		Brown feathery needles, m p 247°	Granules, m p. 197 5°.

cultly soluble greenish-yellow plates. **Salts:** *sodium*, difficultly soluble, 1 part dissolving in 60 parts of cold water or 10 parts of boiling water; aqueous solution exhibits dark green fluorescence; *calcium*, very difficultly soluble large, flat prisms, 1 part dissolving in 20 parts of water at 100°.

1-Naphthylamine-3-sulphonic acid (γ) is formed in small quantities in the preparation of 1:6- and 1:7-naphthylaminesulphonic acids by nitration and subsequent reduction of 2-naphthalenesulphonic acid; it crystallises in colourless needles, soluble with difficulty. **Salts:** *sodium*, easily soluble; *barium* ($1H_2O$), flat, glistening needles.

1-Naphthylamine-4-sulphonic acid (*naphthionic acid*) is prepared by roasting α-naphthylamine hydrogen sulphate at 170–180° with 3% of oxalic acid; it is soluble with difficulty in cold water, 1 part dissolving in 4,000 parts of water at 15° and crystallises with $1/2H_2O$; ferric chloride colours the aqueous solution brown, which darkens on boiling. The acid, when heated with aqueous solutions of alkalies under pressure at 200–250°, yields the corresponding 1-naphthol-4-sulphonic acid. Concentrated sulphuric acid gives rise to disulphonic acid derivatives, while dilute sulphuric acid decomposes it into α-naphthylamine and sulphuric acid. **Salts:** *sodium* ($4H_2O$), large, monoclinic, colourless crystals, easily soluble in water, insoluble in alcohol precipitated from aqueous solutions by sodium hydroxide and salt; the solutions show an intense reddish-blue fluorescence; the solid salt, when shaken with benzaldehyde, yields the *benzylidene* derivative, $CHPh{:}N.C_{10}H_6.SO_3Na$, which crystallises in golden yellow plates and is decomposed by acids; *calcium* ($8H_2O$), characteristic crystals, soluble in water.

1-Naphthylamine-5-sulphonic acid (naphthalidinic acid; Laurent's or L-acid) is prepared by sulphonating α-naphthylamine with concentrated sulphuric acid at 130°. Naphthionic acid is the initial product, but on prolonged heating the 1:5- and 1:6- acids alone are formed. It crystallises from hot aqueous solutions with $1H_2O$ in small plates, 1 part dissolving in 950 parts of water at 15°, and when fused with sodium hydroxide yields 1-naphthol-5-sulphonic acid. **Salts:** *sodium* ($1H_2O$), small, very soluble prisms; *calcium* ($9H_2O$), triangular plates, readily soluble in water; shaken with benzaldehyde it gives difficultly soluble needles of the *benzylidene* compound.

Aqueous and alcoholic solutions of the acid and its salts show a green fluorescence.

1-Naphthylamine-6-sulphonic acid (β) (**Cleve's β-acid**), produced with the 1:7- and 1:3- acids when naphthalenesulphonic acid is nitrated and subsequently reduced, crystallises in needles and plates according to the solvent used; 1 part of the acid dissolves in 1,000 parts of water at 15°, or 100–200 parts of water at 100°; aqueous solutions give with ferric chloride or gold chloride a cornflower-blue or reddish-violet colouration respectively; it is very stable and is not decomposed by dilute acids at 200°. **Salts:** *sodium* ($4H_2O$), rhombic plates; *calcium* ($7H_2O$), slow crystallisation from hot concentrated solutions ($2H_2O$).

1-Naphthylamine-7-sulphonic acid (**Cleve's J-acid**) crystallises in needles or flat prisms, and dissolves in 215 parts of water at 25°; ferric chloride gives an intense blue colouration, which on the addition of acetic acid becomes red. **Salts:** *sodium* ($1/2H_2O$), thin needles, readily soluble in water and methyl alcohol; *zinc* ($4H_2O$), glistening yellow needles, soluble with difficulty in water. This and the former acid are combined with diazo-compounds to produce black azo-dyes for cotton fabrics.

1-Naphthylamine-8-sulphonic acid (**Schöllkopf's or S acid**) is prepared by cold nitration and subsequent reduction of naphthalenesulphonic acid, the 1:5-acid being formed simultaneously; it crystallises in white needles, soluble in 4,800 parts of water at 21° or 240 parts at 100°. Ferric chloride colours the cold saturated solution an intense violet, while gold chloride gives a red colouration changing to violet and violet fluorescence. The acid does not condense with benzaldehyde; the *diazo*-compound forms difficultly soluble greenish-yellow prisms. **Salts:** *sodium*, plates, 1 part dissolves in 88.5 parts of water at 24° or 37.5 parts at 100°; *potassium*, large, shining plates, 1 part dissolves in 28 parts of water at 19° or 6.7 parts at 100°.

The salts of this acid have the peculiar property of clinging tenaciously to ferric and aluminium oxides.

Monosulphonic Acids of β-Naphthylamine.

The most general method of preparing these acids is by heating the corresponding β-naphtholsulphonic acids with strong aqueous ammonia under pressure.

Most of the β-naphthylaminesulphonic acids are of importance.

2-Naphthylamine-5-sulphonic acid (*Dahl's* or *γ-acid*) crystallises

in long, slender needles, soluble in 1,000 parts of cold or 2,000 parts of hot water; the *diazo*-compound forms greyish-yellow, difficultly soluble crystals. **Salts:** *sodium* ($5H_2O$), large, thick plates, very soluble in water; *potassium* ($1H_2O$), small rhombohedra, very soluble in water.

Aqueous solutions of the acid and salts exhibit a reddish-blue fluorescence.

2-Naphthylamine-6-sulphonic acid (*Bronner's* or *β-acid*) forms long, silky, prismatic needles, soluble in 630 parts of water at 100°; the *diazo*-compound is a difficultly soluble greenish-yellow powder. **Salts:** *sodium* ($2H_2O$), white, silky needles, 1 part soluble in 40 parts of water; *calcium* ($6H_2O$), long, silky needles, 1 part dissolves in 225 parts of cold water; *ammonium* ($1H_2O$), silky needles or plates with a violet fluorescence.

Aqueous solutions of the acid and salts exhibit a blue fluorescence.

2-Naphthylamine-7-sulphonic acid (*F-* or *δ-acid*, *Baeyer's acid*, *Casella's acid* F) forms long, silky needles almost insoluble in cold water, soluble in 350 parts of hot water; the *diazo*-compound forms crystals which appear orange-red by reflected light and colourless by transmitted light. **Salts:** *sodium* ($4H_2O$), needles, difficultly soluble in cold, more easily in hot, water; *ammonium*, small, soluble plates; *calcium* ($6H_2O$), blue fluorescent plates, 1 part dissolves in 280 parts of cold water; *magnesium* ($5\ 1/2H_2O$), small, blue fluorescent needles.

Aqueous solutions of the acid and salts exhibit a reddish-violet fluorescence.

2-Naphthylamine-8-sulphonic acid (*α-acid* or *Baden acid*) forms small prisms, 1 part soluble in 1,700 parts of cold water and almost insoluble in alcohol. **Salts:** *sodium*, glistening tablets, insoluble in alcohol; *potassium* ($1/2H_2O$), six-sided plates; *calcium* ($6H_2O$), large, thick plates, 1 part dissolves in 11 parts of water.

Aqueous solutions of the acid and salts exhibit a pale blue fluorescence.

The β-naphthylaminesulphonic acids give characteristic coloured compounds with benzidine and tolidine, which are very useful in characterising the different isomeric acids. The coloured substances obtained with the three most important of the acids are shown in the following table.

Acid $NH_2 SO_3H$	Colours of the	
	Benzidine compound	Tolidine compound
2 5-		Yellow
2 6-	Yellowish-red	Yellowish-red (Benzopurpurin 1 B)
2 7- . .	. . . . .	Bluish-red, insoluble magnesium salt, completely precipitated by acetic acid

Disulphonic Acids of α-Naphthylamine.

1-Naphthylamine-3 : 6-disulphonic acid (*Freund's acid*), prepared by reducing 1-nitronaphthalene-3:6-disulphonic acid, is readily soluble in water and alcohol, insoluble in ether and benzene; when heated with water it yields α-naphthol-3 6-disulphonic acid.

1-Naphthylamine-3 : 8-disulphonic acid (*ξ-naphthylaminedisulphonic acid*), prepared by nitration and subsequent reduction of 1:6-naphthalenedisulphonic acid, crystallises in colourless, glistening scales ($3H_2O$), and is extremely soluble in water; the *diazo*-compound forms white needles. **Salts :** *sodium hydrogen* ($2H_2O$), long needles, 1 part dissolves in 30 parts of cold water; *disodium* ($6H_2O$), easily soluble in water; *copper*, flesh coloured powder almost insoluble in water.

Aqueous solutions of the acid and salts have a green fluorescence.

1-Naphthylamine-4 : 6-disulphonic acid (*Dahl's acid* No .II). When naphthionic acid is sulphonated, 33% of this acid and 66% of 1-naphthylamine-4:7-disulphonic acid are produced; it is readily soluble in hot water, insoluble in alcohol; ferric chloride produces a white turbidity, while gold chloride gives an orange colouration; the *diazo*-compound forms silky, yellow needles. **Salts :** *calcium hydrogen*, readily soluble white needles, forms double salts with other bases; *calcium*, glistening needles, soluble in hot methyl alcohol, in 85% alcohol, but not in absolute alcohol.

Aqueous solutions of the acid and salts exhibit a blue fluorescence.

1-Naphthylamine-4 : 7-disulphonic acid (*Dahl's acid* No. III) crystallises in rosettes of needles, soluble in 20 parts of hot, sparingly soluble in cold, water, insoluble in 85% alcohol; the *diazo*-compound is a yellow, amorphous powder, difficultly soluble in water.

Aqueous solutions of the acid and salts exhibit a blue fluorescence.

1-Naphthylamine-4 : 8-disulphonic acid (δ-or S-*acid*) is prepared by heating α-naphthylaminemonosulphonic acid S with fuming sulphuric acid at 100° till the product is soluble in water; it crystallises in readily soluble rhombohedra. **Salts:** *sodium hydrogen*, difficultly soluble long prisms; *sodium*, colourless needles or ($2H_2O$) compact clear crystals.

Aqueous solutions of the acid and salts exhibit a green fluorescence.

Disulphonic Acids of β-Naphthylamine.

These acids are obtained by heating the corresponding naphtholsulphonic acids with aqueous ammonia.

2-Naphthylamine-3 : 7-disulphonic acid (*δ-naphthylaminedisulphonic acid*) can be prepared by the reduction of 2-nitronaphthalene-3:7-disulphonic acid; it is sparingly soluble in cold, readily in hot water; the *sodium* salt ($1H_2O$) forms small needles, soluble in 50 parts of water at the ordinary temperature.

2-Naphthylamine-6 : 8-disulphonic acid, (*amido*-G-*acid*), prepared by the action of fuming sulphuric acid on β-naphthylamine at 110–140°, is readily soluble in water and when fused with sodium hydroxide yields aminonaphtholmonosulphonic acid; the *diazo*-compound is readily soluble in water; the *calcium* and *barium* salts (normal) are readily soluble in water.

The acid is used in the form of its diazo-compound in the production of wool-blacks for dyeing.

Naphthylaminetrisulphonic Acids.

1-Naphthylamine-3 : 6 : 8-trisulphonic acid is prepared by the sulphonation and nitration and subsequent reduction of naphthalene-β-sulphonic acid. The solutions in alkalies are not fluorescent. **Salts:** *disodium*, easily soluble in water, difficultly soluble in brine; *sodium hydrogen*, fine white needles.

2-Naphthylamine-3 : 6 : 8-trisulphonic acid is prepared by sulphonating β-naphthylaminesulphonic acid (G) with sulphuric acid, containing 40% of sulphur trioxide, at 120°; it is readily soluble in water; the *diazo*-compound forms yellow needles; the *azo*-compound with R salt is red and easily soluble; the *potassium hydrogen* salt forms

sparingly soluble, glistening needles. Aqueous solutions of the acid and salts exhibit an intense sky-blue fluorescence.

Diaminonaphthalenesulphonic Acids.

A few diaminonaphthalenesulphonic acids have come into use recently for the manufacture of dyestuffs, and therefore are of some importance.

1 : 4-Diaminonaphthalene-6-sulphonic acid, prepared by the nitration and subsequent reduction of 1:5- or 1·7-naphthylaminemonosulphonic acid, the first amino group being protected by acetylation, is a crystalline powder readily soluble in water.

1 : 5-Diaminonaphthalene-3 : 7-disulphonic acid, similarly prepared from naphthalene-2:6-disulphonic acid, forms microscopic crystals, insoluble in water; the *sodium* salt is soluble in 20 parts of cold water.

1 : 8-Diaminonaphthalene-3 : 6-disulphonic acid, prepared by the reduction of 1:8-dinitronaphthalene-3:6-disulphonic acid with ammonium sulphide, is slightly soluble in water; ferric chloride colours an aqueous solution reddish-brown. **Salts:** *potassium* ($3H_2O$), thin needles, fairly soluble in hot water; *sodium*, slender needles; *barium hydrogen*, slender, glistening needles, difficultly soluble in hot water; *barium* ($6H_2O$), slender needles difficultly soluble in hot water.

Aqueous solutions of the acid and salts are not fluorescent.

Analysis of Naphthylaminesulphonic Acids.

Various methods have been proposed for the analysis of the naphthylaminesulphonic acids, and used with more or less success.

Two methods much in use are the titration of the free acid or its sodium salt with a standard solution of (1) sodium nitrite or (2) diazobenzene or diazotoluene. The dye formed is salted out and the end-point ascertained by taking a drop of the solution on a filter paper and adding a drop of the diazo-solution to see if any more dye is formed. Even with the most skilful workers the degree of accuracy is not greater than 1–2%.

Another method proposed by Vaubel (*Chem. Zeit.*, 1893, **17**, 1265) depends on the absorption of bromine by some of the sulphonic acids. He finds that they can be divided into 3 classes:

1. Sulphonic acids which absorb 1 atom of bromine.
2. Sulphonic acids which slowly absorb several atoms of bromine.
3. Sulphonic acids which do not absorb bromine at the ordinary temperature.

Class 1 includes the following acids:
1-Naphthylamine-2-sulphonic acid.
1-Naphthylamine-4-sulphonic acid.
1-Naphthylamine-4:6-disulphonic acid.
1-Naphthylamine-4.7-disulphonic acid.
1-Naphthylamine-4:8-disulphonic acid.
2-Naphthylamine-3·6-disulphonic acid.
2-Naphthylamine-5-sulphonic acid (2 Br).
1 6-Diaminonaphthalene-4-sulphonic acid.

Class 2 includes:
1-Naphthylamine-7-sulphonic acid which absorbs 3 Br.
1-Naphthylamine-8-sulphonic acid which absorbs 2 Br.
1-Naphthylamine-3 7-disulphonic acid which absoıbs 2 Br.
2-Naphthylamine-6-sulphonic acid which absorbs 3 Br.
2-Naphthylamine-7-sulphonic acid which absorbs 3 Br.

Class 3 includes:
2-Naphthylamine-8-sulphonic acid.
2-Naphthylamine-6 8-disulphonic acid.

The method of procedure is to add to a weighed quantity of the sulphonic acid an excess of potassium bromide and sulphuric acid, and then a standard solution of potassium bromate until there is an excess of bromine; the estimation is carried out at the ordinary temperature.

In the case of those acids which fall under Class 3, it has been found that 1 atom of bromine is taken up at 65–75° without much loss of bromine, care must be taken, however, to keep the temperature below 75° otherwise the end-point is not easily determined.

Aminonaphthols. $NH_2.C_{10}H_6.OH$.

These substances are unstable bases obtained by the action of reducing agents on the nitro- or nitroso-naphthols, or on certain azo-dyes. The following table shows the leading differences of the principal members of the group:

	4-Amino-1-naphthol	2-Amino-1-naphthol	1-Amino-2-naphthol
Mode of formation .	Reduction of 4-nitro-α-naphthol, or of Orange I, (Vol 5)	Reduction of 2-nitro-α-naphthol, or of nitroso-α-naphthol	Reduction of 1-nitro-β-naphthol, of nitroso-β-naphthol, or of Orange II, (Vol 5)
Characters of free base	Unstable	Needles from water containing sulphur dioxide, soluble with difficulty in water	Colourless scales, slightly soluble in water; oxidised in the air Ethereal solution exhibits violet fluorescence
Reaction on agitating alkaline solution with air.	Dirty green colouration, changing to yellow	Permanent grass-green colour, and green scum soluble in alcohol to pure green solution Or violet naphthaquinonimide— $C_{10}H_6\left\{\begin{matrix}NH\\O\end{matrix}\right\}$	Brown colouration
Reaction with bromine water.	Yellowish-white needles precipitated, even in very dilute solutions	Yellowish or green precipitate (the same with ferric chloride)	
Characters of hydrochloride	Long white needles or acicular plates With bleaching powder yields $C_{20}H_{12}N_3Cl$, which separates from acetic acid solution in needles, melting at 85° and exploding at 130°	White laminæ	White lustrous needles readily soluble in water, but only sparingly in dilute hydrochloric acid
Product of oxidation with chromic acid mixture	Theoretical yield of α-naphthaquinone	β-naphthaquinone	β-naphthaquinone.

Aminonaphtholsulphonic Acids.

The aminonaphtholsulphonic acids are not so important as the naphthylaminesulphonic acids, but many of them have found useful application in the manufacture of dyes.

1-Amino-2-naphthol-6-sulphonic acid is prepared by the reduction of nitrosonaphtholsulphonic acid with tin and hydrochloric acid; it forms long, greyish-white needles, soluble with difficulty in boiling water and ethyl alcohol, insoluble in ether; with diazobenzenesulphonic acid it gives a fuschine red *dye*. **Salts**: *sodium* ($2\ 1/2H_2O$), used as a photographic developer under the name of "*Eikonogen*" (R. Meldola, *J. Soc. Chem. Ind*, 1889, **8**, 958).

Aqueous solutions of the acid and salts oxidise in air, turning brown.

1-Amino-2-naphthol-4-sulphonic acid forms bright yellow needles, insoluble in water, alcohol, ether, and benzene; it is decom-

posed by concentrated hydrochloric acid at 150° with elimination of sulphuric acid, and in alkaline solution is oxidised by the air giving a brown *dye*, which dissolves in hot water with a green colour.

1-Amino-8-naphthol-4-sulphonic acid (S-*acid*), prepared by fusing 1-naphthylaminedisulphonic acid (S) with potassium hydroxide, forms needles readily soluble in water, and combines with 2 molecules of a diazo-compound yielding dark coloured *dyes;* ferric chloride gives an emerald-green colouration; alkaline solutions have an intense blue fluorescence.

2-Amino-1-naphthol-4-sulphonic acid, prepared by the reduction of the corresponding nitrosonaphtholsulphonic acid, forms pearly needles and plates ($1H_2O$), and when warmed with aqueous alkali in the presence of air yields a violet-black *dye.*

2-Amino-8-naphthol-6-sulphonic acid (G- or *γ-acid*), prepared by fusing β-naphthylaminedisulphonic acid with sodium hydroxide in an autoclave, forms white needles difficultly soluble in water, and reduces ammoniacal solutions of copper and silver salts; the *diazo*-compound is a difficultly soluble, lemon-yellow substance, which dissolves in sodium hydroxide solution with a deep blue colour. The salts of the alkali and alkaline earth metals are readily soluble and acted on by light; solutions of the alkali salts have a blue fluorescence.

Disulphonic Acids of the Aminonaphthols.

1-Amino-8-naphthol-2:4-disulphonic acid (SS- or 2S-*acid*), prepared by fusing 1-naphthylamine-2:4:8-trisulphonic acid with sodium hydroxide, is easily soluble in water; ferric chloride colours its aqueous solution a dark green; the *sodium* salt ($1H_2O$) forms small needles; bleaching powder colours its solution brownish-green, becoming reddish-brown with excess of the reagent.

1-Amino-8-naphthol-3:6-disulphonic acid (H-*acid*), prepared by fusing 1-naphthylamine-3:6:8-trisulphonic acid with sodium hydroxide, forms slender needles, sparingly soluble in water; the *diazo*-compound forms sparingly soluble yellow needles which colour a sodium hydroxide solution violet, changing to green on dilution. **Salts:** *sodium hydrogen* ($1\ 1/2H_2O$), slender, white needles, readily soluble in hot water; ferric chloride or bleaching powder solutions give a brownish-red colouration; *barium hydrogen* ($4\ 1/2H_2O$), difficultly soluble silky needles.

Dilute solutions of the acid and salts have a bluish-red fluorescence which on the addition of alkali becomes reddish-violet.

1-Amino-8-naphthol-4 : 6-disulphonic acid (K-*acid*), prepared by a similar method from the corresponding naphthylaminetrisulphonic acid, unites in acid solution with diazotised sulphanilic acid yielding a very soluble intense Bordeaux-Red azo-dye. The *sodium hydrogen* salt forms needles readily soluble in water, the solution of which has an intense bluish-violet fluorescence, changing to greenish-blue on the addition of alkali; ferric chloride and bleaching powder solutions give dirty green and brownish-red colorations respectively.

2-Amino-8-naphthol-3 : 6-disulphonic acid (RR- or 2R-*acid*), prepared by fusing 2-naphthylamine-3 6 8-trisulphonic acid with alkalis, is used for making black colours dyeing cotton direct.

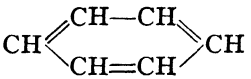
Analysis of the Aminonaphtholsulphonic Acids.

The analysis of the aminonaphtholsulphonic acids can be carried out by the method already mentioned for the analysis of naphthylaminesulphonic acids, namely, by titration with standard solutions of sodium nitrite, diazo-benzene or diazo-toluene.

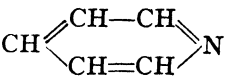
PYRIDINE BASES. $C_nH_{2n-5}N$.

These bases are contained in coal-tar naphtha; in shale-oil; in peat-tar; in tobacco-smoke; and, together with ammonia and methylamine and its homologues, in the product called "Dippel's oil," obtained by the distillation of bones and other animal matters. Pyridine itself has received several technological applications, and is of great interest theoretically in relation to the alkaloids.

Pyridine may be regarded as benzene, in which one of the CH groups has been replaced by N. Thus:



Benzene



Pyridine.

The homologous bases are derived from pyridine by the substitution of CH_3, C_2H_5, etc., for one or more of the hydrogen atoms, and consequently admit of isomeric modification according to the position of the substituted atoms in the chain.

The following is a list of bases of the pyridine series. The b. p.

and sp. gr. are only approximate, as the isomeric modifications exhibit sensible differences in their physical properties.

Formula	Base	B p	Sp gr	
			at 0°	at 22°
C_5H_5N	Pyridine	115–116°	9858	
C_6H_7N	Picoline (2-Methylpyridine)	133–135°	9613	933
C_7H_9N	Lutidine	154°	9443	
$C_8H_{10}N$	Collidine	179°	921	
$C_9H_{13}N$	Parvoline	188°	906	
$C_{10}H_{15}N$	Corridine	211°		974
$C_{11}H_{17}N$	Rubidine	230°		1 017
$C_{12}H_{19}N$	Viridine	251°		1 024

From the above table it is evident that the b. p. rises as the number of carbon-atoms in the molecule increases. For the first four members of the series the sp. gr. diminishes with increase in the molecular weight, but with the higher members the reverse is recorded as being the case. The lower members are miscible with water in all proportions, but collidine and its higher homologues are insoluble, or nearly so, in water.

If a drop or two of pyridine, or one of its homologues, be warmed in a test-tube with a similar quantity of methyl iodide, the product mixed with powdered potassium hydroxide and moistened with water and heat applied, a highly characteristic and peculiar odour is produced, owing to the formation of a pyridine dihydride. It resembles that of a mixture of mustard oil and isonitrile. The least trace of pyridine or its homologues can be detected in this way. A somewhat similar odour is obtained when a quinoline base is treated in the same manner, but the aniline bases and piperidine do not give the reaction. The foregoing test, due to A. W. Hofmann, is modified by de Coninck as follows: 1 c.c. of the base is gradually mixed with 2 c.c. of methyl iodide, the liquid being cooled during the mixing. The crystalline product is dissolved in about 5 c.c. of alcohol, the liquid heated to boiling, and very concentrated potassium hydroxide solution dropped in. A blood-red colour is produced, and the liquid finally becomes dark brown if a pyridine base be present (*Compt. Rend.*, 1886, **102**,

1480). Piperidine, sparteine, cicutine, and the aniline bases give no similar reaction.

The bases of the pyridine series are tertiary monamines, and form with alkyl iodides compounds[1] which are not decomposed by potassium hydroxide, but yield alkaline hydroxides by reaction with silver oxide.

The pyridine bases and their salts exert a soporific action on the higher animals. When inhaled, pyridine acts as a respiratory sedative. It has been successfully used as a heat stimulant and as a topical antiseptic in diphtheria. Penzhold found pyridine to act as a general antiseptic, especially as regards *mycelia*. On the lower animals, pyridine and its homologues act as violent poisons, and have been successfully employed in 0.2% solution for destroying the scab-acarus in sheep, the vine-louse, and other injurious insects The pyridine bases appear to be little, if at all, inferior to nicotine for these purposes, and have also been employed in disinfecting powders.

Isolation of Pyridine Bases.

For the *preparation* of the pyridine bases, bone-oil, or the fraction of coal-tar or shale-oil boiling between 80° and 250°, should be agitated with sulphuric acid diluted with twice its measure of water, the treatment being repeated to ensure the complete solution of the bases. The acid liquid is separated and distilled (or boiled by a current of steam) till the vapours no longer redden a slip of fir-wood moistened with hydrochloric acid, showing that all the pyrrole has been driven off. The liquid is then filtered through linen to separate tarry matters, an excess of sodium hydroxide added, and the whole distilled with steam as long as bases continue to pass over, as indicated by the production of fumes by contact of the vapours with hydrochloric acid. The distillate is allowed to cool, and is then treated gradually with a large quantity of solid potassium or sodium hydroxide, till the pyridine bases separate as an oily layer on the surface of the alkaline liquid.[2] The upper stratum is separated, and, if it contains aniline, fuming nitric acid is cautiously added and the mixture gradually heated to boiling, whereby the aniline is destroyed, while the pyridine bases remain intact.[3] Water is then added, the precipitate filtered off, and

[1] Their methiodides (PyMeI) strongly excite the brain and paralyse the extremities

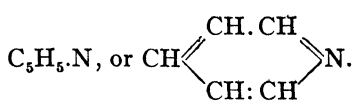
[2] The alkali can be greatly economised, with a loss of some of the higher homologues, by rendering the distillate acid with hydrochloric acid, and concentrating it to a small bulk by evaporation at a gentle heat before adding potassium hydroxide.

[3] Greville Williams destroys aniline and its homologues by heating with potassium nitrite and hydrochloric acid. Häusermann converts the aniline into sulphate, which salt is much less soluble than the sulphates of the other bases

the filtrate again treated with solid potassium hydroxide. The layer of bases is removed, and further treated with solid potassium or sodium hydroxide for several days, or until no more alkali dissolves It is only by prolonged contact with solid caustic alkali that the bases can be freed from water, and it is absolutely necessary to obtain them in a perfectly anhydrous state before attempting to separate them by fractional distillation. This is a very tedious operation, but is greatly facilitated by operating in a vacuum, and by the employment of a Hempel's tube or Henninger's or Glynsky's bulbs. Goldschmidt and Constam (*Ber.*, 1884, **16**, 2976) found that the mixture of bases extracted by vitriol from coal tar boiled between 92° and 200°, and after repeated fractionation a little passed over below 100°, and about one-half between 114° and 117° (pyridine), while above this temperature no constant b. p. was observed. Very little distilled above 160.° The most volatile fraction boiled constantly at 92–93°, treatment of which, with solid potassium hydroxide, caused a separation of absolute pyridine, boiling at 114–115 °

C. Hausermann has pointed out that the amount of sulphuric acid employed in English tar-works for treating 50 and 90% benzols is insufficient to remove the bases. He found up to 0.10% of pyridine in commercial 50% benzene, and 0.25% in the toluene made from this. Hence the nearly pure benzene, toluene, xylene, etc., now largely manufactured, can be employed with advantage for the preparation of the pyridine bases, as the tedious fractionation has already been accomplished. Thus the base extracted by diluted sulphuric acid from toluene will be nearly pure pyridine; from xylene, chiefly picoline; and from burning and solvent naphtha, the higher homologues. English-made toluene yields about 0.5% of pyridine, and a similar amount of picoline can be extracted from commercial xylene. Pyridine is more commonly made from crude heavy naphtha, and picoline from the lighter creosote oils.

Pyridine.



This substance is the lowest and most important member of the pyridine series of bases. It has been used as an antiseptic and germicide, and is employed in Germany for "denaturing" alcohol. Pyridine

is the starting-point in the preparation of several valuable antipyretics, and many of the natural alkaloids are derivatives of it.

The method of preparing pyridine from tars has already been sufficiently indicated. It may be obtained by several interesting synthetical reactions, as by passing a mixture of acetylene and hydrocyanic acid through a red-hot tube: $2C_2H_2 + CHN = C_5H_5N$. Pure pyridine is conveniently obtained in small quantity by distilling nicotinic acid with lime: $C_5H_4N.COOH + CaO = C_5H_5N + CaCO_3$.

Commercial pyridine may be purified by dissolving 200 c.c. in 400 c.c. (or a sufficiency) of strong hydrochloric acid, filtering the liquid if necessary, and then adding 1,000 c c of a 30% aqueous solution of potassium ferrocyanide. The precipitate is filtered off and washed with cold water, in which the hydroferrocyanides of ammonia and the picolines are easily soluble, while the corresponding salt of pyridine dissolves but sparingly. The washed precipitate is treated with a cold, highly concentrated solution of sodium hydroxide, when the pyridine separates as an oily layer; and, thus obtained, it contains a considerable but variable proportion of water, but if desired may be rendered anhydrous by treatment with sticks of potassium or sodium hydroxide, which should be renewed until they cease to liquefy on standing.

The following method of purification has been recommended by Ladenberg (*Annalen*, 1888, **247**, 4): a solution of 135 grm. of mercuric chloride in 1000 c.c. of hot water is added to a solution of 20 grm. of pyridine in 100 grm. of a 10% solution of hydrochloric acid and the precipitated *mercurichloride*, $C_5H_5N,HCl,2HgCl_2$, crystallised from water; it forms compact needles, m. p. 177–178°; the pure double salt is then distilled with a strong solution of sodium hydroxide and the base thus obtained dehydrated in the manner just described.

Pure pyridine is a colourless liquid, b. p. 115.5° /760 mm., D_4^{15} 0.9893, possessing a most powerful and persistent odour, and producing a bitter taste in the mouth and at the back of the throat. The vapour causes severe headache. The b. p. of pyridine is greatly reduced by the presence of water, which it is difficult to separate completely, and which pyridine absorbs with avidity from the air. A mixture of water and pyridine having the composition represented by the formula $C_5H_5N,3H_2O$, has a sp. gr. 1.0219 and boils constantly at 92–93°.

Pyridine dissolves in water in all proportions, but is precipitated from its solutions by excess of strong potassium or sodium hydroxide. It is also miscible with alcohol, ether, chloroform, benzene, and the fatty oils. Pyridine, as compared with its derivatives, is not an active poison; in small doses it has a stimulating effect, while in large doses it has a direct paralysing action on the cardiac muscle.

Pyridine is a powerful base, neutralising acids completely and fuming like ammonia in presence of hydrochloric acid and other volatile acids. It blackens calomel, and precipitates many metallic solutions. Pyridine has no effect on a solution of calcium chloride, but on passing carbon dioxide through the liquid calcium carbonate is precipitated. (No precipitate is produced if aniline be substituted for pyridine in this reaction.) Absolute pyridine has no action on litmus, but in presence of water it turns it strongly blue, though the reaction is not capable of being employed for titrating the base, for which purpose methyl-orange is suitable. On phenolphthalein pyridine has no action.

Pyridine is an extremely stable substance. It is not affected by treatment with chromic or fuming nitric acid, and these reagents may be employed to free it from aniline and empyreumatic impurities.

A substitution product of pyridine, 3 5-*dibromopyridine*, $C_5H_3NBr_2$, is formed by heating a mixture of pyridine hydrochloride and bromine at 200°. It is precipitated by adding water to its solution in strong hydrochloric acid in needles m. p. 109–110°, but commencing to sublime at 100°. It is soluble in ether and is not acted on by alkalies, acids or oxidising agents.

By reduction with tin and hydrochloric acid, pyridine is converted into piperidine, $C_5H_{11}N$, identical with the substance obtained by hydrolysis of piperine, the alkaloid of pepper.

When pyridine is acted on by sodium at 75–80°, it yields γ-dipyridyl, dipyridine, *iso*-nicotine, etc.

4:4′-(γ)-*Dipyridyl*, $\begin{vmatrix} C_5H_4N \\ \\ C_5H_4N \end{vmatrix}$, crystallises from light petroleum in glistening plates, m. p. 111–112°, b. p. 293°/744 mm., and from water in needles containing $2H_2O$, m. p. 73°; it sublimes in long needles and has a bitter taste. A solution of the *hydrochloride*, $C_{10}H_8N_2$, 2HCl, which forms large, monoclinic, transparent rods, when treated with a few drops of a solution of potassium ferrocyanide yields a pale pre-

cipitate which quickly becomes dirty indigo-blue and then dissolves in boiling water to a deep purple-red solution.

Dipyridine, $C_{10}H_{10}N_2$, is an almost odourless, highly refractive, viscid oil, b. p. 286–290° /735 mm. (decomp.), the salts of which are amorphous.

iso-Nicotine, $C_{10}H_{14}N_2$, forms slender needles, m. p. 78°, b. p. above 260° (decomp.); it is very hygroscopic and attacks the skin in the same way as potassium hydroxide. When oxidised with potassium permanganate it yields pyridine-4-carboxylic acid.

Salts of Pyridine.

Pyridine forms well-defined salts, most of which are crystallisable and deliquescent. They are odourless when pure, and can be dried without change at 100°, but become slightly coloured on exposure to air and light.

Pyridine nitrate, C_5H_5N,HNO_3, forms slender, colourless needles, or short thick prisms, very easily soluble in water, but less so in alcohol, and insoluble in ether.

Pyridine sulphate, $(C_5H_5N)_2,H_2SO_4$, is crystalline, and extremely soluble in water and alcohol.

Pyridine Hydrochloride, C_5H_5N,HCl —When pyridine is neutralised with hydrochloric acid, and the solution evaporated at 100°, a syrupy liquid is obtained, which, on cooling, becomes gradually converted into a mass of radiating crystals. The salt deliquesces in moist air, and sublimes unchanged at a high temperature. It is volatile to a very notable extent at 100°, and hence cannot be dried at that temperature without loss. It is readily soluble in water and alcohol, but insoluble in ether.

With platinic chloride, a solution of pyridine hydrochloride yields a yellow precipitate of the *platinichloride*, C_5H_5N,H_2PtCl_6, which crystallises in orange-yellow, triclinic prisms, m. p. 240–242°, and decomposes a few degrees above this temperature; it is readily soluble in boiling water, less so in alcohol and insoluble in ether. An aqueous solution of the platinichloride, when boiled for a short time, yields the *salt* $(C_5H_5N)_2,H_2PtCl_6,(C_5H_5N)_2PtCl_4$, crystallising in golden-yellow leaflets, but if the boiling be prolonged for several hours, the *pyridine platinochloride*, $(C_5H_5N)_2PtCl_4$, separates as a yellow powder, insoluble in water and acids. The platinichloride, when heated with

an excess of pyridine, yields the *platinosochloride* $(C_5H_5N)_2PtCl_2$, which crystallises from alcohol in small needles.

Pyridine picrate, $C_5H_5N,C_6H_2(NO_2)_3OH$, is deposited in beautiful yellow needles when picric acid in aqueous solution is added to a solution of an equivalent weight of pyridine. The salt has a remarkable tendency to carry picric acid down with it, so that if twice the equivalent proportion of picric acid be employed, the product has the percentage composition of an acid salt, Py,2Pc; but its real nature is indicated by its behaviour with ether, which dissolves out the free picric acid, leaving the normal picrate. Pyridine picrate may also be prepared by mixing strong solutions of sodium picrate and pyridine hydrochloride. The salt melts at 162°, and is soluble in 91 parts of cold water, but in less than 6 parts of boiling water. It is readily soluble in hot alcohol, but requires about 100 parts of the cold solvent, and is deposited on cooling in long, slender, interlaced needles of a beautiful yellow colour. It is only very slightly soluble in ether, chloroform, or benzene, and practically insoluble in petroleum spirit, but it dissolves with great facility in pyridine and cresylic acid. It is readily soluble on warming in ether, benzene, or petroleum spirit containing 10% of cresylic acid, and is freely soluble in aqueous solution of pyridine and sodium cresylate (A. H. Allen).

Pyridine picrate has an intensely bitter taste and nauseous pyridic after-taste. A moderate dose, for example 0.2 grm., produces violent vomiting. It is a valuable insecticide.

Pyridine is remarkable for its tendency to form compounds with metallic salts. These substances are more or less liable to decomposition by washing or boiling with water, and lose pyridine when heated to 100° or a somewhat higher temperature.

The *zinc chloride* compound, $2C_5H_5N,ZnCl_2$, separates as a voluminous white precipitate on treating an aqueous solution of zinc chloride with excess of pyridine; it crystallises from hot alcohol with $2H_2O$ in stout rods, sinters at 200°, and is converted by prolonged treatment with water into pyridine and a basic zinc chloride. The zinc chloride compound dissolves in hydrochloric acid to form a double chloride of zinc and pyridine, $2(C_5H_5N.HCl),ZnCl_2$, which forms groups of white lustrous needles soluble in water, almost insoluble in cold alcohol. The *cupric chloride* compound, $2C_5H_5N,CuCl_2$, is precipitated in fine greenish-blue, glistening, silky needles on adding pyridine to an alcoholic solution of cupric chloride; it is soluble in

pyridine, in aqueous solutions of pyridine and in ammonia. With mercuric chloride, a very dilute aqueous solution of pyridine (1–1,000) yields a precipitate which dissolves with extreme readiness in warm water, and separates out, as the solution cools, in long white needles. With mercuric iodide pyridine forms a compound, $2C_5H_5N,HgI_2$, which crystallises from alcohol in beautiful white needles, m. p. 97°.

From acid solutions of pyridine, phosphotungstic acid throws down a very difficultly soluble precipitate.

Detection and Estimation of Pyridine.

The recognition and estimation of pyridine are to a great extent based on the properties already described. In the free state, the smell and basic character of pyridine amply suffice for its recognition in the absence of other basic substances of powerful odour, and it is readily liberated from its salts by addition of sodium hydroxide, and obtained free from every interfering substance by distilling its aqueous solution. It may also be extracted from its aqueous solution by agitation with ether, provided that the liquid be saturated with sodium hydroxide.

From *aniline*, pyridine is distinguished by not giving any coloured product on adding a solution of bleaching powder, though the liquid acquires a new and peculiar odour. Means for distinguishing between pyridine and piperidine are given on page 142.

The presence of pyridine in aqueous solutions containing more than 1% may be detected according to E. Vongerichten (*Ber.*, 1899, **32**, 2571) as follows: An alcoholic solution of 1-chloro-2:4-dinitrobenzene is added to a portion of the liquid under investigation and the mixture gently warmed and shaken; after cooling, the addition of sodium hydroxide solution produces a reddish-violet colouration if pyridine is present.

The following method for the detection of pyridine in "denatured" alcohol has been described (*Chem. Ind.*, 1900, **23**, 25). Sulphuric acid is added to the sample of spirit, which is then evaporated to dryness, the residue neutralised with sodium hydroxide solution, distilled and the distillate treated with a solution of potassium mercuric iodide; if pyridine is present, a yellow crystalline precipitate is obtained which, when treated with potassium hydroxide, gives the characteristic odour of pyridine. According to W. Lang, the traces of pyridine sometimes contained in commercial *alcohol* may be detected and removed by

shaking the spirit with powdered zinc chloride; or, according to W. Kirschmann, by the addition of an acid solution of aluminium sulphate. In the former case, the pyridine is removed in the form of its zinc chloride compound, and in the latter case pyridine alum is formed. (See Vol. 1. page 102.)

The traces of pyridine sometimes present in *fusel oil* may be detected by adding picric acid, which occasions a formation of pyridine picrate.

The presence of *ammonia* in pyridine can be recognized (in the absence of fixed alkalies) by the red colouration produced in the aqueous solution by phenolphthalein, on which pure pyridine has no action. If the indicator be used in considerable quantity, and a low temperature employed (as recommended by J. H. Long, *Analyst*, 1891, **15**, 53), the ammonia can be approximately determined by titrating the aqueous solution with standard acid.

For the detection of traces of pyridine in commercial *ammonia*, H. Ost recommends that the sample should be nearly neutralised, when the odour of pyridine may be recognised. By distilling the nearly neutralised liquid, collecting the distillate in hydrochloric acid, evaporating, and extracting the residue with absolute alcohol, a solution is obtained containing but little ammonium chloride. What is present is removed by boiling off the alcohol and adding platinic chloride solution, when, on evaporating the filtrate and adding alcohol, the pyridine platinichloride crystallises in smooth, ramifying, orange-red prisms, readily soluble in boiling, but very sparingly in cold, water.

Estimation.

A gravimetric method for the estimation of pyridine in aqueous solutions has been described by M Francois (*Compt. Rend.*, 1903, **137**, 324; *J. Pharm. Chim.*, 1903, **18**, 337). The solution, containing not less than 0.1 grm. of pyridine, is treated with 20–30 drops of hydrochloric acid and an excess of auric chloride, in a small beaker; a precipitate is formed and the solution turns deep yellow. The liquid is evaporated to dryness on a water-bath and when all the hydrogen chloride is driven off, the beaker is placed in a desiccator for a short time. The dried residue is treated with pure dry ether, transferred to a filter and washed with ether until the filtrate runs away colourless, and finally transferred to a weighed crucible; any precipitate adhering to the sides of the beaker is dissolved in a little water and the solution

added to the weighed crucible; the water is carefully evaporated on a water-bath and the filter is then incinerated and the ash added to the pyridine aurichloride. The substance is ignited and the residual gold weighed; 196.6 parts of gold correspond to 79 parts of pyridine. Results are quoted in the original which show that the method is accurate.

In the absence of ammonia or other bases, free pyridine may be estimated by titration with standard acid and methyl orange (not litmus). K. E. Schulze (*Ber.*, 1887, **20**, 3391) recommends the following method, based on the use of ferric chloride as an indicator: Normal sulphuric acid is added slowly and with constant agitation to 20 c.c. of an approximately 5% solution of pyridine to which has been added previously 1 c.c. of a 5% aqueous solution of ferric chloride, till the precipitated ferric hydroxide is redissolved; toward the end of the action it is advisable to add the acid at the rate of 1 drop per minute. 1 c.c. of normal acid corresponds to 0.079 grm. of pyridine.

A volumetric method based on the absorption of bromine has been devised by A. Labat (*Zeitsch. anal. Chem.*, 1907, **46**, 60). The conditions described must be strictly observed, since the quantity of bromine absorbed depends on the volume of the solution. An $N/20$ solution of bromine in water is added from a burette to 10 c c. of the solution of pyridine (containing 0.1–5% of pyridine) until an opalescence is produced which persists during 10 secs ; if n = no. of c.c. of bromine-water, then 100 c.c. of the solution contains $\frac{10n}{36} - 0.50$ grm. of pyridine. If more solution be employed for the titration, then for

20 c.c. $x = \frac{10n}{80} - 0.40$ 30 c.c $x = \frac{10n}{116} - 0.39$

40 c.c. $x = \frac{10n}{150} - 0.44$ 50 c.c. $x = \frac{10n}{200} - 0.415$

Two methods for the estimation of pyridine in aqueous ammonia have been described. The first, devised by Pennock and Morton (*J. Amer. Chem. Soc.*, 1902, **24**, 385) is performed as follows: 100 c c. of the sample are neutralised with sulphuric acid (1:5), using methyl-orange as an indicator, care being taken to keep the temperature below 20°. The liquid is then distilled into a flask containing 30 c.c. of water until the total volume equals 100 c.c.; the distillate, which contains all the pyridine and a small quantity of ammonia, is cooled to 10°, phenolph-

thalein added and then a solution of mercuric chloride from a burette until the colour is discharged; 4 more drops of the mercuric chloride solution are run in, whereby all the ammonia is carried down in the precipitate of NH_2HgCl, the latter is filtered off and the filtrate titrated with $N/10$ acid and methyl-orange; 1 c.c. $\equiv$ 0.0079 grm. of pyridine.

The second method, recommended by Milbauer and Stanck (*Zeitsch. anal. Chem.*, 1904, **43**, 215) is as follows: 100–200 c.c. of the sample are diluted with an equal volume of water and then added to dilute sulphuric acid containing a few drops of a solution of *Patent Blue* V. N. as indicator. The strongly acid liquid is evaporated nearly to dryness and mechanically shaken with a sufficient quantity of freshly prepared sodium hydrogen carbonate solution and an equal volume of ether for 10–15 minutes. The ethereal extract is withdrawn and the aqueous portion again treated with ether. The united ethereal extracts are filtered and thoroughly shaken with an excess of $N/10$ H_2SO_4 after the addition of a few drops of *Patent Blue.* Sodium chloride is then added in excess and the liquid titrated back with $N/10$ NaOH solution. Under these conditions the end-point with the indicator is quite sharp. It is advisable to make a third extraction with ether and to titrate the extract as described above to ensure that no pyridine remains in the aqueous layer.

Pyridine bases in ammonium salts are estimated by treating a neutral solution of about 100 grm. of the salt in 30 c.c. of water with sodium hydrogen carbonate and ether as in the case of the ammonia solutions. If only very slight traces of pyridine are present, a larger quantity of the salt is extracted with hot alcohol, the alcoholic extract acidified and distilled, and the residue treated as just described.

Results of experiments are recorded which show that the method is reliable.

Commercial Pyridine.—Pyridine is employed in Germany, in conjunction with wood spirit and turpentine, for "denaturing" spirit. An article intended to be used for this purpose is required to answer to the following official tests.

(1) The colour must not be deeper than straw-yellow. (2) If 10 c.c. of a solution of 1 c.c. of the sample in 100 c.c. of water be shaken vigorously with 5 c.c. of a 5% solution of anhydrous, fused cadmium chloride, a distinct crystalline deposit should appear within 10 minutes. (3) 10 c.c. of the same solution of pyridine should give a white precipitate with 5 c.c. of Nessler's reagent. (4) When 100 c.c. of the sample

are distilled (in a small metal flask provided at the top with a small globe, which is connected with a Liebig's condenser, a thermometer being fitted to the globe, and a moderate heat applied) so that the distillate passes over in separate drops, 90% should have distilled over when the thermometer stands at 140° the barometric pressure being 760 mm. (5) When the sample is mixed with twice its volume of water it must wholly dissolve, and no oily drops must separate even after long standing. (6) Four drops of the sample heated on platinum foil over a Bunsen flame should burn with a sooty flame and leave no residue. (7) When 20 c.c. of the sample are shaken with an equal volume of a solution of sodium hydroxide of sp. gr. 1.4, a layer of anhydrous base, measuring at least 18.5 c.c., should separate out on standing.

The last test is now usually replaced by one prescribing the use of solid potassium hydroxide. 50 c c. of the sample is placed in a graduated cylinder, furnished with a stopper, and a long stick of potassium hydroxide immersed in it. The alkali gradually absorbs the water from the pyridine, and forms a lower layer of saturated solution. A second stick is added as soon as the first has sunk much below the surface of the pyridine, and is followed by a third if the second liquefies completely or considerably. Agitation should be avoided, and care must be taken that the last stick is left in contact with the upper layer of bases until the action is at an end. It is then cautiously removed with a bent wire, or broken down by a glass rod, and the volume of the layer of anhydrous bases carefully observed. By this test, commercial pyridine usually shows from 8 to 10% of water (=92 to 90% of anhydrous bases).

Instead of estimating the water, K. E. Schulze recommends titration of the bases with standard acid (*loc. cit*).

The following test is also employed: *N*-sulphuric acid solution is added to a solution of 1 c.c. of the sample in 10 c c. of water until a drop placed on Congo Red paper produces a distinct blue boundary which disappears almost immediately. Not less than 10 c.c of the acid solution should be used to bring about this result. To prepare the Congo Red paper, filter paper is saturated with a solution of 1 grm. of Congo Red in 1.000 c.c. of water and then dried.

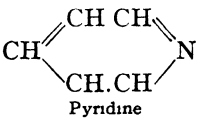
Commercial pyridine, as now produced, consists chiefly of pyridine and picoline. Ammonia is apt to be present in notable quantity, as

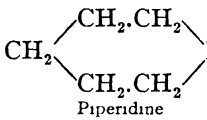
also pyrrole and other strong smelling impurities.[1] A considerable but variable proportion of water is present.

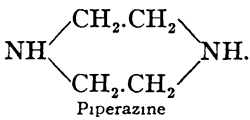
Pyridine intended for pharmaceutical or medicinal use should not be altered by light; a 10% solution in water should not be reddened by phenolphthalein (presence of ammonia); and 5 c.c., to which 2 drops of $N/10$ permanganate have been added, should retain a red colour for at least an hour.

Piperidine. $C_5H_{11}N$.

The relationship of pyridine to piperidine and piperazine[2] is shown by the following formulæ:


Pyridine


Piperidine


Piperazine

It is prepared by the reduction of pyridine with sodium and alcohol or by the electrolysis of a solution of this base in dilute sulphuric acid. It is also obtained by rapidly heating pentamethylenediamine:

$$C_5H_{10}(NH_2)_2,HCl = C_5H_{11}N + NH_4Cl.$$

Piperidine is likewise produced by the hydrolysis of piperine, the alkaloid of pepper, which when boiled with alkalis, yields piperic acid and piperidine, $C_{17}H_{19}NO_3 + H_2O = C_5H_{11}N + C_{12}H_{10}O_4$.

According to W. Johnstone (*Analyst*, 1890, **14**, 41) a small quantity of piperidine is obtained when pepper is distilled with water alone, probably owing to partial decomposition of the piperine by water or an enzyme.

Piperidine is a colourless, limpid liquid, b. p. 106°, $D^{19.6}$ 0.8619, having a peculiar odour, resembling at the same time that of ammonia and pepper, and possessing a very caustic taste. It dissolves in all proportions of alcohol and water, the addition of water to piperidine being accompanied by the evolution of heat.

Piperidine is a powerful base. Its aqueous solution restores the

[1] The pyridine produced at certain works becomes turbid when diluted with more than 40% of water, whereas the best makes are miscible with water in all proportions. On distilling the former brands the disturbing impurity is left in the "tailings."

[2] *Piperazine* is a strong base which absorbs carbon dioxide from the air and forms large, rhombic leaflets, m. p. 104°, b. p. 145–146°. Piperazine has neither caustic nor toxic properties, and passes through the system unchanged, but dissolves uric acid in large amount, forming the neutral urate, $C_4H_{10}N_2,C_5H_4N_4O_3$. *Piperazine phosphate* forms four-sided tabular crystals, which character, and those of the bismutho-iodide, distinguish piperazine from spermine, $C_4H_8N_2$, which otherwise it closely resembles.

blue colour of reddened litmus paper, and behaves like ammonia with metallic solutions, except that the precipitates produced with salts of zinc and copper are not soluble in excess. Piperidine absorbs carbon dioxide from the air, and if the gas be passed into a solution of calcium chloride, to which piperidine has been added, calcium carbonate is precipitated.

Piperidine forms readily crystallisable salts, most of which are soluble; the *hydrochloride*, $C_5H_{11}N,HCl$, forms needles m. p. 237°, the *aurichloride*, $C_5H_{11}N,HAuCl_4$, crystallises from alcohol in four-sided leaflets, sinters at 215°, m. p. 218–229° (decomp.), the *platinichloride*, $C_5H_{11}N,H_2PtCl_6$, forms long, orange needles, m. p. 198–200° (decomp.); it crystallises from alcohol with 1Et.OH in small, orange-yellow needles, m. p. 191° (decomp.).

Piperidine may be distinguished from pyridine by means of the following tests:

Reagent	Piperidine	Pyridine
Freshly prepared solution of gallic acid	Pale rose colouration turning to deep yellow.	Neither colouration nor precipitate
Pyrogallol	Yellow colouration at once, gradually changing to brownish-black	Pale yellow colouration after some time
Catechol	Violet colouration changing to pink and finally to yellow	No colouration
Quinol	Yellow colouration changing to deep brown	No colouration

Piperidine is estimated by titration with standard acid, using either litmus or methyl orange as an indicator 1 c.c. $N/10$ acid $\equiv$ 0.0085 grm. piperidine.

Homologues of Pyridine.

The homologues of pyridine occur with that base in the products of the distillation of bones, coal, shale, etc. Various members of the class have been obtained synthetically.

Picolines. Methylpyridines. C_6H_7N; or $C_5H_4(CH_3)N$.

Three isomeric modifications of picoline exist, differing according to the orientation of the CH_3 group in relation to the N. The picoline of coal tar is chiefly the ortho-modification (1:2), often called α-picoline

mixed with some meta- or β-picoline (1 3) Although the former boils at 129° and the latter at 143°, they cannot be separated by fractional distillation, but may be isolated by taking advantage of the different solubilities of their platinichlorides (*Ber*, 1879, **12**, 2008). Lange (*Ber.*, 1885, **18**, 3436) maintains that α-picoline is preferably separated from bone-oil by means of its sparingly soluble mercurochloride. γ-Picoline (1·4) is produced by the distillation of acrolein-ammonia, or by heating allyl tribromide with ammonia, and by the action of pyridine on methyl iodide. Its presence has been recognised in coal tar.

The picolines are metameric with aniline, $C_6H_5NH_2$, which, however, is a primary amine, whereas the picolines have the characters of tertiary bases. In their odour, solubility, basic properties, and characters of their salts, the picolines closely resemble their lower homologue pyridine, but have a lower density and higher b. p. than the latter substance.

α-Picoline, 2-methylpyridine, is an oil, b. p. 129°/760 mm., D_4^{15} 0 9497; the *aurichloride* forms small prisms, m. p. about 183–184°; the *platinichloride* forms orange-red crystals, m. p. 194° (decomp.); the *picrate* crystallises in needles, m. p. 169–171°.

β-Picoline, 3-methylpyridine, is an oil, b. p. 143°, D_4^{15} 0.9613; the *aurichloride* forms yellow needles, m. p. 182–184°; the *picrate* crystallises from alcohol in glistening needles or leaflets, m. p. 149–150°.

γ-Picoline, 4-methylpyridine, is an oil, b. p. 143°, D_4^{15} 0.9571; the *aurichloride* crystallises in lemon-yellow crystals, sinters at about 185°, m p. 201–203° (decomp.), the *picrate* forms glistening needles, sinters at 155°, m. p. 163°.

Dimethylpyridines. C_7H_9N.

The following dimethylpyridines have been isolated from coal-tar oil, shale oil, etc.

2 : 4-Dimethylpyridine is an oil, b. p. 159–159.5°, D^{14} 0 9380, soluble in 5 parts of cold water, less soluble in hot water; it is not turned red by hydrochloric acid or benzoyl chloride; the *platinichloride*, $(C_7H_9N,HCl)_2PtCl_4$, forms orange-red prisms, m. p. 216° when slowly heated, 223° (decomp.) when heated quickly; the *aurichloride*,

$C_7H_9N,HAuCl_4$ has m. p. 94°; the *picrate* forms slender, pale yellow needles, m. p. 179°.

3:4-Dimethylpyridine has b. p. 163 5–164 5°: the *platinichloride* ($2H_2O$) forms glistening crystals, m. p. 205° (decomp), the *aurichloride*, $C_7H_9N,HCl,2AuCl_3$, forms slender, pale yellow needles, m. p. 160–162°.

2:6-Dimethylpyridine has b. p. 142.5°; the *platinichloride* forms orange-red crystals, m. p. 210° (decomp.); the *aurichloride* crystallises in pale yellow, matted needles, m. p. 79–81°; when dried at 80°, m. p. 122–123°; the *picrate* forms slender, pale yellow needles, m. p. 161°.

2:5-Dimethylpyridine has b. p. 154–155°; it is readily soluble in cold, less in warm, water; the *platinichloride* ($2H_2O$) forms orange-red crystals, m. p. 192–194° (decomp.); the anhydrous salt has m. p. 238° (decomp.); the *picrate* crystallises in yellow needles, m. p. 156–157°.

3:5-Dimethylpyridine has b. p. 169–170°; the *platinichloride* crystallises in dark red needles or leaflets, m. p. 255–256° (decomp.); the *aurichloride* forms yellow needles, m. p. 149°.

Ethylpyridines. C_7H_9N.

2-Ethylpyridine occurs in coal-tar oil, it has b. p. 148.5° (corr.), the *platinichloride* forms orange-yellow plates, m. p. 165–167° (decomp.); the *aurichloride* forms glistening, yellow leaflets, m. p. 121°.

3-Ethylpyridine is obtained, together with the 4-isomeride, by heating brucine or cinchonine with potassium hydroxide; it has b. p. 162–165° and is soluble with difficulty in cold water, the *platinichloride* crystallises in glistening, yellowish-red plates, m. p. 196°; the *aurichloride* forms dark yellow leaflets, m. p. 130°; the *picrate* forms yellow needles, m. p. 128–130°.

4-Ethylpyridine is an oil with an unpleasant odour, b. p. 164–166°; the *platinichloride* forms plates m. p. 213°; the *aurichloride* crystallises in prisms, m. p. 147–148°; the *picrate* has m. p. 168°.

Collidines. $C_8H_{11}N$.

α-Collidine, 2-methyl-4-ethylpyridine, is an oil, b. p. 178°, D^{15} 0.853, the salts of which are amorphous and gummy; the *platinichloride* separates as an oil; the addition of a solution of chromic acid gives a red oil.

β-Collidine, 4-methyl-3-ethylpyridine is an extremely poisonous oil, b. p. 195–196°; the *platinichloride* is an orange-red crystalline powder; the *picrate* has m. p. 148–150°.

γ-Collidine, 2 : 4 : 6-trimethylpyridine, occurs in Scottish shale oil; it is an oil, b. p. 171°, D^{15} 0.917, which becomes brown when exposed to the air; the *aurichloride* forms wholly needles, m. p. 112°; the *picrate* forms long, silky, yellow needles, m. p. 155–156°; the *platinichloride* forms orange-red crystals, m. p. 223–224° (decomp.).

Pyridinecarboxylic Acids.

Pyridine itself is an extremely stable substance, resisting the strongest oxidising agents, but its homologues yield by oxidation a series of acids in which the alkyl groups are replaced by a corresponding number of carboxyl groups. The pyridinecarboxylic acids derive their chief interest from the light they throw on the relationship of the natural vegetable alkaloids to the pyridine bases. Three isomeric pyridine-monocarboxylic acids, $C_5H_4N.COOH$, are obtainable; exactly corresponding to the three isomeric modifications of picoline (methyl-pyridine). The same acids may also be obtained by the action of heat on the di- or tri-carboxylic acids, just as benzoic acid, $C_6H_5.COOH$, is obtained by the action of heat (and lime) on phthalic acid, $C_6H_4.(COOH)_2$. One of them (nicotinic acid) is also obtained by the oxidation of nicotine.

Pyridine-monocarboxylic acids, $C_5H_4N.COOH$,[1] unite in themselves the basic characters of pyridine with those of an acid. Thus they combine with hydrochloric acid, and the resulting compound forms double salts with mercuric chloride, platinic chloride, etc ; while, on the other hand, they form a series of well defined crystallisable salts. The following table exhibits their more important characters:

[1] The bases from coal tar boiling between 130° and 140° are boiled in an apparatus furnished with a reflux condenser with 10 times their weight of potassium permanganate in 2 1/2% aqueous solution, until the permanganate is reduced The manganese oxide is then filtered off, and the clear liquid concentrated to a small bulk It is then neutralised and treated with copper acetate The precipitate is separated, decomposed by hydrogen sulphide and the filtrate decolourised by animal charcoal On further concentration and cooling it deposits colourless needles of picolinic acid The filtrate from the copper precipitate is further evaporated, acidified with acetic acid, and treated at its b p with copper acetate The resulting bluish-green precipitate is separated, boiled rapidly with water, and decomposed by hydrogen sulphide. On evaporation, the filtrate deposits colourless crusts of *iso*-nicotinic acid.

	Picolinic or pyridine-2-carboxylic acid	Nicotinic or pyridine-3-carboxylic acid	*iso*-Nicotinic or pyridine-4-carboxylic acid
Mode of formation	Oxidation of α-picoline by permanganate	Oxidation of β-picoline by permanganate, or nicotine by permanganate, chromic acid or nitric acid	Action of heat on pyridine di- or tri-carboxylic acid Oxidation of γ-picoline
Crystalline character	Prismatic needles	Needles	Needles
M. p.	135°; sublimes in lustrous needles.	232°	Sublimes in small plates without melting, m p in sealed tube 305° (299°) (310°) (317°)
Solubility	Easily soluble in cold or hot water and in alcohol Nearly insoluble in ether, chloroform, benzene, etc	Sparingly soluble in cold, easily in warm water, sparingly in ether or chloroform.	Sparingly soluble in water, very sparingly in ether and benzene
Reaction with neutral lead acetate	No change	No change	
Reaction with ammoniacal lead acetate	No change	White crystalline precipitate	
Reaction with cupric acetate	Slowly deposits shining laminæ and needles of violet-blue colour, and metallic lustre Soluble in hot water	Pale blue-green precipitate, insoluble in a large quantity of water	Green crystalline precipitate on warming
Reaction with ferrous sulphate	Pale reddish-yellow colouration	No change	No change
Characters of hydrochloride— $C_6H_5O_2N,HCl$	Large, lustrous, orthorhombic prisms which become rapidly turbid on exposure to air	Monoclinic prisms, quite permanent in the air	Large glistening, monoclinic prisms
Aurichloride	M p 200°	M p 207°	M p 219°

On heating with lime, the above acids yield pyridine, just as benzoic acid yields benzene under similar conditions. The sodium salts of the α and β acids, when treated in solution with sodium amalgam, give off ammonia, and yield the salt of an unsaturated acid of the fatty series, $C_6H_8O_3$.

Pyridinedicarboxylic Acids. $C_5H_3N(COOH)_2$.—Of the six possible acids of this formula, all are known. They are produced by the oxidation of homologues of pyridine containing two substituted hydrogen atoms, and also by the oxidation of other substances.

Quinolinic acid, pyridine-2:3-dicarboxylic acid, obtained by the oxidation of coal-tar quinoline with permanganate, crystallises in

glistening, short, monoclinic prisms; it sinters and turns brown at 190–195°, sometimes also melting at this temperature, but becomes again solid and then has m. p. 231°; the *copper* salt, $(C_7H_4O_4N)_2Cu,H_2O$, forms microscopic, ultramarine-blue needles. The acid gives a reddish-yellow colouration with ferrous sulphate.

Lutidinic acid, pyridine-2 : 4-dicarboxylic acid, prepared similarly from 2:4-dimethylpyridine, crystallises in leaflets and plates, m p. 239–240°; it is moderately soluble in cold, very soluble in hot water, insoluble in benzene and ether, and gives an intense blood-red colouration with ferrous sulphate; the *copper* salt, $C_7H_3O_4NCu,3H_2O$, is obtained as an insoluble, pale bluish-green, crystalline precipitate; the precipitate from a boiling solution is anhydrous.

iso-Cinchomeronic acid, pyridine-2 : 5-dicarboxylic acid, crystallises from hot water with $1H_2O$ in microscopic leaflets and from cold water with $1\ 1/2H_2O$ in crystals, m. p. 236°; it gives a reddish-yellow colouration with ferrous sulphate; the *copper* salt, $C_7H_3O_4NCu,H_2O$, is precipitated from hot solutions as a pale-blue, crystalline powder.

Dipicolinic acid, pyridine-2 : 6-dicarboxylic acid, crystallises from cold water with $1\ 1/2H_2O$ in long, slender, silky needles, m. p. 226° (decomp.); it gives a reddish-yellow colouration with ferrous sulphate; the *copper* salt, $C_7H_3O_4NCu,2H_2O$, forms dark blue prisms.

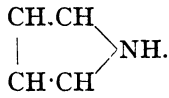
Cinchomeronic acid, pyridine-3 : 4-dicarboxylic acid, is obtained by the oxidation of quinine, cinchonine, etc., by nitric acid and by the oxidation of *iso*-quinoline with potassium permanganate; it crystallises in granules from water and in prisms from acidified solutions, m. p. 258–259° (decomp.); the *copper* salt, $C_7H_3NCu,\ 3\ 1/2H_2O$, is a dark blue, crystalline precipitate which loses $3H_2O$ at 100°

Dinicotinic acid, pyridine-3 : 5-dicarboxylic acid, has m. p. 323° (decomp); it is almost insoluble in water.

Pyridinetricarboxylic acids, $C_5H_2N(CO_2H)_3$, are obtained by the oxidation of certain alkaloids. Thus cinchonine, cinchonidine, quinine and quinidine, when oxidised by an alkaline solution of potassium permanganate, yield *α-carbocinchomeronic* or *pyridine-2:3:4-tricarboxylic acid*, which crystallises with $1\ 1/2H_2O$ in transparent, rhombic plates, loses its water of crystallisation at 115–120° and then has m. p. 249–250° (decomp), when heated rapidly; it yields cinchomeronic acid when heated at 170° and gives a pale-red colouration with ferrous sulphate. Berberine, when oxidised by nitric acid, yields

berberonic or *pyridine-2:4:5-tricarboxylic acid*, which crystallises with $2H_2O$ in triclinic prisms, loses $1H_2O$ when exposed to the air, turns red at 215° and has m. p. 235°; it yields with ferrous sulphate a blood-red colouration and with lead acetate an insoluble precipitate.

Pyrrole.



This associate of the pyridine bases in coal tar and bone oil is widely distributed in nature, since chlorophyll, the green pigment of plants, and hæmoglobin, the pigment of blood, are derivatives of pyrrole. It is best prepared by shaking bone oil with dilute sulphuric acid and fractionating the insoluble portion. The portion, b. p. 98–150°, is heated with potassium hydroxide solution so long as ammonia is evolved and then steam distilled; the portion of the distillate, b. p. 115–130°, is heated with an excess of solid potassium hydroxide until two layers form, it is then allowed to cool, the oil poured off and the potassium pyrrole, C_4H_4NK, washed with ether and decomposed by water; the liberated pyrrole is separated by steam distillation and fractionated.

Pyrrole is a colourless liquid, b. p. 130–131°, D_4^{21} 0.96694, with a pungent taste, and odour resembling chloroform. It is but little soluble in water, and insoluble in alkalies, but dissolves in dilute acids alcohol, and ether. It is indifferent to most reagents, but appears to possess feebly-marked basic properties. The only definite salt is the *picrate*, which forms unstable red needles melting at 71°.

Pyrrole turns brown in the air, and when warmed with acid forms a red substance known as pyrrole red. A piece of pine-wood, moistened with hydrochloric acid and exposed to the vapour of pyrrole becomes faintly red, and after some time, carmine-red.

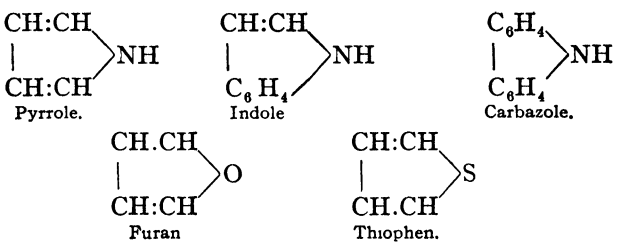
When 5 grm. of pyrrole are added to a solution of 10 grm. of isatin in 5,500 c.c. of water and 50 grm. of sulphuric acid at 5°, an indigo-blue substance, *pyrrole-blue A*, $C_{24}H_{16}O_3N_4$, is obtained, which dissolves in concentrated sulphuric acid to a violet solution changing to bluish-black. A similar compound, *pyrrole-blue B*, $C_{24}H_{16}O_2N_4$, is obtained by adding a solution of 0.75 grm. of pyrrole in 10 c.c. of glacial acetic acid to a mixture of 100 c c. of a 1% solution of isatin in glacial acetic acid and 40 c.c. of 15% sulphuric acid cooled to 0°, and diluting the

mixture after 5 minutes with 10 c.c. of ice-water; the dried precipitate, after boiling 2 or 3 times with pyridine, is obtained as a glistening, blue powder resembling cantharidine; it dissolves in boiling glacial acetic acid to a blue solution and in concentrated sulphuric acid to a violet-red solution which changes to blue and on the addition of water deposits a disulphonic acid.

Small quantities of pyrrole may be detected by boiling a short time with 2 c.c. of a solution of alloxan; if pyrrole is present, a violet-blue colouration is produced which becomes red when the solution is cooled with cold water and on the addition of aqueous sodium hydroxide turns green changing rapidly to an intense blue.

A 4% solution of formaldehyde containing a few drops of sulphuric acid, when treated with pyrrole in the cold, yields in a few minutes a white substance which decomposes without melting when heated, is insoluble in all organic solvents and turns red when exposed to the air.

If a solution of phenanthraquinone in acetic acid be treated with pyrrole and a little dilute sulphuric acid, a brown precipitate is formed, which dissolves in chloroform with a beautiful violet-red colour. When an aqueous solution of benzoquinone is treated with pyrrole and dilute sulphuric acid, a dark green precipitate is formed, insoluble in ether. These reactions indicate the close relationship between pyrrole and thiophen, which itself has the constitution of a thiofuran. Many of the reactions of pyrrole are also produced by carbazole, which is an imino-diphenyl. Indole has a constitution between pyrrole and carbazole. Thus:



Methylpyrroles.

Two isomeric methylpyrroles exist in bone-oil. To isolate these, the fraction of bone oil, b. p. 140–150°, is converted into the potassium derivative and this is heated at 200° in a current of carbon dioxide.

Two isomeric *homopyrrolecarboxylic acids* are formed. The α-acid forms leaflets, m. p. 169.5° and a *lead* salt very soluble in water, while the β-acid forms crystalline crusts, m. p. 142.4° and a nearly insoluble *lead* salt. On distilling the respective acids with lime the corresponding homopyrroles are regenerated. The α-compound, *2-methylpyrrole*, is a liquid, b. p. 147–148°/750 mm., while the isomeride, *3-methylpyrrole*, is a liquid, b. p. 142–143°/742.7 mm.

2:5-Dimethylpyrrole also occurs in bone oil; it is a liquid, b. p. 169°, with a sharp, unpleasant odour, gives a cherry-red colouration with a pine-shaving moistened with hydrochloric acid, and a brownish-red colouration with ferric chloride.

Tetraiodopyrrole, C_4I_4NH, has been introduced into medicine under the name of "*iodol.*" It is prepared by the action of a solution of iodine in potassium iodide on a solution of pyrrole containing potassium hydroxide, and forms a tasteless, pale yellow, crystalline powder, having a faint, thymol-like odour. Iodol decomposes without melting at 140–150°, is soluble in 5,000 parts of water, moderately soluble in light petroleum and dilute acids, readily soluble in ether and hot alcohol. Iodol is not decomposed by boiling water, but is turned black by hydrochloric acid; an alcoholic solution does not give a precipitate with mercuric chloride.

An *additive* compound, $C_4I_4NH,C_{10}H_{18}O$, is formed by warming cineol (eucalyptol) with iodol; it forms yellowish-green crystals, m. p. 112° (decomp.).

Iodol contains 90% of iodine and possesses antiseptic and local anesthetic properties analogous to those of iodoform, over which its slight odour and freedom from toxic properties give it the preference. Iodol can be recognized by the green colour of its solution in sulphuric acid, changing to dirty violet and by the bright red colour produced when an alcoholic solution is warmed with nitric acid.

A compound of iodol with egg albumin has been introduced recently into medicine for internal administration; it is an odourless, tasteless, pale yellow powder, insoluble in the ordinary solvents, and soluble only in dilute alkalies

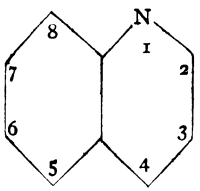
Quinoline and its Allies.

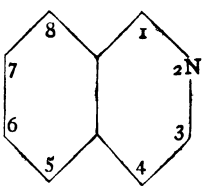
The interesting base which gives its name to the quinoline series bears the same relation to naphthalene that pyridine bears to benzene;

that is, it is derived by the substitution of an atom of nitrogen for one of the CH groups of naphthalene:

Benzene,	C_6H_6	Pyridine,	C_5H_5N
Naphthalene,	$C_{10}H_8$	Quinoline,	C_9H_7N

Just as two isomeric naphthols exist, so two isomeric quinolines are possible and have been obtained. Quinoline (I) is obtained with its homologues by distilling quinine, cinchonine and other alkaloids with lime or potassium hydroxide; it also exists, together with *iso*-quinoline (II) in coal-tar.

I. 

II. 

Quinoline, Chinoline, Leucoline. C_9H_7N.

This base is generally prepared by Skraup's method, which consists in shaking together aniline (76 pts.), glycerol (240 pts), sulphuric acid (200 pts.), and nitrobenzene (48 pts) or arsenic acid (114 pts). When the aniline sulphate has dissolved, a reflux condenser is fitted to the flask, which is heated at 130° until action commences when the flame is removed. When the action is at an end (3 hours), the product is cautiously diluted with water, boiled to get rid of traces of nitrobenzene, then rendered alkaline and the quinoline and aniline distilled in a current of steam. The oil obtained is separated from the aqueous layer, dehydrated over potassium hydroxide, and fractionally distilled, whereby a separation of the bases is effected tolerably readily, aniline boiling at 184°, and quinoline at 239°. To purify the latter it is again fractionally distilled, and boiled with weak chromic acid mixture (to oxidise any aniline); or the quinoline is dissolved in six parts of water, and strong sulphuric acid added in the exact quantity necessary to combine with the base. After cooling, the liquid is filtered, and the insoluble hydrogen sulphate washed with alcohol till snow-white, and then decomposed by potassium hydroxide.

If arsenic acid be used instead of nitrobenzene, after the steam distillation the distillate is treated with excess of hydrochloric acid and then with sodium nitrite, warmed, saturated with sodium hydroxide

and again steam distilled. The distillate is then extracted with ether. (Knüppel, D. R. P. 14976).

Quinoline is a colourless, mobile liquid, m. p. —19.5°, b. p. 237°/746 mm. (Skraup), 240–241°/750 mm. (Kretsky), 238°/760 mm. (Kahlbaum), $D^{\circ}_{\circ}$ 1.1081, D^{20}_{20} 1.0947, having a penetrating and peculia taste and an after-taste slightly resembling peppermint oil. It has a faint aromatic odour, like that of bitter-almond oil. Quinoline evaporates completely, but slowly, at the ordinary temperature, so that the grease spot formed by it on paper is not permanent. It resinifies on exposure to air and when left standing over water forms a mixture having the composition $C_9H_7N, 1\,1/2H_2O$, which becomes turbid at blood temperature. Quinoline containing traces of water boils at 227–228°.

Quinoline is very sparingly soluble in cold water, but more freely so in hot. It is miscible in all proportions with alcohol, ether, carbon disulphide, and fixed and volatile oils; and is also easily soluble in chloroform, amyl alcohol, benzene and light petroleum.

Quinoline has well-marked basic characters, and forms an extensive series of salts, most of which are crystallisable and deliquescent. It precipitates ferric and aluminium solutions, and at a high temperature decomposes ammonium salts.

Salts: the *hydrochloride*, C_9H_7N,HCl, forms small aggregates of hygroscopic crystals, m. p. 93–94°; the *platinichloride*, C_9H_7N,H_2PtCl_6, crystallises from hot dilute hydrochloric acid with $2H_2O$ in yellow needles, m. p. 225°, and from hot water with $1H_2O$ in small yellow needles, m. p. 218°; the *picrate* forms bright yellow needles, m. p. 205°; the *tartrate*, $3C_9H_7N,4C_4H_6O_6$, forms large, flat, rhombic needles, m. p. 125° (decomp.), and is readily soluble in water and hot alcohol; it is used as an antipyretic and antiseptic, being specially useful in the case of intermittent fevers; the *salicylate*, $C_9H_7N,C_7H_6O_3$, is a greyish-white powder soluble in water, alcohol and ether; it is employed as an antiseptic and antineuralgic; the *thiocyanate*, $C_9H_7N,HCNS,xH_2O$, is used for certain venereal diseases, but finds greater application in the form of the bismuth double salt, $(C_9H_7N,HCNS)_2,Bi(SCN)_3$, under the name of "*crurin*," a reddish-yellow powder insoluble in alcohol, ether and water; it is usually taken in the form of tablets containing 50% of starch.

Quinoline forms additive compounds with many organic substances

Thus, an ethereal solution of the base and iodoform, when kept a few hours, deposits the *additive product*, $3C_9H_7N,CHI_3$, in the form of large needles, m. p. 65°, insoluble in water, acids and alkalies, soluble in benzene and light petroleum; it is used as an antiseptic and antipyretic. The *additive product* with *iso*-amyl iodide, $C_9H_7N,C_5H_{11}I$, forms yellowish-green crystals, m.p. 184–185°; if the quinoline employed contains lepidine (as is the case with quinoline made from cinchonine) the additive product, when dissolved in aqueous potassium hydroxide, gives a beautiful, but not very permanent, blue colour owing to the formation of *Quinoline-blue*, $C_{28}H_{35}N_2I,1\ 1/2H_2O$. The latter substance crystallises in green needles with a metallic reflex, m. p. 100°, when heated quickly, and dissolves in alcohol to a beautiful blue solution. Both quinaldine and lepidine give this reaction.

Quinoline (2 mols) heated with resorcinol (1 mol.) at 100° yields a *substance* which crystallises in silvery plates, m p. 102°, and is readily soluble in alcohol, ether and chloroform, but insoluble in light petroleum; it is used as an antiseptic and antipyretic.

Reaction of Quinoline and its Salts.

Quinoline salts in aqueous solution are precipitated milky white by alkali hydroxides and ammonia, the precipitate being somewhat soluble in excess. From the alkaline liquid, the quinoline can be readily extracted by ether, chloroform, or petroleum spirit.

Iodised iodide of potassium gives a reddish-brown precipitate even in dilute solutions of quinoline salts (1 in 25,000). Potassio-mercuric iodide only precipitates quinoline from tolerably strong solutions (1 in 3,000), the precipitate being yellowish-white and amorphous, but converted into delicate amber-yellow needles on addition of hydrochloric acid. This reaction is characteristic. Phosphomolybdic acid, in presence of nitric acid, produces a yellowish-white precipitate in quinoline solutions.

Potassium ferrocyanide colours solutions of quinoline salts greenish, and on addition of hydrochloric acid a reddish-yellow amorphous precipitate is thrown down, if the liquid be not too dilute.

Quinoline is precipitated by picric acid, but not by tannic acid or ferric chloride; and its salts, in the solid state, yield no colour reactions with nitric acid or strong sulphuric acid, either alone or in association with oxidising agents.

A solution of a quinoline salt, when treated with a solution of potassium dichromate, yields a yellow, crystalline precipitate of the *dichromate*, $C_9H_7N,H_2Cr_2O_7$, which crystallises from water in glistening needles, m. p. 164–167°.

Quinoline possesses powerful antiseptic properties; 0.2% of the tartrate is said to completely prevent the lactic fermentation of milk, the decomposition of urine and gelatin, and the development of bacteria in cultivation fluid. Even in concentrated solution it does not coagulate albumin, and in the proportion of 1% it completely destroys the coagulability of the blood. On the other hand, quinoline is remarkably inactive to yeast-cells, and does not affect alcoholic fermentation, even when present in considerable quantity.

Quinoline has been used in medicine as an antipyretic, the adult dose of the tartrate being from 7 to 12 gr. It is said by some not to produce any unpleasant after-effects, but by others to cause irritation of the stomach and collapse. It is not found in the urine of those who have taken it internally.

An aqueous solution of quinoline is used as a gargle for diphtheria and dysentery.

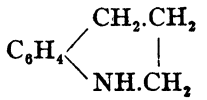
Commercial quinoline is often very impure and quite unfit for medicinal use. C. Ekin (*Pharm. Jour.*, 1882 [*iii*], **12**, 661) has described a specimen which had a deep brown colour and an odour like oil of bitter almonds. On treating it with hydrochloric acid a large proportion remained insoluble, and was evidently unconverted nitrobenzene, while the soluble part gave the reactions of aniline.

Pure quinoline should be colourless or only faintly yellow, and have the correct b. p. If mixed with 40–50 times its own weight of water, it should give a filtrate which is not coloured violet by a solution of bleaching powder.

The salts of quinoline should be completely soluble in water, and the free base in a slight excess of hydrochloric acid. The neutral solution should be free from bitter taste (which indicates the presence of impurity derived from cinchonine), and should not give a coloured precipitate with alkali hydroxides.

Estimation of Quinoline.

Quinoline can be titrated fairly accurately with standard acid, if methyl-orange be employed as an indicator.

Tetrahydroquinoline. 

When quinoline is acted on by nascent hydrogen, it is first converted into *dihydroquinoline*, C_9H_9N, a solid substance, m. p. 161°, and subsequently into *tetrahydroquinoline*, a liquid, b. p. 251°, the *hydrochloride* of which, $C_9H_{11}N,HCl$, forms prisms, m. p. 180–181°, while the *platinichloride* forms reddish-yellow crystals, m. p. 200°. Both these reduction-products yield nitrosamines, and can be alkylated, and hence are secondary bases. Tetrahydroquinoline possesses stronger antipyretic characters than quinoline itself, and this property is exhibited still more strongly in certain of its derivatives, several of which have received some application in medicine (see below).

Alkylquinolines.

A large number of the higher homologues of quinoline are produced on distilling alkaloids with potassium hydroxide while many have been isolated from coal tar and shale oils.

From the acid tar produced in the purification of shale oil, Robinson and Goodwin (*Trans. Roy. Soc. Edin.*, 1879, **28**, 561; 1880, **29**, 265) obtained the following bases of the quinoline series.

Base	Formula	B. p.
Tetracoline	$C_{12}H_{13}N$	290–295
Pentacoline	$C_{13}H_{15}N$	305–310
Hexacoline	$C_{14}H_{17}N$	325–330
Heptacoline	$C_{15}H_{19}N$	345–350
Octacoline	$C_{16}H_{21}N$	360–365

Quinaldine, 2-methylquinoline, C_9H_6MeN, sometimes forms 25% of coal-tar quinoline; it is a colourless liquid with a faint quinoline-like odour, b. p. 244–245°/750 mm., the salts of which are mostly soluble in water; the *dichromate*, $(C_{10}H_9N)_2,H_2Cr_2O_7$, forms yellowish-red needles, soluble with difficulty in cold, readily soluble in hot, water. When heated with phthalic anhydride and zinc chloride, quinaldine gives rise to a beautiful yellow dye, *quinophthalone*, $C_6H_4(CO)_2{\cdot}CH.NC_9H_6$, m. p. 235°. The sodium salt of the sulphonic acid of the latter substance is the *Quinoline Yellow* of commerce.

Quinaldine has been used as an antipyretic and antiseptic, but has a much weaker effect than quinoline.

Lepidine, 4-methylquinoline, is obtained together with quinoline when cinchonine is distilled with potassium hydroxide. It is a liquid with an odour like quinoline, b. p. 261–263°, D^{20} 1.0862, and solidifies to a crystalline mass at 0°. It is readily soluble in water and miscible with alcohol, benzene, ether, and light petroleum in all proportions. It closely resembles quinoline in its antipyretic and antiseptic properties.

8-Hydroxyquinoline, $OH.C_9H_6N$, is of importance since many antipyretics and antiseptics are derived from it (see below). It is obtained by fusing quinoline-8-sulphonic acid with sodium hydroxide, also by Skraup's reaction from 2-aminophenol, 2-nitrophenol, glycerol and sulphuric acid.

8-Hydroxyquinoline crystallises in long, glistening prisms, having a saffron-like odour, m. p. 75–76°, b. p. 266.6°(corr.)/752 mm.; it is volatile in steam, sublimes slowly at the ordinary temperature, is soluble with difficulty in ether and cold water, but readily soluble in alcohol, benzene, chloroform and dilute sodium hydroxide. The solutions in acids and alkalies are yellow; the colourless alcoholic solution becomes yellow on the addition of water. An aqueous solution gives with ferric chloride an intense green colouration and with ferrous sulphate a red colouration followed by a black precipitate.

An alcoholic solution of 8-hydroxyquinoline, when treated with copper acetate, yields a greenish-yellow crystalline precipitate of the *copper* salt, $(C_9H_6ON)_2Cu$. The *picrate*, $C_9H_7ON,C_6H_2(NO_2)_3OH$, forms yellow prisms, m. p. 203–204°; it is difficultly soluble in cold alcohol and almost insoluble in benzene

Carbostyril, 2-hydroxyquinoline, $C_9H_6(OH)N$, is used in medicine and in large doses has a similar action to curare. It is obtained by the reduction of *o*-nitrocinnamic acid and crystallises with $1H_2O$ from a hot 1% aqueous solution in long, feathery crystals and from alcohol in large prisms, m. p. 199–200°. It dissolves in aqueous alkalies forming salts which are decomposed by carbon dioxide.

Antipyretics and Antiseptics Derived from Quinoline.

A considerable number of substances related to quinoline, and mostly allied to tetrahydroquinoline, have been introduced from

time to time as antiseptics, antipyretics and febrifuges. Some of these are very powerful in their action and appear likely to receive a permanent place in medicine; but they are not periodics, and cannot be substituted for quinine in cases of ague or intermittent fevers. The following are some of the most important of the antipyretics and antiseptics derived from or related to quinoline

M-Kairoline is the hydrogen sulphate of 1-methyltetrahydroquinoline, $C_6H_4\begin{cases}CH_2.CH_2\\ \quad\quad|\\ NMe.CH_2\end{cases}$, obtained by acting on tetrahydroquinoline with methyl iodide, the free base has b. p. 245°.

A-Kairoline has a similar constitution but contains an ethyl instead of a methyl group.

M-Kairine is the hydrochloride of 8-hydroxy-1-methyltetrahydroquinoline, $OH.C_6H_3\begin{cases}CH_2.CH_2\\ \quad\quad|\\ NMe\ CH_2\end{cases}$. The corresponding ethyl compound is known as A-*Kairine* On adding an alkali hydroxide to the aqueous solution of a kairine, the penetrating characteristic odour and bitter taste of the free base are easily recognised, while the alkaline solution rapidly becomes coloured and deposits a brown humus-like substance. When the aqueous or alcoholic solution of a kairine is treated with an oxidising agent, such as potassium dichromate and an acid, it gives a series of colours ranging from violet-blue to purple, or sometimes greenish. Without the addition of an acid, the solution becomes dark purple, and on standing a violet precipitate is formed, which dissolves in alcohol with black colour. A drop of ferric chloride, added to a dilute and neutral solution of kairine, instantly produces a violet colouration, rapidly changing to brown, with precipitation. An excess of ferric chloride added to a strong solution of kairine produces a nearly black precipitate. Sodium nitrite and dilute sulphuric acid produce an orange or red colour in kairine solutions. Potassium ferrocyanide gives a voluminous precipitate, and phosphotungstic acid a pale yellow precipitate.

The kairines act as powerful antipyretics. Their use is almost obsolete, as their action is somewhat uncertain; and they are said to be liable to produce vomiting, cyanosis, and collapse.

Thalline is the commercial name of another antipyretic having the

constitution of 6-methoxytetrahydroquinoline, $OMe.C_6H_3\begin{cases}CH_2.CH_2\\ \quad\quad |\\ NH.CH_2\end{cases}$

Thalline is prepared by heating *p*-aminoanisole and *p*-nitroanisole with glycerol and sulphuric acid and reducing the product with nascent hydrogen. The free base crystallises in large, colourless prisms, m. p. 42°, b. p. 283°, possesses a bitter, saline and pungent taste, and is sparingly soluble in water, but readily soluble in alcohol, benzene, choloroform or ether.

Thalline sulphate, $(C_{10}H_{13}ON)_2,H_2SO_4,2H_2O$, is the most common variety of commercial thalline. It crystallises from alcohol in long, colourless needles having an aromatic, bitter, saline taste and a faint odour resembling coumarin. It dissolves in 7 parts of cold water and 100 parts of 90% alcohol; the solutions give an acid indication and become brown on exposure to light. A very dilute aqueous solution of commercial thalline gives with ferric chloride a yellow colouration, changing to emerald-green (destroyed by reducing agents) and passing in a few hours to deep red. The reaction is extremely delicate. A green colour is also produced by auric chloride, silver nitrate, mercuric nitrate, chlorine water, etc., and, in acid solution, also by a solution of bleaching powder and potassium ferricyanide. Strong sulphuric acid dissolves thalline sulphate without colouration, but on addition of nitric acid the liquid becomes deep red, and immediately afterward yellow-red. Fuming nitric acid colours a dilute aqueous solution red. Sulphuric acid and sugar give a red colouration. Iodine colours the solution dark brown, then dingy green. Ammonia forms a white precipitate of the free base, readily taken up by ether on agitation. If not too dilute, solutions of thalline sulphate yield precipitates with the general reagents for alkaloids.

If to an aqueous solution of *β*-naphthaquinone a small quantity of the solution of a thalline salt be added, and then a drop or two of sodium hydroxide solution, a fine cherry-red colouration is produced, becoming more brilliant on adding nitric acid. The colouring matter is extracted by ether or chloroform.

Thalline tartrate occurs in commerce as a faintly yellow crystalline powder. It dissolves in 10 parts of cold water, and the solution gives the same reactions as the sulphate. In alcohol it is very sparingly soluble. The salt contains 52.2% of thalline.

The salts of thalline become altered by exposure to light.

Thalline salts are powerfully antipyretic, and have been employed in yellow fever. They cause profuse perspiration, and are apt to produce depression, etc. Hence their internal use is practically obsolete. Thalline acts as a direct blood-poison, its antithermic properties being due to the destruction of the red corpuscles. It has found considerable application in the treatment of gonorrhœa. The sulphate is official in the *German Pharmacopœia* of 1890.

Exhibition of thalline causes a dark colouration of the urine. A derivative, which also gives a green colour with ferric chloride, but differs from thalline in being extracted by agitating the acidified urine with light petroleum, should first be removed, and then the unaltered portion of the thalline can be isolated by rendering the urine alkaline with ammonia, and agitating with ether or benzene. Very small quantities of thalline can in this way be recognised in urine

An *additive* product of thalline (or its sulphate) and iodine has been introduced for the treatment of carcinoma.

Thermifugin, another antipyretic, is sodium hydroxy-1-methyl-tetrahydroquinolinecarboxylate, $CO_2Na.C_6H_2(OH)\begin{matrix}CH_2.CH_2\\ |\\ NMe.CH_2\end{matrix}$ It forms colourless crystals which dissolve readily in water; the solutions become brown when kept.

Analgen, 5-acetylamino-8-ethoxyquinoline,

$$\begin{matrix}CH:C(NHAc).C.CH:CH\\ |\qquad\qquad\quad ||\quad |\\ CH{\cdot}C(OEt).-C-N:CH,\end{matrix}$$

forms colourless crystals, m. p. 155°; it is readily soluble in alcohol, sparingly soluble in water (7 in 1,000), an acidified aqueous solution is yellowish-red.

The corresponding benzoyl compound, $NHBz.C_9H_5(OEt)N$, is known as *benzanalgen, quinalgen* and *labordin;* it is a white, tasteless, crystalline powder, m. p. 208°, readily soluble in very dilute acids; the solutions are coloured.

The analgens find application in the treatment of lumbago, rheumatism and neuralgia.

Loretin is the name given to 7-iodo-8-hydroxyquinoline-5-sulphonic acid, $\begin{matrix}CH:C(SO_3H)\\ |\\ CI=C(OH)\end{matrix}\!\!>C_5H_3N,$

an odourless, tasteless, reddish-yellow, crystalline powder, which turns brown at 200° and liberates iodine at 260°. It is readily soluble in alcohol, ether and water and dissolves without decomposing in hot concentrated sulphuric acid. The sodium salt is compressed into tablets, which dissolve in 11 parts of hot water to a yellow solution, and is used in this form for making disinfecting baths.

A 5–10% solution of loretin in collodion is employed for coating wounds.

A non-poisonous compound of loretin with iodoform has been introduced for the treatment of wounds

A mixture of the sodium salt of loretin (10 pts) with bismuth nitrate (4 pts), occurring as an insoluble, yellow powder, finds application as an antiseptic and astringent.

Griserin is a mixture of 20% of sodium hydrogen carbonate and an iodohydroxyquinolinesulphonic acid; it dissolves in water, but is insoluble in alcohol, ether and chloroform. It is employed as an antiseptic in the treatment of tuberculosis and other infectious diseases.

Quinosol is the double sulphate of potassium and 8-hydroxyquinoline, $(OH.C_9H_6N)_2,H_2SO_4,K_2SO_4$. It is a crystalline, sulphur-yellow powder which dissolves readily in water Ferric chloride gives an intense dark-green colouration. Quinosol is employed in gynecological operations.

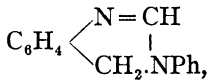
Argentol is the silver salt of a hydroxyquinolinesulphonic acid, $OH.C_9H_5N.SO_3Ag$. It is employed in the form of an ointment with vaseline or lanoline for the treatment of ulcers, wounds and various skin diseases.

Diaptherin is a compound formed by the union of 2 mols. of 8-hydroxyquinoline with 1 mol. of *o*-phenolsulphonic acid, $OH.C_6H_4.SO_3H,2C_9H_6N.OH$. It forms transparent, yellow crystals, m. p. 85°, is soluble with difficulty in absolute alcohol and moderately soluble in water. Ferric chloride produces a bluish-green colouration which becomes yellow on the addition of hydrochloric acid. Diaptherin is non-poisonous; it is used as an antiseptic and for the treatment of rheumatism.

Quinazolines.

The quinazolines may be regarded as derivatives of quinoline formed by the replacement of the 3CH group by N.

A substituted dihydroquinazoline, namely 3-phenyl-3:4-dihydroquinazoline,



has acquired some practical interest as the base of *"orexin,"* a preparation said to have valuable tonic, stomachic and appetising properties on which, however, some doubt has been thrown (*Pharm. J.*, 1890 [*iii*], **20**, 709, 825, 977; **21**, 43). The usual dose of orexin is from 2 to 10 gr.

Orexin, which occurs as a hydrochloride having the composition $C_{14}H_{12}N_2,HCl,2H_2O$, is prepared by acting on the sodium derivative of formanilide with *o*-nitrobenzyl chloride, and reducing the *o*-nitro-benzylformanilide thus obtained with tin and hydrochloric acid.

Orexin (hydrochloride) crystallises with $2H_2O$ in white needles, m. p. 80°. When kept in a desiccator for some time they become anhydrous, and then melt at 221°. Orexin has a bitter taste, and somewhat intense, burning after-taste. The powder induces violent sneezing. Orexin dissolves readily in water (13 pts) and alcohol, but not in ether. On adding an alkali to the aqueous solution the free base is separated as an oil which becomes crystalline when kept, or as a white flocculent precipitate readily soluble in ether and chloroform. A solution of orexin yields with mercuric chloride a white precipitate soluble in hot water, and redeposited in white needles on cooling. Potassium dichromate gives a yellow precipitate soluble on heating, and redeposited on cooling in golden-yellow needles. Bromine-water is decolourised with formation of a yellowish amorphous precipitate. Orexin reduces potassium permanganate in the cold.

On heating oxerin in a test-tube with about twice its measure of zinc-dust, the strong characteristic odour of phenyl-isonitrile is produced. On treating the residue with hydrochloric acid, and adding bleaching-powder solution to the filtered liquid, a blue colouration is obtained, owing to the previous formation of aniline.

Orexin tannate has been introduced for administering to children. It is a tasteless powder which becomes brown and acquires an unpleasant taste at 100° and decomposes completely at a higher temperature. It is almost insoluble in water, and only slightly soluble in alcohol and ether, but readily soluble in very dilute hydrochloric acid (0.3%)

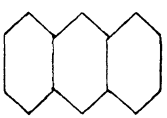
from which solution it is precipitated unchanged by strong acid and dilute aqueous alkali.

Orexin tannate gives a bluish-black colouration with iron salts and when treated with aqueous ammonia becomes clotted while the liquid assumes a wine-red colouration. A further test for orexin tannate is as follows: 0.5 grm. is dissolved in 3 c.c. of 30% acetic acid, rendered alkaline with sodium hydroxide and shaken with ether. The residue obtained by evaporating the ethereal extract is dissolved in concentrated sulphuric acid, addition of nitric acid to which should then produce a green colouration which frequently appears to be red on the edges. If the solution be diluted and treated with sodium hydroxide it should become yellow and yield a yellow precipitate.

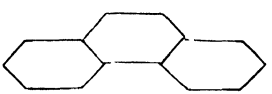
Orexin tannate when heated with zinc dust yields phenyl *iso*-cyanide, benzonitrile and aniline.

ACRIDINE AND ITS ALLIES.

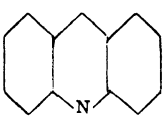
Acridine and its isomeride phenanthridine bear the same relation to anthracene and phenanthrene respectively that quinoline bears to naphthalene, and pyridine to benzene (compare). The following formulæ show their constitution and relationship to anthracene and phenanthrene:



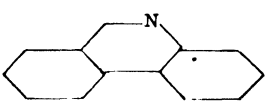
Anthracene



Phenanthrene



Acridine



Phenanthridine.

Acridine. $C_{13}H_9N$.

Acridine has been prepared synthetically by heating concentrated formic acid or chloroform with diphenylamine and zinc chloride, and also by various other reactions. Acridine is contained in coal tar, and may be extracted from the fraction boiling between 300° and 360°, or from crude commercial anthracene, by agitating it with dilute sulphuric acid, precipitating the acid liquid with potassium chromate,

purifying the acridine chromate by recrystallisation, precipitating the base by ammonia, and recrystallising it from hot water. The hydrochloride may also be employed for the purification of acridine.

Acridine crystallises in colourless or brownish-yellow leaflets, broad needles or rhombic prisms, m. p. 110°; it sublimes in needles, distils without decomposition above 360°, and is volatile in steam.

Acridine is very slightly soluble in cold, but more readily in boiling, water, crystallising on cooling in long needles. It is readily soluble in alcohol, ether, benzene, carbon disulphide, etc.

Dilute solutions of acridine (and its salts) exhibit a strong blue fluorescence, which is green in more concentrated solutions, and disappears if they are very strong.

The most characteristic property of acridine is its intensely irritating effect on the skin and mucous membrane. Violent sneezing and coughing are produced by inhaling the smallest particle of the dust or vapour. The base and its salts attack the tongue even in minute quantities, and even very dilute solutions cause acute stinging when applied to the tongue or skin.

Acridine has been employed as an insecticide, and compositions containing it have been patented for coating the bottoms of vessels. It is highly probable that the preservative properties of coal-tar creosote oil are partially due to the presence of acridine.

Acridine is a very stable substance. Sulphuric acid has no action upon it, except at a very high temperature, and potassium hydroxide does not react below 280°. Concentrated nitric acid converts acridine into nitro-derivatives Most other oxidising agents act with difficulty or not at all on acridine, but by the action of potassium permanganate it is converted into *quinoline-2:3-dicarboxylic* or *acridinic acid.* The latter substance crystallises with $2H_2O$ in slender needles, decomposes at 120–130°, and is sparingly soluble in water.

The addition of a 10% solution of cobalt nitrate to a boiling solution of 1 grm. of acridine in 500 c c. of bleaching powder solution (1·5) and subsequently boiling for one hour leads to the formation of *9-acridone,* stout, yellow needles, m. p. 354° (decomp.).

Salts of Acridine.

Acridine is a feeble base. It forms no carbonate, and its salts are more or less decomposed by boiling with a large quantity of water.

Acridine hydrochloride, $C_{13}H_9N,HCl,H_2O$, forms yellow plates.

The solution in water exhibits a bluish-green fluorescence and gives a yellow crystalline precipitate, m. p. 235°, of the *mercurichloride*, $(C_{13}H_9N,HCl)_2,HgCl_2$, on the addition of mercuric chloride. With platinic chloride it yields the *platinichloride*, $(C_{13}H_9N)_2,H_2PtCl_6$, in minute, sparingly soluble, yellow needles.

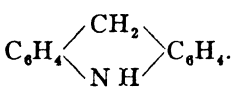
Acridine nitrite, $(C_{13}H_9N)_2,HNO_2,3H_2O$, obtained by the interaction of acridine hydrochloride and sodium nitrite, forms long, yellow, silky needles, m. p. 151°. It loses $2H_2O$ at 70–80°.

Acridine sulphite, $(C_{13}H_9N)_2,H_2SO_3$, is precipitated in yellowish-red or brownish needles, very slightly soluble in water, on mixing solutions of sodium sulphite and acridine hydrochloride, and adding hydrochloric acid.

Acridine Picrate, $C_{13}H_9N,C_6H_3O_7N_3$, is obtained as a canary-yellow precipitate, consisting of microscopic, yellow, prismatic needles with a faint green reflex; it commences to decompose at 208°. The picrate is almost wholly insoluble in cold water; 10 c.c. of the saturated solution in alcohol and benzene at 17 5° contain 0 004 grm. and 0.001 grm. of the salt respectively. Acridine has been suggested by Anschutz (*Ber.*, 1884, **17**, 438) as a suitable reagent for the estimation of picric acid, the hydrochloride being used as a precipitant for metallic picrates, and a solution of the free base in benzene for the picric acid compounds of hydrocarbons.

Conversely, in the absence of substances forming picrates soluble with difficulty in benzene or water, acridine may be estimated by precipitating and weighing as the picrate.

Hydroacridine. Dihydroacridine.



This substance is formed (together with a white substance insoluble in alcohol) by the reduction of acridine in alcoholic solution by sodium amalgam. It forms prisms, m. p. 169°, insoluble in water, slightly soluble in cold alcohol, very soluble in hot alcohol or ether. It dissolves in concentrated sulphuric acid, and is precipitated unchanged on dilution with water. Argentic and cupric oxides reconvert it into acridine. Hydroacridine is the analogue of piperidine (page 141) and tetrahydroquinoline (page 155).

Phenanthridine.

Phenanthridine crystallises in slender needles, m. p. 104°, and distils above 360°. The vapour when inhaled induces violent sneezing. Aqueous solutions exhibit a blue fluorescence. It is thus seen that phenanthridine presents the closest resemblance to acridine; it may be distinguished from the latter substance, however, by adding sodium sulphite to a solution of the hydrochloride containing excess of hydrochloric acid; phenanthridine does not yield a precipitate while acridine gives a precipitate of reddish-brown needles. The *mercurichloride*, $C_{13}H_9N,HCl,HgCl_2$, crystallises in small prisms, m. p 197°.

A boiling solution of phenanthridine in bleaching powder solutions, when treated with cobalt nitrate, yields *phenanthridone* which crystallises in long, silky needles, m. p. 293° (corr.).

THE VEGETABLE ALKALOIDS.

By THOMAS A. HENRY, D. Sc. (Lond.)

When Serturner in 1817 (*Gilbert's Annalen*, 1817, **55**, 56) published his paper entitled "Morphia, a new salt-forming substance, and meconic acid, as the chief constituents of opium," he described morphia as a "vegetable alkali" and drew attention to its relationship to ammonia. Between 1817 and 1835 about 25 substances exhibiting similar basic properties and most of them possessing well-marked physiological action were isolated from plants and it became convenient to group them together under one name and for this purpose the term alkaloid came into use about 1833

Since that date this name has been used to designate the class of naturally occurring nitrogenous organic compounds, possessing basic properties. Konigs (*Studien uber die Alkaloide*, p. 31, Munich, 1880) suggested the limitation of the term to pyridine derivatives of natural occurrence and there was at first some inclination among chemists to adopt this view. More recently, however, it has been felt undesirable to exclude such substances as caffeine and theobromine, which are purine derivatives and hygrine, which is a pyrrolidine derivative from the application of the name, and the term is now used by most writers in its original significance. It is convenient to separate the alkaloids into 2 groups according to their origin, viz., (1) animal alkaloids and (2) vegetable alkaloids, and this article is concerned only with the second of these groups, which is by far the more important.

Distribution and Mode of Occurrence.—Alkaloids have been isolated from both the great classes of plants, but whereas those obtained from the Cryptogams (so-called flowerless plants) can be numbered on the fingers of one hand, those occurring in the Phanerogams (flowering plants) run into hundreds. The existence of alkaloids in the flowering plants is, however, limited to comparatively few natural orders, the richest in these substances being the *Papaveraceæ*,

Leguminosæ and *Ranunculaceæ*. Other striking groups in this respect are the *Solanaceæ*, furnishing the tropine series of alkaloids, and *Rubiaceæ* yielding the cinchona alkaloids. The usual statement that the *Compositæ*, one of the largest and most cosmopolitan of orders, yields very few alkaloids is negatived by Greshoff's statement that out of 150 plants of this order examined, 50 yielded alkaloids (*Ned. Tijd. Pharm.*, 1900, **12**, 137). No alkaloid has yet been obtained from the *Graminaceæ*, and only one, stachydrine, from the *Labiatæ*.

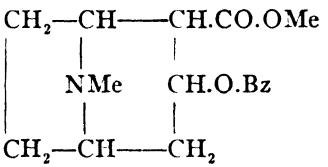
As a general rule alkaloids may occur in all parts of a plant, though where several are present one of these may be found in greatest abundance in a particular part and occasionally when only one alkaloid is present this may be restricted to certain parts. The amount of alkaloid found in different parts of the same plant may vary widely (compare Feldhaus, *Arch. Pharm.*, 1905, **243**, 328) thus, in the several parts of a specimen of Stramonium from Madras examined recently at the Imperial Institute, the following percentages of alkaloids were found:

	Roots	Leaves	Seeds
Total alkaloids	0 159	0 420	0 186
Approximate ratio of hyoscyamine to scopolamine	Alkaloids not identified	5 1	3 1

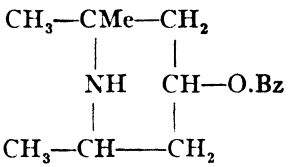
Alkaloids usually occur in plants in the form of salts of malic, oxalic, succinic, tannic or some other vegetable acid and in a few cases as salts of special acids; thus the aconite alkaloids occur chiefly in combination with aconitic acid, the opium alkaloids largely with meconic acid, the cinchona alkaloids with quinic acid and so on. Choline is widely distributed among plants in the form of the complex substances known as lecithins. Salts with inorganic acids are not infrequent, thus morphine occurs naturally in opium in part as morphine sulphate.

Mode of Formation in Plants.—It seems likely that the simplest group of vegetable alkaloids represented by such substances as asparagine, glutamine, leucine, arginine, choline, muscarine and betaine result from the decomposition of proteins, and this view finds support in the fact that choline especially is widely distributed in the vegetable kingdom. This method of formation may also apply in the case of the purine bases of vegetable origin such as xanthine, caffeine, theo-

bromine, etc., and Pictet assigns a like origin to alkaloids having a pyrrolidine nucleus, *e. g.*, nicotine, atropine, cocaine and hygrine (*Arch. Sci. Phys. Nat.*, 1905 [IV], **19**, 329) and suggests that alkaloids having a pyridine or quinoline nucleus may result from molecular transformation of methylpyrroles or -indoles, since he has shown that by the action of heat, 1-methylpyrrole is converted into pyridine and 1-methylindole into quinoline (*Ber.*, 1904, **37**, 2792). The suggestion has also been made that alkaloids may result directly from condensation of certain aldehydes or ketones with ammonia or amines In support of this it is urged that these classes of compounds are known to occur in plants and that some of the reactions alluded to can take place at atmospheric temperatures and are therefore not precluded from occurring in plants. Further, such reactions give rise to substances having heterocyclic nuclei and in some cases exhibiting physiological action quite similar to that shown by some groups of naturally occurring alkaloids. Perhaps the most interesting case of this kind is diacetonamine obtained by condensing acetone with ammonia This substance can be converted by simple reactions into β-eucaine, which is quite similarly constituted with cocaine and is an efficient substitute for this alkaloid in medicine. The two substances are represented by the following formulæ·



Cocaine



β-Eucaine

Function in Plants.—As regards the function of alkaloids in plants, two views have been held. The first supposes that they are ultimate products of metabolism and play no further part in the life of the plant. In support of this view it is urged that alkaloids are found in greatest abundance in the bark of the stem or root, in seeds, fruit rinds and other parts, which are thrown off by the plant and are, therefore, convenient receptacles for such waste products or excreta (Guareschi, *Einfuhrung in das Studium der Alkaloide*, Berlin, 1896. Compare Pictet, *loc. cit.*). On the other hand, it has been shown especially by Lotsy in Java, in the case of cinchona, that alkaloids are formed in the leaves apparently as normal products of assimilation,

and are produced in greatest amount in those parts in which metabolism is proceeding most rapidly and that they are apparently ultimately consumed in large measure by the plant and are therefore to be regarded as plastic products.

It is of considerable industrial interest to note that by the application of modern cultural methods, including plant selection, appropriate manuring, and collection at the proper period, the yield of a plant not only in "total alkaloids," but in any particular alkaloid peculiar to that plant, may be greatly increased. For examples of this kind of work, reference may be made to van Gorkom's *Scheikundige Bijdragen tot de Kennis der Java-Kina*, 1908 (de Bussy, Amsterdam) and Chevalier's paper on Solanaceous plants (*Compt. Rend.*, 1910, **150**, 344).

Nomenclature.—It is impossible as a rule to apply systematic names to the alkaloids obtained from plants since in the great majority of cases their chemical constitution is unknown and even where it is known the systematic names are too cumbrous for general use. The names given to alkaloids have reference as a rule to their botanical origin or to their physiological properties, thus: *aconitine* obtained from *Aconitum Napellus* and *morphine* in allusion to the soporific properties of this alkaloid. In recent years the tendency has been to adopt the first system of naming only. For secondary alkaloids, either occurring with or derived from the principal alkaloid, it is becoming customary where possible to express more or less clearly in the names assigned to them their relationship to the principal alkaloid, thus Quinine; Hydroquinine: Cocaine (methylbenzoylecgonine); Cinnamylcocaine (methylcinnamylecgonine): Aconitine (acetylbenzoylaconine); Benzaconine (benzoylaconine); Aconine: Pelletierine; *iso*-Pelletierine; Methylpelletierine. Where the relationship is unknown the names of the secondary alkaloids are commonly formed from that of the first by the insertion of the particles *in, en, id, ig, is, at,* etc.; thus gelsemine, gelsem*in*ine; yohimbine, yohimb*en*ine; pilocarpine, pilocarp*id*ine; and so on.

It will be clear from the foregoing that the chief characteristic of the nomenclature of alkaloids is the lack of system which prevails and where attempts have been made, *e. g.*, by Ciamician and Silber (*Ber.*, 1896, **29**, 481), and by Willstätter (*ibid.*, 1897, **30**, 2692) to re-name groups of closely related alkaloids on a systematic basis, chemists have shown no tendency to adopt the names suggested. Fortunately, however, in English the convention has been generally

accepted that the names of all alkaloids in common with those of other nitrogenous bases must end in *ine*. This serves to distinguish them from the glucosides and so-called "neutral principles," the names of which end in *in*.

There is a similar lack of system in the naming of the alkaloidal salts; thus since the alkaloids resemble ammonia and the amines and form salts by combining with the whole molecule of acid presented to them (NH_3,HCl; B,HCl; B,H_2SO_4; B,$H_2C_2O_4$, where B represents an ordinary monacidic alkaloid) their compounds with the halogen acids are called hydrochlorides, hydrobromides, and so on, but the application of the rule stops there and the salts with other acids receive the ordinary designations, *e. g*, quinine sulphate, nitrate oxalate, picrate, etc.

One of the characteristic properties of the haloid salts of the alkaloids is that of forming double salts especially with the haloid salts of the heavy metals. There are conveniently called platinichlorides, [B.HCl]$_2$,$PtCl_4$, aurichlorides B.HCl,$AuCl_3$, mercurichlorides, B $HgCl_2$, etc. In certain cases the free alkaloids may also combine with a molecule of auric chloride forming substances of the type B.$AuCl_3$. These are now generally called auric-chloride compounds, while the prefix *aurochlor-* is used for a class of substances, the first member of which was prepared by Dunstan and Shepheard (*Trans. Chem. Soc.*, 1893, **63**, 195) from caffeine. In these a gold chloride residue, $AuCl_2$, replaces an atom of hydrogen in the molecule of the alkaloid, thus aurochlorcaffeine has the formula $C_8H_9(AuCl_2)O_2N_4$.

Isolation and Purification of Alkaloids.—As a preliminary to the identification or estimation of an alkaloid it is almost always necessary to prepare it in a fairly pure form. The method to be used in isolating alkaloids from plant material will depend on the nature of the latter, and in the case of cinchona bark, opium, coca leaves, and other well-known products, suitable methods of isolation are described in the special sections of this volume, devoted to the particular alkaloids. The present description is therefore to be regarded only as applying to plant materials, for which special methods have not been worked out.

A test for the presence of alkaloids should first be made and this may be carried out with 30 to 100 grm. of material by the alcohol extraction process outlined below, a Soxhlet or other apparatus (Vol. 1, page 77) being used. Most alkaloids and alkaloidal salts are soluble

in alcohol, but a few of them dissolve with difficulty and some are practically insoluble in this solvent. If therefore no alkaloid or very little is taken out by alcohol, the following methods of extraction should be tried *in turn* and the extracts obtained tested for the presence of alkaloids by the usual alkaloidal reagents, of which at least two, *e. g.*, Mayer's reagent (potassium mercuric iodide) and phosphomolybdic acid should be used, as no one reagent gives precipitates with all alkaloids (see page 172).

1. Extract the ground plant with cold water.

2. Mix the ground plant, first slightly moistened with water, with one quarter its weight of slaked lime, dry the mixture either in a desiccator or by gently warming; then extract with the following solvents in the order indicated. light petroleum, ether, chloroform, alcohol.

3. Extract a fresh portion of the ground plant with dilute (1%) hydrochloric acid by maceration or percolation in the cold.

If all these methods fail to extract alkaloid, it may be assumed that the material under investigation is free from such substances.

Assuming that the preliminary test has shown the presence of alkaloidal substance it is generally necessary to accumulate enough of this for identification. The preliminary test will have indicated already the best method of extraction but it should be remembered that many alkaloids are decomposed by long continued contact with lime or magnesia, or when allowed to remain in excess of dilute acid so that wherever possible extraction of plant material directly with a neutral organic solvent such as alcohol is to be preferred even if such a method is slow. Where it is found essential to use an acid extracting medium, alcohol containing a small amount of the necessary acid (acetic or tartaric being preferable to mineral acids) should be used in place of dilute aqueous acids, if possible.

The following process of extraction is capable of very general appli cation though it requires modification for particular cases.

The plant product is ground to a fine powder and moistened with 95% alcohol and allowed to stand for several hours. It is then packed in a Toogood's glass percolator (obtainable from Messrs. Toogood Ltd., South Wark Street, London, S. E.), the rubber bung and siphon tube of which have been replaced by a well-fitting cork carrying a tube with a plain glass stopcock. The latter is closed and 95% alcohol poured into the percolator until the ground plant is just covered.

The mouth of the percolator is covered with a ground glass plate and the whole set aside during 24 hours. The stopcock is then opened and the extract run off completely. It can be replaced by more alcohol and the process repeated until the ground plant is exhausted. In order to avoid the passage of small particles into the stopcock the end of the latter inside the percolator should be covered by a plug of cotton wool to serve as a filter, before putting in the ground drug If the material under examination is tough and difficult to grind into fine powder, it is advantageous to place a layer of about 2 in. of fine washed sand at the bottom of the percolator before putting in the ground plant and if the material shows any tendency to clog so that the extract does not run freely, the alcohol should be allowed to drain, and the ground plant taken out, mixed with its own weight of washed sand, and repacked in the percolator.



FIG 1.—Toogood percolator

This method of extraction may also be used for the other solvents indicated, care being taken that a percolator with a closely-fitting ground glass lid is selected when more volatile solvents such as chloroform or ether are to be used. These percolators are supplied in various sizes, holding about 4 oz. and upwards of ground plant. Their form is shown in Fig. 1.

This process answers well in most cases for roots, stems, leaves, flowers, husks of fruits and similar materials. If these contain much resin or colouring matter, soluble in alcohol, it is often advantageous to mix the ground material with lime or magnesia previous to extraction but this should not be done unless it is quite certain that the alkaloid (or alkaloids) present is unaffected by long continued contact with these materials. If there is any doubt on this point, the extraction should be made without lime or magnesia and the obnoxious matter got rid of at a later stage. Many seeds contain considerable quantities of fat or oil and it is advantageous to remove this prior to extraction with alcohol. For this purpose the ground seed may be extracted with light petroleum in which very few of the naturally occurring alkaloidal salts are soluble If the seeds are very rich in oil it is often advan-

tageous to re-grind them after partial extraction by light petroleum in order to facilitate complete removal of the oil. In all cases the light petroleum extract should be tested for alkaloids, and if it contains any, the alkaloid should be recovered by extraction with dilute acid as described below.

Which ever solvent is employed the extraction must be continued until the plant is exhausted, this point being ascertained by testing a few drops of the liquors by one of the general alkaloidal reagents. (See page 185.)

The extraction liquors are next concentrated by distilling off the solvent, which should be done under reduced pressure if water or alcohol has been used as a solvent. If an acid liquid has been used for extraction, dilute ammonia should be added before concentration until the liquor is just faintly acid but still free from any precipitate. The concentrated product requires treatment, which varies with the extracting medium used.

1. Aqueous extracts are usually very much coloured by extractive matter and these should, after concentration to a low bulk, be clarified with lead acetate, the precipitate filtered off, and the filtrate treated with hydrogen sulphide to remove the excess of lead acetate. The filtrate from the lead sulphide is then ready for final extraction as described below.

2. Alcoholic extracts generally contain oil, chlorophyll, and occasionally much resinous matter. They should be concentrated almost to dryness and the residue extracted with water slightly acidified with dilute hydrochloric acid and the mixture set aside to deposit resin, etc. Galenical preparations such as the tinctures, liquid extracts, etc., of the pharmacopœia, may generally be treated in like manner. If they contain much alcohol this should be distilled off and the residue treated as above. In either case if the liquid decanted from the insoluble part of the alcoholic extract is dark coloured, it should be clarified with lead acetate as described under (1), but if not it may be filtered and is then ready for final extraction.

3. Extracts in light petroleum, ether or chloroform are usually fairly clean and in these cases the whole of the solvent should be removed by distillation. The residue should then be taken up with successive portions of dilute (0.5%) hydrochloric acid until all the alkaloid is dissolved out. The mixed acid liquors should be filtered if necessary, and treated as described below.

The slightly acid aqueous liquid obtained as described in paragraphs 1, 2, or 3 above should contain the whole of the alkaloid (or alkaloids), present in the plant extracted. Where only strongly basic alkaloids, the salts of which are not dissociated by water are concerned, it is possible to remove traces of impurities from this acid liquid by shaking with ether or chloroform, but, this may lead to loss of weakly basic alkaloids, and if this preliminary purification is attempted, the ethereal or chloroform solution should be tested for alkaloids, and the latter recovered if present.

Most of the alkaloids are almost insoluble in water and in alkaline solutions and consequently on adding an alkali (usually dilute ammonia in *slight* excess, but in some cases a dilute solution of sodium hydroxide must be used) to this acid liquid, the alkaloids are precipitated, generally as flocculent white powders, which may either be filtered off or, better, may be dissolved in an immiscible organic solvent by shaking successive portions of this with the alkaline aqueous liquid. The extraction should be made complete by testing a few drops of the aqueous liquid from time to time with Mayer's or other suitable reagent (see page 185) and continuing the extraction with fresh portions of solvent until all the alkaloid is removed, unless there is reason to suppose the presence of an alkaloid soluble in water, but not in organic solvents in which case another method of isolation must be used (see below). The immiscible organic liquid to be used depends on the solubility of the alkaloid (or alkaloids) Most alkaloids are soluble in chloroform, but ether is a better medium to work with when possible and light petroleum or benzene or mixtures of two of these are often useful solvents to employ; amyl alcohol is the most useful solvent for morphine. (For a full description of the method of applying immiscible solvents see Vol. 1, pages 79–82.) At this stage it is often possible to effect at least a partial separation when several alkaloids are present by the use of two or more solvents in turn.

The solution in the organic solvent should be run into a second separating funnel and allowed to stand until as much as possible of the adherent aqueous liquid has separated and this should be run off. A few pieces of fused calcium chloride should be placed in the alkaloidal solution and this set aside for a few hours to dry. It may then be run into a tared flask, the funnel washed out with a little more of the dry solvent and the washings added to the bulk. The solvent should be distilled off, the last traces being removed by exposure in a vacuous

desiccator and finally the alkaloidal residue should be dried at 100° until of constant weight, and weighed. This roughly quantitative work is of course not essential in accumulating material for identification, but it is always useful to record the actual weight obtained in such an operation as that described.

A few of the alkaloids are more soluble in water than in any organic solvent and in such cases separation by immiscible organic solvents is not feasible. Recourse must then be had to precipitation with a reagent such as potassium mercuric iodide or solution of iodine in potassium iodide (see pages 189). The precipitates so obtained are allowed to drain, suspended in as little water as possible and decomposed by hydrogen sulphide in the case of the potassium mercuric iodide precipitate, or by sodium thiosulphate for the periodide precipitate. In either case the alkaloid remains in the water as a hydriodide and may be recovered by evaporating the aqueous liquid over calcium chloride in a vacuous desiccator.

The residue obtained by any of these processes is the "total alkaloid" of the plant and is generally a mixture of alkaloids. The presence of a particular alkaloid in it may be established in some cases by applying a series of tests known as "colour tests" (see page 189, and the special sections). But, wherever possible, it is best to separate the constituents of the mixture and identify these by the usual physical constants, such as crystalline form, m. p., specific rotation, etc., either of the alkaloids themselves or of their most characteristic salts. For this purpose the alkaloidal residue is usually converted into the corresponding salts by exactly neutralising it with an appropriate acid and then every effort is made to induce this mixture to crystallise. Crystallisation may often be induced by dissolving the *dry* mixture of salts in *dry* alcohol in a *dry*, clean, stoppered bottle, and adding *dry* ether until a faint cloudiness is apparent. The liquid is then set aside when crystallisation may take place. If only amorphous products separate at first, the clear liquid should be decanted into a second bottle and a little more of the precipitant added. If after several trials in this way no crystals separate, the solvent should be distilled off, the various fractions assembled and dissolved in water and a fresh salt tried in like manner. It is convenient to try the salts in the following order: hydrochloride, hydrobromide, hydriodide, sulphate, nitrate, oxalate, picrate. If the hydrochloride does not crystallise, it should be re-dissolved in water and fractionally precipitated with gold

chloride, in which case a series of crystalline aurichlorides may be obtained, or, if this fails, with platinic chloride. The aurichlorides and platinichlorides are often useful as means of identification as they frequently crystallise readily and their m. p and composition often afford conclusive evidence of the identity of a particular alkaloid. The free alkaloid may be recovered from the aurichlorides or platinichlorides by suspending these in water and decomposing them by hydrogen sulphide, when the alkaloidal hydrochlorides remain in solution and may be used for the preparation of other salts or the free bases. When the mixture of alkaloids or salts has been induced to crystallise, it must be recrystallised in the usual manner until of definite m. p. or until several fractions of definite m. p. are obtained.

Isolation of Liquid Volatile Alkaloids.—The general method already described will suffice for these alkaloids, since they usually occur in plants in the form of salts, which are soluble in alcohol or water and their presence is as a rule easily recognised by their characteristic odour, which becomes apparent when alkali is added to a plant extract containing them. They can be obtained from the concentrated alcoholic extract by distilling off the alcohol as completely as possible on the water-bath, adding water and excess of potassium hydroxide to the residue and distilling in steam. They are best obtained pure by converting into salts and re-crystallising these until of constant m. p. Typical methods for the isolation of volatile alkaloids are described under coniine (page 211), nicotine (page 237), and sparteine (page 230).

Isolation of Alkaloids for Medico-legal Purposes.—The methods generally used are those of Stas-Otto, Dragendorff or Kippenberger. They do not differ in principle from those already described, but since in such cases the alkaloids are usually present only in small quantity and are generally mixed with much organic matter, special care has to be exercised in isolating them. Full details are given in "*Poisons, Their Effects and Detection*," by A. and M. Wynter Blyth (London, Charles Griffin and Co., 1906). The Stas-Otto process consists in treating the finely minced material with strong alcohol in the proportion of 1,000 c.c. to each kilogram of material to be extracted. The alcohol is distilled off and the aqueous residue shaken with light petroleum to remove fat. Any alkaloid removed by the light petroleum is recovered by shaking this with dilute acid, and is returned to the aqueous extract. The latter is then evaporated to dryness and the

residue extracted with absolute alcohol, which dissolves all the alkaloid. The residue from this is then submitted to tests for identification. It is almost always impure and may give reactions with the usual alkaloidal precipitants although no alkaloid is present.

In Kippenberger's process (*Zeit. Anal. Chem.*, 1895, **34**, 294) the matter is digested with a solution of tannin (100 grm.) in glycerol (500 grm.) at 40° for two days. The extract is heated to 50° to coagulate proteins, cooled and filtered. It is next shaken with light petroleum to remove fat and the last traces of petroleum removed from the extract by heating on the water-bath. The fat-free extract is then acidified and shaken with chloroform, which removes colchicine, papaverine, narcotine, delphinine and aconitine.

The extract is next made alkaline with dilute solution of potassium hydroxide and again extracted with chloroform which removes brucine, strychnine, emetine, veratrine, narcotine, codeine. thebaine, atropine, sparteine and nicotine.

The extract may still contain narceine, morphine, and curarine. The first two can be isolated by passing carbon dioxide into the extract to convert the alkali hydroxide into carbonate and then extracting with a mixture of alcohol and chloroform. Finally, curarine can be extracted by agitation with a mixture of equal volumes of ether and chloroform. This process also provides for the isolation of certain glucosides and neutral principles such as strophanthin, picrotoxin, cantharidin, etc., which are toxic.

The alkaloidal residues obtained as described may then be examined in detail.

Kippenberger's process may be usefully combined with the Stas-Otto process by applying it to the final alcoholic extract obtained by the latter method.

The processes to be used in separating and identifying the alkaloidal constituents of the purified residues obtained in these various ways are identical with those given elsewhere in this volume, notably pages 183 to 185, and the succeeding special sections.

Estimation of "Total Alkaloids."—As a rule the estimation of the "total alkaloids" only of a plant product is attempted, though in a few cases more or less successful methods have been devised for the separation and estimation of the principal, as distinct from the secondary alkaloids. For examples of such methods see under opium (page 370), cinchona bark (page 510), strychnos seeds (page 461), etc.

Methods for the estimation of the total alkaloids in the principal drugs, etc., are described in the succeeding sections relating to these products, and it is only necessary here to refer to certain general methods that have been suggested as applicable to many plants.

The general process of extraction already described can be applied to the estimation of total alkaloids. For this purpose a quantity of the ground plant, large enough to yield an alkaloidal residue weighing from 0.2 to 0.4 grm. should be taken and completely extracted in a Soxhlet extraction apparatus, with a suitable solvent, selected as a result of the preliminary extraction experiments. In the case of estimations the extraction must be carried out completely and rapidly and it is often possible to use methods, which would be objectionable in carrying out extraction on a larger scale. Thus for the purposes of an estimation it is unimportant that an alkaloid may undergo isomeric change in contact with alkali or acid since the weight of alkaloid obtained will be the same. Consequently preference should be given to methods in which solvents such as light petroleum, ether or chloroform, which extract little more than the alkaloid, can be used as primary extracting liquids, since in that way preliminary purification of the extract by lead acetate, etc., can be avoided. It is often convenient, however, when material is scarce to use the residues obtained in the course of several estimations for further investigation and in such cases care must be taken that the processes used do not occasion change in the alkaloid. The residues obtained in estimation should not only be weighed but should if possible be titrated in order to avoid the possible inclusion of non-alkaloidal material. This titration is usually best carried out by dissolving the alkaloidal residue in a known quantity of standard acid and then titrating back with standard alkali. Care is needed in the selection of an indicator for the titration (see page 181).

A number of authors have proposed general methods for the estimation of alkaloids and of these the following may be referred to.

Keller's Process (*Schweiz. Woch. fur Chem. und Pharm.*, 1894, **32**, 44).—From 12 to 25 grm. of the finely powdered drug, dried over soda-lime or sulphuric acid, is placed in a flask with a measured quantity of ether or a mixture of ether and chloroform in the proportion of 3:1. Enough of the solvent should be used to cover the drug completely. The mixture is set aside for 5 to 10 minutes with occasional agitation. To it is added 10% ammonia solution in excess (usually 10 c c.) and the maceration continued with occasional careful agitation

during 30 minutes. At the end of that time a little water is added and the mixture shaken for 2 or 3 minutes until the drug runs together and the ether will separate, fairly free from particles. The ethereal solution is then run off into a second flask and allowed to stand until it is quite clear when an aliquot part, usually 50 to 100 c c., is measured into a separating funnel where it is shaken successively with 25, 15, and 10 c c. of 0.5 to 1.0% hydrochloric acid, the acid extract being run as completely as possible into a second separating funnel Excess of 10% ammonia solution is added to the acid liquid and the alkaloids, thus liberated, extracted by shaking with three successive portions of a mixture of equal parts of ether and chloroform or with ether alone. The whole of the ethereal liquid is run through a small filter-paper into a tared flask, the solvent distilled off and the residue dissolved once or twice in small quantities of dry ether and the latter evaporated to facilitate drying. Finally the residue is dried during 15 minutes at 100° and weighed. It is then dissolved in 5 to 10 c.c of dry alcohol and titrated with $N/20$ hydrochloric acid using hæmatoxylin as indicator.

Ekroos (*Arch. Pharm.*, 1898, **236**, 328) has employed a modification of Keller's process in which sodium hydroxide is used in place of ammonia for cinchona bark.

Linde (*Arch. Pharm.*, 1899, **237**, 392) has usefully summarised the objections to Keller's and the related processes thus:

1. It is assumed that the ethereal solvent extracts all the alkaloid or at least contains it in equal concentration within and without the drug.

2. The amount of ethereal solution used is assumed to contain an aliquot part of the whole of the alkaloidal matter present, no allowance being made for change in the volume of the solvent through solution of fat or soap formed by the action of ammonia on the fat.

3. The repeated agitations with (*a*) ethereal liquid and (*b*) acid are unnecessary and equally good results are obtained with smaller volumes of liquid and a single agitation, especially if the alkaloids are salted out by the addition of some brine.

4. It is better to measure the liquids than to weigh[1] them.

5. In Ekroos' process the use of sodium hydroxide solution in place of ammonia solution is objectionable since it would liberate any amines present and would form soaps with any oil or fat in the drug.

[1] In the above account of Keller's process the quantites of solvent to be used are given in c c in place of grms as Keller originally gave them

In addition to the foregoing a number of processes based on the use of alkaloidal precipitants have been suggested. Particulars of these are given on pages 185 to 197.

For the estimation of pure alkaloids in solutions or preparations of these, ordinary volumetric and gravimetric processes are available and are described in the sections relating to the particular alkaloids

Volumetric Estimation of Alkaloids.—Pure alkaloids may often be estimated volumetrically by titration with $N/10$ acid and it is sometimes convenient to ascertain the purity of an alkaloidal residue by titration, as a partial check on the accuracy of a process of estimation. Most alkaloids behave as monacidic bases even when they contain 2 atoms of nitrogen. Further, they show many anomalies in their behaviour with the usual indicators.

Phenolphthalein.—Comparatively few of the alkaloids show a normal alkaline reaction to this indicator in aqueous solution, the exceptions being atropine, homatropine, hysocyamine, scopolamine, conicine, conhydrine and sparteine, all of which according to Astruc (*Compt. Rend.*, 1901, **133**, 98) behave to it as monacidic bases. Ecgonine, benzoylecgonine, morphine and narceine in benzene solution, according to the same author, behave as monobasic acids to phenolphthaleïn and can be titrated with $N/10$ potassium hydroxide, though the results are only approximate in the cases of morphine and narceine. Kippenberger states that sparteine alone can be satisfactorily titrated in presence of phenolphthaleïn (*Zeit. Anal. Chem.*, 1900, **39**, 202, 290). Owing to this general neutrality of free alkaloids to phenolphthaleïn, it is possible in many cases to estimate the amount of acid in combination with alkaloids by titrating with $N/10$ alkali in presence of phenolphthalein. For this purpose the free acid should first be neutralised by the addition of $N/10$ sodium hydroxide solution using methyl-orange as indicator. The acid in combination with the alkaloid may then be ascertained by titration with $N/10$ alkali and phenolphthalein. This process is of course inapplicable in the case of alkaloids, which give an alkaline reaction with the latter indicator (see above). According to Runne (*Apoth. Zeit.*, 1909, **24**, 662, 1910, **25**, 137) the acid in quinine hydrochloride may be estimated in this way in aqueous solution, but with quinine sulphate, codeine phosphate or cocaine hydrochloride the titration is best conducted in alcohol or a mixture of alcohol and water, while the process is not suitable for morphine salts.

Methyl-orange.—Most of the salts of alkaloids with mineral acids are neutral to this indicator and consequently the alkaloids may be titrated directly with $N/10$ acid in its presence. The exceptions according to Astruc (*loc. cit.*) are caffeine, which is neutral to all the usual indicators, and aconitine (see below), veratrine, strychnine and brucine, which are only feebly basic to methyl-orange. Sparteine is diacidic to methyl-orange as are also quinine, cinchonine, cinchonidine and quinidine. Methyl-orange according to Kippenberger (*loc. cit.*) gives approximate results only with atropine, emetine and coniine. Allen has observed that in titrating an alkaloid with methyl-orange, it is rarely convenient to employ an aqueous solution of the base A solution of the alkaloid in proof or rectified spirit is generally suitable, and the indicator is fairly sensitive under such conditions. But when the alkaloid is much coloured, as is frequently the case in the assay of the bases directly extracted from their sources, it becomes difficult or impossible to observe the end of the action. Under such circumstances, Allen overcame the difficulty by dissolving the alkaloid in a little ether, and placing the solution in a small stoppered cylinder, together with a few c.c. of water, coloured with a drop of methyl-orange solution (1:1,000). On then gradually dropping in the standard acid and agitating thoroughly after each addition, it is easy to observe the end point, as the colouring-matter remains in the upper ethereal stratum, and presents a marked contrast to the red colour of the aqueous liquid. By operating in this manner and employing $N/50$ hydrochloric acid, Allen obtained perfectly satisfactory estimations of aconitine, etc., even when working on so little as 0.030 grm.

Lacmoid.—This substance has been used by van Itallie (*Analyst*, 1889, **14**, 118) for the titration of atropine, hyoscyamine and coniine by $N/10$ hydrochloric acid and by Messner for the mixed alkaloidal residue obtained from cinchona extract (*Zeit Angew. Chem.*, 1903, **16**, 444). Kippenberger (*Zeit. Anal. Chem.*, 1900, **39**, 202, 290) states that it gives good results with atropine, cocaine, morphine, codeine, papaverine, narcotine, nicotine, coniine and veratrine when these are dissolved in standard sulphuric acid and titrated back with $N/50$ alkali.

Rosolic Acid.—This has been used for alkaloidal titrations by Dieterich (*Pharm. J.*, 1887 [*iii*], **17**, 888) but appears to be of doubtful value, though according to Astruc (*loc. cit.*) conicine, conhydrine, sparteine, atropine and the related alkaloids, the cinchona alkaloids,

and morphine, codeine, and thebaine behave as monacidic bases in its presence.

Iodesin.—According to Kippenberger (*loc. cit.*) this gives good results with thebaine, codeine, emetine and coniine (compare Linde, *Arch. Pharm.*, 1900, **238**, 102 and Tapis, *ibid.*, 1902, **240**, 390). It has been used with good results in estimating nicotine (*q. v.*). It is also used in the assay processes of the *United States Pharmacopœia;* Eighth Revision, for estimating the alkaloids of nux vomica and physostigma and as an alternative indicator for the coca and pilocarpine alkaloids.

Hæmatoxylin. This indicator is used in three important assay processes.

Properties of the Alkaloids.—The most characteristic property of the alkaloids as a class is their marked physiological action and in this connection it is sufficient to recall the fact that a large proportion of the crude drugs of vegetable origin recognised in the pharmacopœias owe their medicinal value to the alkaloids they contain. A considerable amount of work has been done in attempting to correlate the molecular structure of the alkaloids with their physiological action but up to the present no useful generalisations have been made in this subject. In some cases it seems that the change from a substance, which is relatively inert in a physiological sense to one of great physiological activity is conditioned by quite minor changes in constitution, *e. g*, the change from tropine to atropine, and aconine to aconitine implies merely the introduction of acyl and alkyl groups. Most of the alkaloids are bitter to the taste, strychnine and the cinchona alkaloids being notable examples of this. In addition, certain of the alkaloids have a more marked action on the nerve endings in the tongue and, as in the case of cocaine, produce a numbing effect, and in those of the aconitine group, a pronounced tingling sensation. These characteristic actions are useful tests for these alkaloids but they must be applied with great care. Certain of the alkaloids exert a marked action on the nerves, which contract or dilate the pupil of the eye and this action is occasionally employed as a test for their presence. Such tests are, however, best regarded as merely confirmatory of evidence obtained by chemical reactions. The test is best made by placing a drop of the alkaloidal solution to be examined, as nearly neutral as possible, on the eye of a rabbit, dog or cat, when, in a time varying from a few minutes to about half an hour, a marked

contraction or dilation of the pupil will be observed. The principal alkaloids causing such action are as follows:

A. The pupil is *dilated* by:

1. Atropine, hyoscyamine and scopolamine, and preparations of belladonna, henbane and stramonium; solanine; and extracts from Solanaceous plants generally.
2. Cocaine and preparations of coca.
3. Coniine and preparations of hemlock and other umbelliferous plants.
4. Cytisine and laburnum extracts.
5. Gelsemine and preparations of gelsemium.
6. Sparteine and preparations of broom.
7. Certain ptomaines.

Mydriasis, or dilation of the pupil, is so striking a characteristic of atropine and the associated bases that these are often grouped together as the "mydriatic alkaloids." The mydriasis is only observed in the eye to which the alkaloid is applied.

B. The pupil is *contracted* by:

1. Physostigmine (eserine) and preparations of the Calabar bean.
2. Pilocarpine and preparations of jaborandi.
3. Muscarine, neurine.

A similar effect on the pupil is produced by the poisons when taken internally or hypodermically in sufficient quantities. Sometimes, as in the case of morphine and preparations of opium, the pupils are contracted during the early stages of the poisoning, but dilated subsequently, especially after death. Nicotine and preparations of tobacco in some cases cause contraction, and in others dilation, of the pupil. In poisoning with aconitine alternate contraction and dilation of the pupil is sometimes observed.

Most of the alkaloids are crystalline solids at ordinary temperatures but a few have only been obtained in an amorphous condition, *e. g.*, pilocarpine, while others, especially those free from oxygen such as coniine, nicotine, and sparteine, are liquid and volatile.

Some of the solid crystalline alkaloids may be sublimed, *e. g.*, caffeine, and many of them yield characteristic sublimates, which may be used as a means of recognising them (compare Winter Blyth, "*Poisons and Their Detection*," 1909.

Alkaloids are usually sparingly soluble in water and may be pre-

cipitated from solutions of their salts in water by the addition of alkalies, but some alkaloids, *e. g.* morphine, re-dissolve in excess of the stronger alkalies, such as sodium or potassium hydroxide. Most of them are soluble in alcohol, chloroform or amyl alcohol, many are soluble in ether or benzene, and a few are soluble in light petroleum.

The salts of alkaloids are generally soluble in water or alcohol; but a few are insoluble in water and have been used for the estimation of the respective alkaloids, *e. g.*, quinidine hydriodide, cinchonidine hydrogen tartrate; those of the less basic alkaloids are hydrolysed by excess of water and consequently the free alkaloid may often be extracted in such cases by shaking an acid solution of the alkaloid with an immiscible solvent (compare Hill, *Pharm. J.*, 1900 [*iv*], **10**, 185; Schaer, *ibid.*, p. 308; Kippenberger, *Zeit. Anal. Chem.*, 1900, **39**, 202, 290; Proelss, *Apoth. Zeit.*, 1901, **16**, 434; Springer, *ibid.*, 1902, **17** 225, Simmer, *Arch. Pharm.*, 1906, **244**, 672).

Most alkaloids are colourless, berberine and sanguinarine being two notable exceptions.

The alkaloids are generally optically active, most of them being lævorotatory. In some cases the salts show an optical rotation opposite in kind to that of the free base (*e. g.*, nicotine and aconitine).

REACTIONS OF THE ALKALOIDS.

General Precipitants.—Alkaloids as a class give precipitates with a considerable number of reagents, especially with compounds of some of the heavy metals. The three precipitants of most general applicability are a solution of iodine in potassium iodide, a solution of phosphomolybdic acid, and a solution of the double iodide of mercury and potassium, but no one of these will precipitate every alkaloid. With the exception of tannin, which should be applied in a neutral or faintly alkaline solution, the precipitants should usually be added to a solution of the base slightly acidified with sulphuric or acetic acid, but in some cases (as in the precipitation of certain picrates) the solution should be distinctly acidified with sulphuric acid. As tests for the presence of alkaloids, a drop or two of the selected precipitant may be added to a few drops of the liquid under examination, contained in a watch-glass.

Picric Acid, $C_6H_2(NO_2)_3.OH$ [*Hager's Reagent*] (*Zeit. Anal. Chim.*, 1870, **9**, 110; 1881, **20**, 415).—When used as a test for alkaloids, picric

acid is best employed in saturated, cold, aqueous solution. The alkaloidal solution should be rendered distinctly acid with dilute sulphuric acid, except in cases where the alkaloidal picrate is only thrown down in neutral solution. The precipitated picrates have usually a pale yellow colour, and are generally crystalline or become so on standing, the forms in many cases being characteristic.

Picric acid produces no precipitate in 0.02% solutions (acidified with sulphuric acid) of caffeine, coniine, morphine or theobromine; and aconitine, atropine, nicotine, and veratrine are precipitated in fairly concentrated solutions only. Atropine and morphine are precipitated from tolerably concentrated neutral solutions. Copious precipitates are produced by picric acid in acidified solutions of berberine, delphinine, emetine, the cinchona alkaloids, opium alkaloids (except morphine and pseudomorphine), strychnine and brucine. Picric acid is especially suitable for the precipitation of the cinchona alkaloids and Hager has devised a process of assaying cinchona bark based on that fact (see page 489). The following are the limits of dilution at which precipitation occurs, and the characters of the precipitates, according to T. G. Wormley:

Alkaloid	Character of precipitate	Limit of precipitation
Nicotine .	Amorphous, changing to crystalline tufts, soluble in nicotine	1 40,000
Conine . . .	Amorphous, or liquid globules becoming crystalline, soluble in coniine and acetic acid	1 500
Morphine . .	Amorphous	1 500
Codeine	Amorphous	1 2,000
Narceine	Amorphous, soluble in acetic acid	1 5,000
Strychnine .	Amorphous, quickly assuming characteristic crystalline forms	1 20,000
Brucine	Amorphous, becoming crystalline	1 10,000
Aconitine .	Amorphous, insoluble in ammonia	1 5,000
Atropine.. .	Amorphous, changing to characteristic crystalline forms, soluble in weak acids, including acetic	1 1,000
Veratrine	Amorphous, soluble in weak acids, including acetic	1 · 5,000
Jervine .	Amorphous	1 1,000
Solanine ...	Gelatinous, soluble in excess of picric acid solution	1 : 1,000
Gelsemine . . .	Amorphous	1 · 500

The alkaloids may be recovered from their picrates by mixing the moist precipitate with sodium carbonate, drying the mixture, and extracting with alcohol; or the picrate may be shaken with ammonia and a suitable immiscible solvent.

Picrolonic acid (*4-nitro-1-p-nitrophenyl-3-methyl-5-pyrazolone*) has been found useful as a reagent for alkaloids, since it yields characteristic crystalline picrolonates with coniine (m. p. 195.5°), nicotine (m. p. 213°), strychnine (m. p. 275°), brucine (m. p. 256°), morphine (m. p. 186.5°), codeine (m. p. 219°), atropine (m. p. 194°), quinine (m. p. 225°) and hydrastine (m. p. 220°). They consist usually of 1 mol. of base combined with 1 mol. of acid, but quinine combines with 2 mol. of the acid. No compounds with aconitine, cocaine, or caffeine have been obtained. The reagent is best applied in alcoholic solution (Warren and Weiss, *J. Biol. Chem.*, 1907, **3**, 327). It does not seem well suited for the estimation of alkaloids (compare Matthes and Ramstedt, *Arch. Pharm.*, 1907, **245**, 112; *Zeit. Anal. Chem.*, 1907, **46**, 565).

Tannic acid precipitates the great majority of the vegetable alkaloids. The precipitates are usually soluble in weak acid or ammonia solution.

The tannates of aconitine, brucine, caffeine, colchicine, morphine, physostigmine (eserine), and veratrine are dissolved by dilute acetic acid and quinine tannate by somewhat stronger acid. The tannates of aconitine, berberine, brucine, caffeine, cinchonine, colchicine, narcotine, papaverine, thebaine, solanine, strychnine, and veratrine resist more or less perfectly the action of cold dilute hydrochloric acid. The tannates of aconitine, physostigmine (eserine), quinine, solanine, and veratrine are not dissolved by cold dilute sulphuric acid. Aconitine, physostigmine (eserine), and veratrine are completely precipitated by tannic acid from solutions strongly acidified by sulphuric acid, but only partially from slightly acidified solutions.

An alkaloid may be recovered from its tannate by mixing the moist precipitate with freshly precipitated lead carbonate or hydroxide, drying the mixture, and boiling it with alcohol or other suitable solvent, which, on evaporation, will often leave the alkaloid in a characteristic crystalline form.

Phosphomolybdic Acid (Sonnenschein's Reagent).—This is one of the most useful general reagents for alkaloids and is also used for separating them from foreign matter. It is prepared by acidifying

a warm solution of ordinary sodium phosphate with nitric acid, and adding an excess of ammonium molybdate solution. The yellow precipitate is separated, washed with water, acidified with nitric acid, and dissolved in a hot solution of sodium carbonate. The solution is evaporated to dryness and ignited at a low red heat till all ammonium salts are volatilised, the residue moistened with nitric acid, and again ignited. The product, consisting of sodium phosphomolybdate is dissolved in 10 times its weight of a mixture of 1 vol. of strong nitric acid (sp. gr. 1.42) with 9 vol. of water.

Sonnenschein's reagent gives yellow, usually amorphous precipitates with nearly all alkaloids, and as most of the precipitates are very insoluble, a negative reaction with the reagent affords in many cases positive proof of the absence of alkaloids; but, on the other hand, ammonium salts and other non-alkaloidal substances are also precipitated by Sonnenschein's reagent. The phosphomolybdates are decomposed by ammonia, in some cases with the production of a white precipitate of the liberated alkaloid, which can usually be dissolved by agitation with a suitable solvent, but when the alkaloid is readily oxidisable, treatment of the phosphomolybdate with ammonia is attended with the formation of a blue or green colouration indicating reduced molybdic acid. This occurs in the cases of aconitine, atropine, berberine, codeine, colchicine, coniine, morphine, nicotine, physostigmine (eserine), etc. When this occurs the alkaloid is best recovered by mixing the moist precipitate with potassium or sodium carbonate, and extracting rapidly with strong alcohol.

Phosphotungstic acid, Scheibler's reagent, is used in a similar manner to Sonnenschein's reagent and gives similar reactions with alkaloids. It is prepared by dissolving 100 parts of sodium tungstate and 60 to 80 parts of sodium phosphate in 500 parts of water, and adding nitric acid till the reaction is acid; or ordinary sodium tungstate may be digested with half its weight of phosphoric acid of 1.13 sp gr. and allowed to stand for some days when phosphotungstic acid will separate in crystals. Scheibler's reagent precipitates 1:200,000 solution of strychnine and 1:100,000 solution of quinine. The alkaloids may be recovered from their phosphotungstates in the same manner as from their phosphomolybdates (see above).

Metatungstic acid, silicotungstic acid [R. Godeffroy], and **Phosphoantimonic acid** [Schultze] have been proposed as precipi-

tants for alkaloids, but the advantages claimed for them have not led to their general adoption.

Bromine dissolved to saturation in strong *hydrobromic acid* has been recommended as a general reagent for alkaloids by T. G. Wormley. It is probable that hydrochloric acid might be substituted for the hydrobromic acid, without detriment to its efficacy. *Wormley's reagent* produces yellow amorphous precipitates in solutions of many alkaloids, and crystalline precipitates with atropine, hyoscyamine, and veratrine, the microscopic appearance of the precipitate being in each case characteristic. C. L. Bloxam (*Chem. News*, 1883, **47**, 215) has pointed out that certain of the alkaloids give characteristic colour reactions when bromine water is added drop by drop, to their solutions in dilute hydrochloric acid. Thus brucine is stated to yield a violet colour, and strychnine the same on boiling; narcotine a rose pink, and the same with quinine, changed in the latter case to the characteristic grass-green colour on adding ammonia. With excess of bromine, strychnine, brucine and narcotine readily give yellow precipitates; while quinine, morphine and cinchonine are only precipitated with difficulty or from strong solutions.

Iodine, dissolved in a solution of potassium iodide [*Wagner's reagent*], yields reddish or red-brown precipitates with nearly all the alkaloids, even in very dilute solutions. The precipitates are formed more readily in solutions slightly acidified with sulphuric acid. The quantity of the reagent used should be insufficient to colour the solution yellow. Precipitation is so general and occurs in such dilute solutions, that a negative reaction is almost conclusive proof of the *absence* of ordinary alkaloids, though precipitation is not conclusive proof of the *presence* of an alkaloid. The precipitates from aqueous solutions are usually amorphous, though codeine, narceine and strychnine are exceptions. In alcoholic solutions the precipitates are sometimes not formed, or are deposited very slowly; but when produced they are often of different character from those yielded in aqueous solutions, and in some cases are crystalline. The precipitates are mostly periodides of the alkaloids, the formulæ in some cases being very complex. Thus with quinine there is first a formation of BHI,I; with more of the reagent BHI,I_4 is obtained; while in alcoholic solution, in presence of free sulphuric acid and with an excess of the reagent, the curious quinine iodosulphate or herepathite $B_4,3H_2SO_4,2HI,I_4,3H_2O$ is produced. The periodides of the alkaloids have been studied ex-

tensively by Prescott and Gordin (*J. Amer. Chem. Soc.*, 1898, **20**, 706; 1899, **21**, 231; *Arch. Pharm.*, 1899, **237**, 380, 439), who have based on their formation a general method of estimating alkaloids depending on the addition of the alkaloidal solution to a known volume of standard iodine, and the titration of the excess of iodine left in solution, after complete precipitation of the alkaloid periodide. For a further development of the method see Gordin (*Pharm. Archives*, 1899, **2**, 313; *Arch. Pharm.*, 1900, **238**, 335; 1901, **239**, 214, 645) but compare Kippenberger (*Zeit. Anal. Chem.*, 1903, **42**, 101). The alkaloids may be recovered from their periodides by treating the precipitate with sulphurous acid, a sulphite and dilute sulphuric acid, or sodium thiosulphate, and then adding an alkali and shaking with a suitable immiscible solvent. Treatment with sodium thiosulphate, avoiding excess, is a convenient means of purifying the periodides from adhering foreign matter. The reduced solution is filtered and again treated with Wagner's reagent, when the periodide is obtained in a condition of purity.

The strength of Wagner's reagent may vary within wide limits, but ordinary $N/10$ solution of iodine is suitable for general use.

Cadmium potassium iodide [*Marmé's reagent*], employed in solutions acidified with sulphuric acid, gives with alkaloids precipitates which are at first amorphous, but which often become crystalline on standing. They are soluble in alcohol, and in excess of the cadmium solution.

Bismuth potassium iodide [Dragendorff's reagent], is best made by mixing 16 volumes of the *British Pharmacopœia* solution of bismuth citrate with 1 of strong hydrochloric acid (sp. gr. 1.16), and adding potassium iodide equal in weight to the hydrochloric acid used (J. C. Thresh). The resulting liquid has an orange colour, and when added to solutions of alkaloids, strongly acidified with sulphuric acid, forms orange-red precipitates, which appear to be, in most cases, wholly insoluble in cold water. The following are the limits of delicacy according to Thresh (*Pharm. Journ.*, 1880 [*iii*], **10**, 641, 809): Strychnine, 1 in 250,000, quinine 1 in 200,000; quinidine 1 in 150,000. cinchonidine 1 in 125,000, narcotine 1 in 50,000; brucine and aconitine 1 in 40,000; atropine 1 in 25,000; morphine and narceine, 1 in 20,000; codeine 1 in 17,500; apomorphine 1 in 12,500; berberine 1 in 6,000; caffeine 1 in 3,000. (See also F. Mangini *Gazetta*, 1882, **12**, 155). Thoms (*Ber. Deut. Pharm. Ges.*, 1905, **15**, 85) has recently used this

reagent for the isolation of alkaloids as part of a general method of estimation, the alkaloids being regenerated by the action of sodium carbonate and hydroxide, extracted with ether and titrated with $N/100$ hydrochloric acid in presence of iodeosin.

Potassium mercuric iodide [Mayer's reagent] is prepared by dissolving 6.775 grm. of dry crystallised mercuric chloride and 25 grm. of potassium iodide separately in water, mixing the solutions so obtained, and diluting the mixture to 1,000 c.c. The solution thus obtained is $N/20$ and of convenient strength for general use, though of only one-half the strength originally proposed by F. F. Mayer (*Chem. News*, 1863, **7**, 159). A. B. Prescott has pointed out (*Chem. News*, 1882, **45**, 114, 123) that the proportions of mercuric chloride and potassium iodide used in making Mayer's solution correspond to $HgCl_2+6KI$, which might be supposed to react to form $2KI,HgI_2+2KI+2KCl$, but the reactions of the solution point rather to the formula $KI,HgI_2+3KI+2KCl$. Nevertheless the proportion of potassium iodide cannot be greatly reduced without precipitation of mercuric iodide; but a permanent solution can be obtained with mercuric chloride, potassium iodide, and potassium bromide, used in the proportion indicated by the formula $HgCl_2+4KI+KBr$. Mayer's reagent precipitates the great majority of alkaloids, and in some cases from very dilute solutions. Applied, as it always should be to solutions rendered distinctly acid by hydrochloric or sulphuric acid, ammonia does not interfere, but the solution to be tested must not be more than slightly alcoholic, and must not contain acetic acid. The precipitates yielded by alkaloids with the reagent are usually yellowish-white in colour, and curdy or flocculent. They are more or less soluble in alcohol, ether, acetic acid, iodides, and sometimes in an excess of the reagent. Certain other organic matters besides alkaloids are also precipitated by Mayer's reagent, which therefore loses much of its value when applied to unpurified solutions.

It is occasionally employed as a means of making an approximate volumetric estimation of the alkaloid present in a solution; but unfortunately the composition of many of the precipitates obtained with it varies to a serious extent with the concentration of the solution, the proportion of acid present, and the excess of the reagent.

With strychnine, the composition of the precipitate produced by Mayer's reagent approximates to BHI,HgI_2; with morphine it appears to be a variable mixture of $B(HI)_4,(HgI_2)_3$ and $B(HI)_6,(HgI_2)_3$;

while with quinine the precipitate is not far from the composition $B_2(HI)_3,(HgI_2)_3$. These formulæ refute the statement made by Mayer and reproduced by various writers, that the precipitates are of definite composition, containing either 1, 2, or 3 molecules of the base. It has been proved by Lyons that the precipitates nearly always contain a smaller proportion of mercury (often less than three-fourths) than has been assumed to be present in them. The subject has also been investigated by Prescott (*Chem. News*, 1882, **45**, 114, 123).

If Mayer's reagent is added until precipitation ceases, there will always be a large excess of the reagent present. This excess bears a relation to the dilution of the liquid, and the more dilute the solution, the larger the volume of Mayer's solution requisite to effect complete precipitation. Hence, in order to render titration with Mayer's reagent of any value, it is essential that the solutions operated on shall be nearly of uniform concentration, and that the reagent be added in exactly the same manner. It is further desirable, whenever possible, to make an experiment, side by side with the alkaloidal solution, with a known weight of the same alkaloid in a state of purity, to avoid any assumption as to the behaviour of the volumetric solution with the alkaloid in question.

The following is the usual method of performing the titration of an alkaloid with Mayer's reagent: The solution, which should be distinctly acid and contain, as a rule, o 5% of the alkaloid, is treated with the reagent as long as a distinct precipitate is produced. As there is no definite end-point and no satisfactory indicator has been as yet devised,[1] it is necessary to filter a portion of the solution to ascertain if the precipitation is complete. A minute filter, about half an inch in diameter, supported on a ring of platinum wire, may be used. A drop or two of the filtered liquid[2] is placed on black glass, or on ordinary glass or black paper, and a drop of the volumetric solution added from the burette, when the faintest turbidity will be readily perceived. Before the end of the titration all the trial-filters and test drops are returned to the solution containing the main quantity of the precipitate.

[1] Mayer proposed to ascertain the excess of the reagent by titrating back with standard silver nitrate solution, without filtering, using potassium chromate as an indicator As pointed out by Lupinski, the suggestion ignores the accumulation of chlorides and iodides in the solution, as also the fact that some of the precipitates react but slowly with silver nitrate Recently Heikel has suggested (*Chem Zeit*, 1908, **32**, 1149, 1162, 1186, 1212) that titration with Mayer's reagent may be much improved by adding the reagent in excess to the alkaloidal solution, titrating the excess by adding a known excess of potassium cyanide and estimating the unused cyanide by titration with standard silver nitrate, in presence of ammonia

[2] A convenient form of filter-tube for the purpose has been described by Bird (*Pharm. Jour.*, 1887 [*iii*], **17**, 826)

The end of the action is the point at which the reagent ceases to produce a precipitate, and it is worthy of notice that, before this point is reached, a condition of equilibrium is attained, in which the solution is liable to be precipitated by the addition of either alkaloidal solution or the mercury reagent.

Lyons has investigated the behaviour of various alkaloids with Mayer's solution, noting the effect of concentration and the volume of the reagent required to precipitate completely a definite weight of alkaloid; in addition, the volume required to produce an *apparent* excess of the mercury reagent (so that the liquid would give a precipitate with more of the alkaloidal solution); and also the actual excess of Mayer's solution used, as estimated from the quantity of mercury present in the solution.

Lyon's results are given in the following table, reproduced from his *Manual of Pharmaceutical Assaying*. The mercurial solution was $N/20$, and 0.1 grm. of alkaloid was employed in each case:

From a study of this table by Lyons, it appears that while a notable excess of the reagent is generally needed to effect complete precipitation, the weight of the precipitate is in many cases considerably below the amount indicated by theory. Better results in this respect are obtainable by allowing the liquid with the suspended precipitate to stand for some time. Lyons states that, under these circumstances, the atropine precipitate becomes dense and crystalline and in part adheres to the beaker, in which it can be washed by decantation, dried, and weighed, the amount thus found falling little short of the theoretical weight of 0.245 grm. for 0.100 of alkaloid.

Alkaloid	Solution		Volume of reagent in c c			Weight of alkaloid precipitated by 1 c c of reagent	Weight of fresh precipitate after drying at 100°
	Condition	Concentration	For apparent excess	For complete precipitation	Used in excess		
Aconitine	...	1 200		7 1	2 0	.0141	.180–.190
Atropine	.....	1 200	7 0	13 1	3 0	.0077	216– 220
Atropine	..	1 400	6 0	14 0	3.5	0072	
Atropine	...	1 600	6 0	15 0	3 6	0067	192– 200
Berberine	.. .	1 200		3 8		0263	
Berberine	....	1 400		3 9		0257	
Berberine	.	1 600		4 6		0218	200– 215
Brucine	Nearly neutral	1 200		8 0	1 7	0125	
Brucine	Nearly neutral	1 400		8 8		0114	
Brucine	Acid	1 400		9 8		0102	
Brucine	Nearly neutral	1 600		9 2		0109	
Cinchonidine		1 100	12 4	13 8	1 0	0073	.
Cinchonidine		1 200	12 4	13 5	0 7	0074	330– 375
Cinchonidine	.	1 200		15 6	2 6	.0064	
Cinchonine	.	1 100	.	12 8	0 8	0078	.
Cinchonine	..	1 100		14 0	1 2	0072	
Cinchonine	Neutral	1 200	7 9	10 8		0093	333– 345
Cinchonine	Acid	1 200	8 0	14 2		0071	
Cinchonine	Neutral	1 400	8 0	12 4	2 4	0082	
Cinchonine	Acid	1 400	9 6	14–18		007 to 0086	
Cocaine	. .	1 200		12 8		0078	246
Cocaine	. .	1 400	10 0	14 4	4 6	0069	
Cocaine	.	1 600		16 0	5 2	0063	
Colchicine		1 200	3 2	9 2		0109	160
Colchicine		1 400	4 2	11 4		0088	
Colchicine	..	1 600	5 0	12 6		0080	
Colchicine	..	1 800	4 0	14 6		0067	
Emetine		1 200	8 0	9 4	0 4	0106	256
Emetine	.	1 400	8 8	10 2	1 0	0098	
Emetine		1 600		10 6	0 6	0094	
Gelsemine		1 200	5 8	10 4		0096	185– 200
Gelsemine	.	1 400	6 5	12 0		0084	
Hydrastine	..	1 200		7 4		0135	200– 210
Hydrastine		1 400		8 0		0125	
Hydrastine		1 600		8 4		0119	
Hyoscyamine		1 200		8 5		0116	220– 250
Morphine		1 200	7 0	4 91		0128	190– 240
Morphine		1 400		8 9	0 6	0110	
Pilocarpine	.	1 200	4 8	16 8		0060	240– 350
Pilocarpine	.	1 200		20 0		0050	
Quinine	Neutral	1 200	11 6	16 4		0061	
Quinine	Acid	1 200	12 4	18 0		0056	310– 335
Quinine		1 400	12 8	16 8		0060	
Quinine		1 600	12 2	20 0		0050	
Strychnine		1 200		11 0	0 6	0091	260– 275
Strychnine	Neutral	1 400	11 6	12 0		0084	
Strychnine	Acid	1 400	11 6	12 2		0082	
Strychnine		1 600	11 2	11 9	0 6	0087	

The following data showing the behaviour of alkaloids with Mayer's reagent are tabulated from the descriptions of Dragendorff (*Plant Analysis* and *Analyse Chimique de quelques Drogues Actives*):

Alkaloid	Dilution of solution	Milligrams of alkaloid pptd by 1 c c	Correction for solubility Mgrms for 10 c c filtrate	Observer	Conditions of precipitation
Aconitine		13 45	0 5	Dragendorff	.
Pseudaconitine		19 4		Dragendorff	
Atropine	1 200	4 85		Dragendorff	} Ample time required
Atropine	1 330	4 14	0 5	Dragendorff	} for precipitation
Hyoscyamine	1 200	3 49		Dragendorff	.
Emetine		9 45		Dragendorff	.
Conine	1 200	6 25		Dragendorff	} Faintly acid only
Conine		2 10		Mayer	} KCl present
Nicotine		2 02		Dragendorff	} Sol strongly acidified
Strychnine		8 35		Dragendorff	. .
Strychnine		8 30		Mayer	} Sol faintly acid only
Brucine		9 85		Dragendorff	
Brucine		11 65		Mayer	} Sol strongly acid
Colchicine	1 600	15 85		Dragendorff	
Morphine		10 00		Dragendorff	.
Narcotine		10 65		Dragendorff	.
Veratrine		14 80	0 7	Masing	} Slightly acid solution
Veratrine		13 50		Mayer	
Physostigmine (eserine)		6 87	1 0	Masing	.
Berberine		21 25		Beach .	.
Chelidonine		8 37		Masing	.
Sanguinarine		7 42		Masing	
Quinine		5 40			
Cinchonine		5 10			.

Hereth (*Pharm. Record*, 1886, p. 209) has proposed an improved method of operating with Mayer's reagent, which allows ample time for the precipitate to form. A number of equal portions of the solution to be tested are treated with the mercurial solution in volumes increasing regularly by 0.1 c.c., and allowed to stand 8 or 10 hours. Trial-portions of each mixture are then removed and tested with 2 drops of Mayer's solution, when a particular mixture will be found to have the mercurial solution in slight excess, while in the previous mixture it is deficient. The true amount lies between the two, and it is easy to ascertain the exact volume required.

Gordin has pointed out that although the composition of alkaloidal precipitates with Mayer's reagent varies, yet the amount of hydriodic acid in relation to the amount of alkaloid in the precipitate remains constant and has based on this observation a method for the estimation of alkaloids volumetrically. The alkaloid is dissolved in a known quantity of standard hydrochloric acid and precipitated completely by Mayer's reagent (or iodine solution, see page 189). The acid remaining in solution is then estimated. The relation between alkaloid and acid carried down being known from a preliminary estimation

made with the pure alkaloid, the quantity of acid carried down and consequently the quantity of alkaloid in the precipitate can be calculated (*Ber.*, 1899, **32**, 2871, and *Arch. Pharm.*, 1900, **238**, 335; 1901, **239**, 214, 645) but compare Kippenberger (*Zeit. Anal. Chem.*, 1903, **42**, 101).

Strychnine, brucine, and quinine are among the alkaloids yielding the least soluble precipitates with Mayer's solution. With atropine, coniine, nicotine, and morphine the reaction is far less delicate, and solanine, colchicine, caffeine and theobromine are not precipitated at all.

Mercuric chloride, $HgCl_2$ gives, with certain alkaloids, precipitates of which the crystalline form or m. p. is characteristic. As a rule the precipitates have the composition represented by the formula B. HCl, $HgCl_2$, though the atropine compound has the formula B.HCl,2$HgCl_2$, and are less insoluble than those produced by Mayer's reagent.

Auric chloride, $AuCl_3$, gives yellow precipitates of alkaloidal aurichlorides with hydrochloric acid solutions of many of the alkaloids. They are generally represented by the formula B HCl,$AuCl_3$. Compounds of the type B.$AuCl_3$ are also known and others such as aurochlorcaffeine, in which the group $AuCl_2$ replaces 1 hydrogen atom in the alkaloid (Dunstan and Shepheard, *Trans. Chem. Soc.*, 1893, **63**, 201, and Dunstan and Harrison, *ibid.*, 443). Auric chloride has the advantage that ammonium salts are not precipitated by it; but the precipitates are unstable, the yellow colour in many cases rapidly changing to reddish-brown, while the supernatant liquid occasionally acquires an intense red colour, especially if the alkaloidal solution is impure. The aurichlorides of the Solanaceous alkaloids are particularly useful for the separation and identification of members of this group.

Platinic chloride, $PtCl_4$, is a useful reagent for many alkaloids, with the hydrochlorides of which it combines to form platinichlorides. In some instances these double salts have the formula B,H_2PtCl_6 and in other cases B_2,H_2PtCl_6. Some of the cinchona bases form platinum salts of both series.

The platinichlorides of the alkaloids vary in colour from pale yellow, through orange and red, to brownish-red. They are mostly sparingly soluble in water, and hence are usually formed as precipitates on adding platinic chloride to a solution of the alkaloid acidified with hydrochloric acid. Xanthine, caffeine, colchicine, and pelletierine are among the alkaloids not precipitated. Of the rest the platinichlorides

of quinine, cinchonine, morphine and strychnine are among those dissolved by hydrochloric acid. The m. p. of the alkaloidal platinichlorides are often characteristic.

Potassium permanganate, $KMnO_4$, produces characteristic reactions with certain of the alkaloids. Beckurts and List have examined the behaviour of a number of them, by adding a solution of the reagent, drop by drop, to a cold saturated aqueous solution of the hydrochloride of the base. Immediate reduction of the permanganate, with separation of brown manganese di-oxide, was observed with the hydrochlorides of quinine, cinchonidine, cinchonine, cinchonamine, brucine, veratrine, colchicine, coniine, nicotine, physostigmine (eserine), codeine and thebaine. The solutions of atropine, hyoscyamine, pilocarpine, berberine, piperine, and strychnine were coloured red, the reagent being only gradually reduced.

With morphine hydrochloride the permanganate produced a white crystalline precipitate of oxydimorphine, which, when filtered off and dried, could be recognised by its characteristic reactions. Apomorphine hydrochloride immediately reduced the reagent, with production of an intense green colour.

On adding a few drops of $N/10$ solution of potassium permanganate to a concentrated solution of narceine hydrochloride a reddish precipitate is immediately formed, which is very stable in the cold and in the absence of an excess of the reagent, but is decomposed on heating or by addition of more permanganate. Solutions of papaverine hydrochloride, and of narcotine, if diluted with hydrochloric acid, at first behave similarly, but the precipitates are much less stable than narceine permanganate, and soon discolour and decompose with separation of manganese di-oxide.

Geisel (*Pharm. Zeit.*, 1886, **31**, 132) has pointed out that cocaine gives a comparatively stable permanganate, which forms a purple-violet precipitate of characteristic microscopic appearance The precipitate forms only slowly in dilute solutions, and undergoes gradual decomposition. Dunstan and Carr (*Pharm. Journ*, 1896 [*iv*], **2**, 121) have shown that aconitine yields a characteristic, crystalline unstable permanganate, which may be used as a test for this alkaloid.

Colour Tests.

Many of the alkaloids give brilliant and in some cases character-

statements made in the literature regarding the colours afforded by alkaloids with such reagents vary widely; thus it is not uncommon to find one author asserting that a particular alkaloid yields a blue colour with some reagent, while a second author asserts that it remains colourless in contact with the same reagent. For this reason the reaction should be compared with that yielded by the pure alkaloid treated side by side with the specimen under investigation. The reagents, which have been proposed as colour-tests for alkaloids are very numerous, and have not always been chosen or applied with discretion, nor with a due regard to purity. The colour reactions may be classified as (1) those produced by dehydrating agents, such as strong sulphuric acid, phosphoric acid, and zinc chloride,[1] (2) those given by oxidising agents not of themselves yielding colours, such as nitric acid, chlorine, bromine or bleaching powder, or sulphuric acid and oxidising agents such as potassium chlorate, perchlorate, or permanganate; (3) those given by oxidising agents, which themselves yield a coloured product by reduction, such as iodic acid and reagents containing chromic, molybdic, tungstic or vanadic acids; and (4) colourations produced by certain special reagents, such as ferric chloride, hydrochloric acid, and sulphuric acid with sugar (see Morphine, page 366).

As a rule the best method of observing the colour indication of an alkaloid is to apply a drop of the reagent by means of a glass rod to a minute fragment of the solid alkaloid placed on a porcelain plate or in a flat porcelain dish. An alkaloidal residue obtained by careful evaporation in a porcelain capsule of an alcoholic, ethereal, chloroform or other solution may be very conveniently employed for observing colour indications.

Concentrated hydrochloric acid gives colour indications with a few alkaloids. Thus, *reddish* colours are yielded with physostigmine (eserine), veratrine (cevadine), protoveratridine (on warming), veratridine (on warming), and veratroidine, and a *yellow* with thebaine. On addition of chlorine water after hydrochloric acid, berberine gives a red colour Nicotine yields an amorphous hydrochloride and coniine a crystalline salt on evaporating the solution in hydrochloric acid.

[1] In using zinc chloride, Czumpelitz directs that the substance to be examined should be first carefully dried, moistened with a solution of 1 grm of fused zinc chloride in 30 c c of water, and dried again If thus treated, strychnine takes a scarlet colour, thebaine a yellow, narceine an olive-green, delphinine a red-brown, berberine a yellow, veratrine a red and quinine a pale yellow The presence of brucine prevents the colouration of strychnine, the tinge produced being a dirty yellow. (*Giornale Farm. Chim*, 1881)

Concentrated sulphuric acid gives colour indications with a number of alkaloids, the colouration varying with the degree of heat applied. The following reactions have been observed when the acid is dropped on the solid alkaloid, without applying heat: No colour, or a faint straw tint only, is yielded by pure aconitine, atropine, caffeine, chelidonine, cinchonidine, cocaine, codeine (violet on warming), hyoscine (scopolamine), hyoscyamine, gelsemine, morphine (purple to brown on warming), nicotine, pilocarpine (blue, Reichard, *Pharm. Centr- H*, 1907, **48**, 417), quinine, quinidine, staphisagrine, brucine, strychnine, physostigmine (eserine), and theobromine. *Yellowish* colourations are given by colchicine, gnoscopine, jervine, and by many other alkaloids in presence of impurities. *Reddish* colours are produced either immediately or gradually with impure aconitine, apomorphine, cocaine (impure), coniine (pale red?), gelsemine (impure), meconidine, narceine (changing to black), narcotine (yellowish-red changing to violet and blue), physostigmine, rhoeadine, veratridine, protoveratridine, veratrine, veratroidine, solanine (violet), taxine and thebaine. *Bluish* colourations are yielded by cryptopine, curarine (after a time), and papaverine (rose to violet on heating) (Hesse, *J. pr. Chem.*, 1903 [*ii*], **68**, 190). *Greenish* colours are given by beberine, berberine, emetine (brownish to green), piperine, pseudomorphine, protoveratrine (green to blue or violet), jervine, and sometimes by rhoeadine.

Some characteristic changes of colour can be obtained by gradually warming the capsule in which the test is being made, by placing it over a small beaker of boiling water. The ultimate result is usually browning and charring of the alkaloid, but the intermediate indications are often of value.

Many substances besides alkaloids give more or less brilliant colour changes with strong sulphuric acid. Thus *red* colourations (often of a brilliant hue) are obtained with amygdalin, columbin, cubebin, elaterin, hesperidin, phloridzin, populin, salicin, sarsaparillin, senegin, smilacin, syringin, and many tannins.

In applying sulphuric acid as a colour test for alkaloids it must be remembered that the presence of a very minute quantity of nitric acid, often present as an impurity, greatly modifies the colourations produced by many of the alkaloids. Thus, if the treatment with sulphuric acid (without applying heat) be followed by the addition of a very minute quantity of nitric acid (at the end of a glass rod drawn out

to a point), or a minute fragment of solid potassium nitrate, the following results will be obtained:[1]

No colour with atropine, caffeine, cinchonidine, cinchonine, nicotine, pilocarpine, quinidine, quinine, staphisagrine, strychnine, or theobromine; *red* colouration with brucine, curarine, narcotine (reddish-violet or blood-red), physostigmine (eserine), cytisine (orange-yellow), thebaine, and veratrine (gradual change to cherry-red). Special and peculiar changes of colour are produced with this test by morphine, codeine, and colchicine, and by atropine (on further addition of sodium hydroxide), and are described in the respective sections on these alkaloids.

Strong nitric acid, of 1.40 to 1.42 sp. gr., gives more or less characteristic colour indications with a number of alkaloids. A drop of this acid should be applied by means of a glass rod to a minute fragment of the alkaloid, or to a residue left on evaporating a solution on white porcelain. No colouration is yielded by aconitine (when pure), atropine (the residue on evaporation is coloured violet by alcoholic sodium hydroxide solution), cytisine, caffeine, cinchonidine, cinchonine, coniine, gelsemine (impure, greenish), quinidine, quinine, strychnine, nicotine or theobromine. *Yellowish* colours are obtained with impure aconitine (colour varies from yellow to red and brown), codeine (orange-yellow), morphine (yellow to red), narceine, narcotine, papaverine (orange), piperine (orange), rhoeadine, thebaine, and veratrine. *Red* shades are produced by impure aconitine (colour varies from yellow to red and brown), apomorphine, beberine (red to red-brown), berberine (red-brown), brucine (blood-red), papaverine (orange-red), pseudomorphine (orange-red), curarine and physostigmine (eserine). Gelsemine yields a deep bluish-green residue on evaporation. *Blue* colours are said to be given by colchicine and solanine (pink, Cazeneuve and Breteau, *Compt. Rend*, 1899, **128**, 887).

Sulphomolybdic Acid [Fröhde's Reagent].—This is one of the most useful of the oxidation tests for alkaloids but it must be borne in mind that the colours produced are those of lower oxides of molybdenum, and that various other substances besides alkaloids readily reduce molybdic acid with the formation of these coloured oxides. The reagent itself, if strongly heated, acquires a blue colouration. It

[1] Erdmann applies this test by mixing 6 drops of nitric acid of 1 25 sp. gr. with 100 c.c. of water, and adding 10 drops of the dilute acid so obtained to 20 grm. of sulphuric acid. From 8 to 10 drops of the solution so prepared, or *Erdmann's Reagent*, is added to 1 or 2 mg. of the solid to be tested, and the colour observed after 20 or 30 minutes

is prepared by dissolving 5 mg. of molybdic acid or ammonium molybdate in 1 c c. of strong sulphuric acid. No colouration is obtained with cinchonidine, cinchonine (blue, Reichard, *Pharm. Zeit.*, 1905, **50**, 314), coniine, delphinine, scopolamine, hyoscyamine, atropine, nicotine, strychnine, caffeine, or theobromine. *Yellowish* colourations are given by aconitine, colchicine, and piperine. *Reddish* shades of colour are produced by brucine, narceine (red, changing to blue), solanine, thebaine (orange), and veratrine (gradual production of a cherry-red colour). *Bluish* colours are given by codeine (gradual production of deep blue), morphine (violet-blue, then dirty green, changing to deep blue), narceine (yellowish-brown, changing to red and blue), papaverine (bluish violet), staphisagrine (violet-brown). *Greenish* colourations are produced by apomorphine (green to violet), beberine (brown-green), berberine (brown-green), emetine (red, changing to green, and turned blue by hydrochloric acid), quinine (pale green), and quinidine (pale green).

Ferric chloride gives a few characteristic colourations, the most important being the blue indication with morphine, bluish-green with the mixed ipecacuanha alkaloids (Allen and Scott-Smith, *Analyst*, 1902, **27**, 345), and the garnet-red colouration with colchicine. A freshly made mixture of ferric chloride and potassium ferricyanide (free from ferrocyanide), in aqueous solution, has a yellowish-brown colour; but in presence of certain alkaloids it is immediately coloured blue (or green) owing to the production of Prussian blue. This reaction was at first regarded as characteristic of the ptomaines, but it is produced by any readily oxidisable alkaloid, and hence is given immediately by morphine, aconitine, physostigmine (eserine), etc., and after a short time by hyoscyamine, emetine, colchicine, nicotine, and many of the tar-bases.

Oxidation colour changes are also produced by reagents having a more powerful oxidising action than the foregoing. Thus strong sulphuric acid may be employed in conjunction with potassium nitrate, chlorate, perchlorate, permanganate, dichromate, and ferricyanide, or with metallic peroxides, such as those of manganese (MnO_2), lead (PbO_2), ruthenium (RuO_3), uranium (U_2O_3), and cerium (Ce_3O_4). The most important of the colour indications obtained with such reagents are those given by strychnine, atropine, pereirine, quebrachine, curarine, and gelsemine (*q. v.*).

Reactions of the Alkaloids with Acids.

Many of the alkaloids are strongly alkaline and most of them form well-defined salts but in other cases the basicity is feeble and no salts are formed with acetic and other weak acids and the salts with the stronger acids are dissociated in presence of water. Very few of the alkaloids form carbonates and consequently the alkali carbonates or alkali hydrogen carbonates (bi-carbonates) may generally be used to liberate them from aqueous solutions of their salts. Most of the alkaloids, even those containing more than 1 atom of nitrogen, behave as monacidic bases and form only one series of salts with acids, thus B HCl. Quinine and the cinchona alkaloids generally are, however, notable exceptions and behave as diacidic bases (compare Veley, *Trans. Chem. Soc.*, 1908, **93**, 2114, 1909, **95**, 1 and 758). This subject of the basicities of the alkaloids is of great importance in connection with their volumetric estimation and has been dealt with already (page 181).

In preparing salts of the alkaloids cold dilute solutions of the mineral acids only are used and when the concentrated acids are applied or when the alkaloids are heated, especially under pressure, with even dilute solutions of the mineral acids, much more profound changes occur. The concentrated acids generally give rise to characteristic colour changes, which are often employed as a means of identifying the alkaloids (see page 198). Apart from the more or less complete decomposition which generally accompanies these colour changes the chemical changes induced by mineral acids on alkaloids are of three types.

1. Dehydration.—This occurs with alkaloids containing at least 2 hydroxyl groups and results in the formation of the anhydro- or *apo*-alkaloids, typical cases being the production of *apo*-morphine from morphine and *apo*-atropine from atropine, represented by the following equations:

$$\underset{\text{Morphine}}{C_{17}H_{19}O_3N} = \underset{\textit{Apo}\text{-morphine}}{C_{17}H_{17}O_2N} + H_2O$$

$$\underset{\text{Atropine}}{C_{17}H_{2}\ O_3N} = \underset{\textit{Apo}\text{-atropine}}{C_{17}H_{21}O_2N} + H_2O$$

2. Elimination of Methoxyl Groups.—A considerable number of the alkaloids contain methoxyl groups, *e. g.*, codeine, quinine, narcotine, and papaverine. These alkaloids when heated with concentrated hydrochloric or hydriodic acid produces methyl chloride or methyl

iodide and leave as a residue a hydroxy-base Thus the alkaloid narcotine may be regarded as the trimethyl-derivative of *nor*-narcotine, the latter being the ultimate product of the action of hydriodic acid on narcotine, thus.

$$\underset{\text{Narcotine}}{C_{19}H_{14}O_4N(OMe)_3} + 3HI = \underset{\text{Nor-narcotine}}{C_{19}H_{14}O_4N(OH)_3} + 3MeI.$$

The quantity of methyl iodide eliminated in such reactions may be estimated by Zeisel's method (Perkin, *Trans Chem Soc.*, 1903, **83**, 1367). Methyl groups attached to nitrogen are also eliminated as methyl iodide by the action of heat on the alkaloidal hydriodides and a development of Zeisel's method has been applied to the estimation of such groups (Herzig and Meyer, *Ber.*, 1894, **27**, 319).

3. Hydrolysis.—Many of the natural alkaloids have the constitution of esters and when heated with alkalies or mineral acids undergo hydrolysis yielding acids and alcohols or phenols A few are at once alkaloids and glucosides, and on hydrolysis by acids yield a sugar and secondary alkaloids.

Typical actions of this kind are the following:

$$\underset{\text{Atropine}}{C_{17}H_{23}O_3N} + H_2O = \underset{\text{Tropine}}{C_8H_{15}ON} + \underset{\text{Tropic Acid}}{C_9H_{10}O_3}.$$

$$\underset{\text{Cocaine}}{C_{17}H_{21}O_4N} + 2H_2O = \underset{\text{Ecgonine}}{C_9H_{15}O_3N} + \underset{\text{Benzoic acid}}{C_7H_6O_2} + \underset{\text{Methyl alcohol}}{CH_3OH}.$$

The decomposition of narcotine by the action of dilute acids, water or alkalies at 140° may also be regarded as of this class though this alkaloid is not strictly of the ester type.

$$\underset{\text{Narcotine}}{C_{22}H_{23}O_7N} + H_2O = \underset{\text{Opianic acid}}{C_{10}H_{10}O_5} + \underset{\text{Hydrocotarnine}}{C_{12}H_{15}ON_3}$$

With Alkalies.

Dilute alkalies decompose alkaloidal salts and liberate the free alkaloids. In a few cases where the latter contain phenolic hydroxyl groups, derivatives are formed with sodium or potassium hydroxide and consequently such alkaloids dissolve in excess of the alkali. This occurs with morphine and codeine.

The action of hot dilute alkalies on the alkaloids is much the same as that of hot dilute acids and leads, for example, to the hydrolysis of the ester alkaloids. Thus the hydrolysis of atropine, cocaine or narcotine referred to above may be equally well brought about by the action of dilute alkalies, or in some cases by the action of water alone. The action of alkali hydroxides on hyoscyamine in alcohol changes

this alkaloid into its racemic isomeride, atropine. The action of fused alkali hydroxides on alkaloids in some cases gives rise to colour changes similar to those produced by strong acids. The heating of alkaloids with dry alkali hydroxides leads generally to profound decomposition and the production of simple products such as pyridine or quinoline.

With Methyl Iodide.

The majority of the alkaloids are tertiary amines and, as such, combine with 1 molecule of methyl iodide forming the alkaloidal methiodides which are quaternary ammonium iodides and are occasionally characteristic. The chief interest of these derivatives is that they form the starting-point of a series of decompositions, which have thrown considerable light on the structure of certain alkaloids. For this series of reactions the name "exhaustive methylation" has been coined and its importance in alkaloidal chemistry may be conveniently illustrated by the following series of reactions in the tropine group.

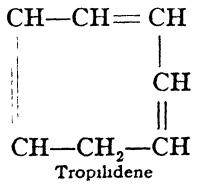
$C_7H_{12}ON.Me$ (Tropine) —by loss of water→ $C_7H_{10}N.Me$ (Tropidine) —by addition of methyl iodide→

$C_7H_{10}NMe_2I$ (Tropidine methiodide) —by action of moist silver oxide→ $C_7H_{10}NMe_2OH$ (Methyltropidine ammonium hydroxide) —by action of heat→

$C_7H_9NMe_2$ (Methyltropidine) —by addition of methyl iodide→ $C_7H_9NMe_3I$ (Methiodide) —by action of moist silver oxide→

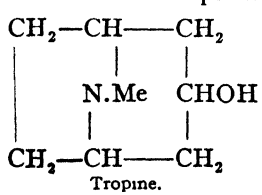
$C_7H_9NMe_3OH$ (Methyltropidine methyl ammonium hydroxide) —by action of heat→ C_7H_8 (Tropilidene) + NMe_3 (Trimethylamine) + H_2O (Water)

Tropilidene has been shown by Willstatter (*Ber.* 1901, **34**, 129) to have the formula



Tropilidene

This series of reactions is best accounted for on the assumption that tropine is represented by the formula



Tropine.

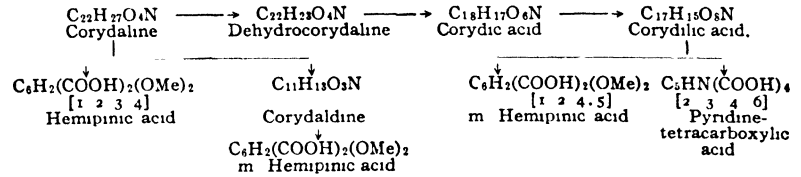
and from this the formula of atropine is readily derived, by the replacement of the hydrogen of the hydroxyl group by a tropic acid radical.

This process of "exhaustive methylation" has been employed in the examination of a number of alkaloidal substances and is one of the most useful methods of investigation for this group.

A few of the alkaloids are secondary amines and these react with methyl iodide to form tertiary amines, so that with these bases "exhaustive methylation" starts a stage lower down. The reaction was first investigated by Hofmann and by Ladenburg for the secondary base piperidine (*Ber.*, 1881, **14**, 494, 659, 1346; 1882, **15**, 1024; 1883, **16**, 2057).

Behaviour with Oxidising Agents.

The study of the oxidation of alkaloids has in a number of cases thrown light on their constitution particularly in the group of *iso*-quinoline alkaloids including berberine, narcotine, hydrastine, papaverine, narceine, corydaline, etc. As an example of the importance of this method of investigation, reference may be made to the work of Dobbie and Lauder on corydaline (*Trans Chem Soc*, 1902, **81**, 145). By oxidation with dilute nitric acid and permanganate this base yields the following series of derivatives:



The ultimate products of oxidation therefore indicate the presence in the molecule of corydaline of 2 benzene rings and 1 pyridine nucleus, and it is mainly on the basis of the study of the oxidation products that the formula proposed by Dobbie and Lauder (*loc. cit.*) for corydaline is founded (compare Haars, *Arch. Pharm.*, 1905, **243**, 165). Similarly interesting results were obtained by Goldschmiedt in the study of the oxidation products of papaverine and by Ladenburg, Merling and others in the oxidation of tropine. Certain of the alkaloids in the *iso*-quinoline group under the action of oxidising agents undergo a kind of hydrolysis into base and acid, thus when narcotine is treated with dilute nitric acid, it decomposes into cotarnine and opianic acid according to the equation (cf. page 203)

$$C_{22}H_{23}NO_7 + H_2O + O = C_{10}H_{10}O_5 + C_{12}H_{15}NO_4$$

Narcotine. Opianic acid. Cotarnine.

Behaviour with Other Reagents.

The foregoing paragraphs give a brief resumé of the chief reagents which have so far proved most useful in the investigation of this group of compounds. In addition many of the alkaloids yield characteristic derivatives on reduction by the usual reagents, such as sodium amalgam, tin and hydrochloric acid, etc. When distilled with zinc dust, bases of the pyridine and quinoline series are usually obtained, depending on the nuclear structure of the alkaloid distilled.

A number of the alkaloids contain hydroxyl groups and furnish esters when treated with acid anhydrides or chlorides. Hygrine is an example of an alkaloid containing a carbonyl group and ketonic compounds such as tropinone, cotarnone, etc., are not uncommon among the oxidation products of the alkaloids. These substances yield derivatives with the usual reagents for carbonyl groups, such as hydroxylamine, phenylhydrazine, etc.

CLASSIFICATION OF THE ALKALOIDS.

Much attention has been devoted in recent years to the investigation of alkaloids and considerable progress has been made in ascertaining the constitution of the more important members of the group. On the basis of this work some of the principal alkaloids may be classified according to their nuclear structure as follows:

Pyridine Group.—Coniine and the other alkaloids of hemlock, piperine, nicotine, and the other alkaloids of tobacco, arecoline and the other alkaloids of areca nut.

Pyrrolidine Group.—Atropine, hyoscyamine and the Solanaceous alkaloids, cocaine and the other coca alkaloids, pelletierine and the other alkaloids of pomegranate root bark. Sparteine.

Quinoline Group.—Quinine, cinchonine and the other cinchona alkaloids, strychnine and brucine.

iso-**Quinoline Group.**—Papaverine, narcotine, hydrastine, narceine, berberine, corydaline.

Phenanthrene Group.—Morphine, codeine, thebaine.

Purine Group.—Caffeine, theobromine.

Glyoxaline Group.—Pilocarpine.

Amino-acid Group.—Asparagine, leucine.

Choline Group.—Choline, muscarine, betaine, sinapine.

Alkaloidal Glucosides.—Solanine.

Alkaloids of Unknown Constitution.—Aconitine, veratrine, yohimbine.

VOLATILE BASES OF VEGETABLE ORIGIN.

By FRANK O. TAYLOR.

Certain plants contain bases which differ from the ordinary vegetable alkaloids, in being volatile, liquid at ordinary or only slightly raised temperatures, and in containing no oxygen. While resembling each other in the above respects, the volatile bases present little further resemblance.

The volatile alkaloids are not numerous, being limited to the following substances, and a few others which have been but imperfectly investigated.

a. *Arecoline* and other alkaloids of areca nuts
b. *Coniine* and the associated alkaloids of hemlock.
c. *Hygrine*, the volatile alkaloids of coca.
d. *Lobeline*, the alkaloid of lobelia.
e. *Lupinined*, other alkaloid of lupines.
f. *Nicotine*, the volatile alkaloid of tobacco.
g. *Pelletierine* and other alkaloids of the pomegranate tree.
h. *Piturine*, the volatile alkaloid of pituri.
i. *Sparteine*, the volatile alkaloid of broom.
j. *Spigeline*, an alkaloid in *Spigelia Marylandica*

Piperidine, a volatile alkaloid said to exist naturally in pepper as a decomposition product of piperine, has already been described. (See page 141.)

For the *estimation* of volatile alkaloids (*e. g*, coniine in hemlock, and nicotine in tobacco), A. Loesch (*Jour. Amer Chem. Soc.*) recommends that a weighed quantity of the substance should be boiled in water acidified with hydrochloric acid, the residue pressed and washed with water. The solution and washings are evaporated to one-fourth, and then distilled with slaked lime (using a good condenser). When the liquid passing over is no longer alkaline to litmus, the distillation is

exactly neutralised with sulphuric acid, evaporated to dryness at 100°, and the powdered residue exhausted with alcohol, which leaves the ammonium sulphate undissolved, while the sulphate of conine (and other alkaloids) pass into the solution. The filtered liquid is evaporated to dryness and the residue shaken three times with potassium hydroxide solution and ether, the ethereal liquid separated and shaken with a known volume of standard sulphuric acid, the ether distilled off or separated, and the excess of sulphuric acid determined by titration. By this process, Loesch found 5.25% of nicotine in tobacco leaves, and 0.06% of coniine in the common hemlock plant.

This distillation method is not, however, to be specially recommended, as some of the more commonly used methods of alkaloidal extraction by volatile solvents are usually to be preferred.

ARECA OR BETEL-NUT ALKALOIDS.

The Areca palm is indigenous to the Sunda Islands but is cultivated in the warmer parts of India and the Philippines, and is an article of commerce. Extensive use is made of it in the Far East as a masticatory together with lime and the leaves of the betel pepper. According to von Bibra, betel chewers number upward of 100,000,000. The nut is used in India and China as a vermifuge and has from there been introduced into the European and American Materia Medica.

From the seed of *Areca catechu*, Jahns (*Ber.*, 1888, **21**, 3404) has isolated four alkaloids, the principal one, Arecoline, being volatile.

Arecoline, $C_8H_{13}O_2N$, is a colourless, odourless, oily liquid, strongly alkaline in reaction, and soluble in all proportions in water, alcohol, ether and chloroform. It boils at about 220° (Jahns) or at 209° (Pictet) and is volatile with steam. In chemical character and physiological action it resembles *pelletierine*, it being a valuable tænicide. On hydrolysis it yields methyl alcohol and *arecaidine* and is therefore *methyl-arecaidine.*

For the preparation of arecoline, macerate the powdered drug with cold milk of lime in successive portions till exhausted; filter, exactly neutralise with sulphuric acid and evaporate nearly to dryness; take up with a little water and filter off the calcium sulphate, again concentrate to a thin syrup, make alkaline and extract with ether. It may now be purified by extracting from the ether with dilute sulphuric acid, again rendering alkaline after concentration, taking up with ether, converting into hydrobromide and recrystallising this salt from

absolute alcohol. The yield is about 0.07 to 0.1%. The residual aqueous solution from which the first ether extract was made may now be acidified with sulphuric acid and the other alkaloids precipitated with bismuth-potassium iodide. For details of their separation and purification see the original reference to the *Berichte*, on page 208.

Another method of preparation is by extracting the drug three times with cold water containing 2 grm. concentrated sulphuric acid per kilo of drug. Concentrate the acid solution to a weight about equal to the drug used; add bismuth-potassium iodide, being careful not to add excess, in which the alkaloidal precipitate is soluble, and let stand several days. Filter off, wash, and boil with barium carbonate and water; filter, concentrate to thin syrup, mix with barium oxide and extract arecoline with ether. From the residue the other alkaloids may now be obtained.

Arecoline hydrobromide, $C_8H_{13}O_2NHBr$, occurs in colourless, needle-like prisms, permanent in air, melting at 167–168°, readily soluble in water, in hot—but less freely in cold—alcohol, but difficultly soluble in ether and chloroform. This salt is official in the *German Pharmacopœia*.

The hydrochloride forms slender, deliquescent needles, melting at 157–158°, readily soluble in water, alcohol and a mixture of alcohol and ether. It seems to form several double salts with cadmium chloride.

The sulphate, nitrate and acetate resemble the hydrochloride.

The aurichloride, $C_8H_{13}O_2N$, $HAuCl_4$, is a yellow oil, sparingly soluble in cold water.

A platinichloride, $(C_8H_{13}O_2N)_2$, H_2PtCl_6, is precipitated from mixed alcoholic solutions of arecoline hydrochloride and platinum chloride by addition of ether. Forms rhombic crystals from water, melting with decomposition at 176°.

The most delicate reaction of arecoline is the formation of a pomegranate red precipitate with bismuth-potassium iodide, in the form of microscopic crystals. Phosphomolybdic acid produces a white precipitate. Potassium-mercuric iodide throws down from not too dilute solutions, a yellow, oily precipitate which very slightly crystallises. Iodine produces brown drops and picric acid a resinous precipitate, both of which finally become crystalline. Gold chloride produces an oily precipitate which does not crystallise. Platinic chloride, mercuric chloride and tannic acid give no precipitate.

Dr. Marmé (*Pharm. Zeit.*, 1889, **34**, 97) investigated the physio-

logical action of arecoline and found that is caused a slowing of the heart action, tended to produce a cessation of respiration, and in large doses caused tetanic cramps which quickly gave place to partial paralysis. Its most notable action in small doses is an increased peristaltic action of the bowels.

Arecoline separates unaltered from the secretions and excretions, from which it can be recovered. It can best be identified by its behaviour with bismuth-potassium iodide and its physiological action upon the heart of a frog. Its chief medicinal value lies in its vermicide and tænicidal properties.

Froehner states that the dosage of arecoline should be about 0.1 grm. for horses and 0.25 grm. for oxen.

Homarecoline or ethyl-arecaidine, $C_9H_{15}O_2N$, may be produced by substituting the ethyl group for the methyl. The product closely resembles arecoline and is poisonous.

Arecaine, $C_7H_{11}O_2N.H_2O$, occurs in colourless, non-hygroscopic crystals which lose their water of crystallisation at 100° and melt with decomposition at 213°. It is freely soluble in water, giving a solution neutral in reaction, and also in dilute alcohol; almost insoluble in absolute alcohol, by which it is dehydrated, and practically insoluble in ether, chloroform and benzol. Arecaine forms a crystalline *hydrochloride,* $C_7H_{11}O_2N$, HCl, and other crystalline salts soluble in water and alcohol, and of acid reaction; an *aurichloride,* $C_7H_{11}O_2N$, $HAuCl_4$, melting at 186–187°, and a *platinichloride,* $(C_7H_{11}O_2N)_2, H_2PtCl_6$, melting at 213–214° with decomposition, both of which are crystalline.

For method of preparation see *Ber.*, 1888, **21,** 3404. The yield is about 0.1%.

In chemical properties arecaine is related to *trigonelline* and resembles it in being a betaine-like substance.

Arecaine is *n-methylguvacine* and was obtained by Jahrs by treating *guvacine* dissolved in methyl alcohol with sodium and subsequently heating to 140–150° with potassium methyl sulphate. It seems to have very little physiological action as compared with arecoline.

Its solution in dilute sulphuric acid gives the following reactions: With bismuth-potassium iodide, an amorphous, red precipitate which becomes crystalline; with potassium-mercuric iodide, a yellow, crystalline precipitate; with potassium iodide, a dark crystalline precipitate in the acid but not in a neutral solution. Phosphomolybdic and tannic acids give a slight turbidity, while picric acid gives no precipitate.

Arecaidine, $C_7H_{11}O_2N,H_2O$, isomeric with arecaine, is present in very small quantity in areca nuts and may be prepared by saponifying arecoline. (See *Jahr's, Ber.*, 1890, **23,** 2972.) From 60% alcohol it crystallises in colourless quadratic or hexagonal plates, permanent in the air but which lose their 1 mol. of water of crystallisation at 100° and melt with decomposition at 222–224°. Arecaidine is readily soluble in water and dilute alcohol, slightly soluble in absolute alcohol and almost insoluble in ether, chloroform and benzene.

Arecaidine forms crystallisable salts and is precipitated by auric and platinic chlorides, forming an *aurichloride* melting at 197–198° and a *platinichloride* melting at 208–209°.

If dry hydrochloric acid is passed into a suspension of finely powdered arecaidine in methyl alcohol till the latter is saturated, arecoline is formed and this reaction may be used for separating arecaine and arecaidine, as the hydrochloride of the former is produced.

Jahns' researches indicate that arecaidine is methyl-tetrahydronicotinic acid By reason of the presence of a carboxyl group it has a weakly acid character and is said to form salts with both acids and bases.

Guvacine, $C_6H_9ON_2$, is so called from Guvaca, the Indian name for areca palm. (See *Jahr's, Ber.*, 1891, **24,** 2615.) It crystallises in lustrous scales, neutral in reaction, which melt at 271–272° with decomposition. It is soluble in water and dilute alcohol but less readily than arecaine or arecaidine; insoluble in absolute alcohol, ether, chloroform and benzene. Its salts are crystalline, of acid reaction, and are soluble similarly to the base. The *hydrochloride* forms broad, flat prisms, anhydrous, soluble in water but sparingly soluble in dilute hydrochloric acid; the *nitrate* occurs in lustrous prisms and the *sulphate* as silvery scales. The *aurichloride* crystallises in flat prisms melting at about 194–195° and the *platinichloride* forms hexagonal prisms containing 4 H_2O, m. p. with decomposition about 211°.

Coniine.[1] Conia Conicine.

$$C_8H_{17}N,C_5H_{10}(C_3H_7)N, \text{ or } CH_2\left\{\begin{matrix}CH_2\ CH(C_3H_7)\\CH_2\ CH_2\end{matrix}\right\}NH.$$

[1] Coniine has been prepared synthetically by the reducing action of sodium on a boiling alcoholic solution of allyl-pyridine, $C_5H_4(C_3H_5)N$, itself obtained from α-picoline and paraldehyde The artificial base thus prepared is identical in all its properties with the natural alkaloid, except that it is optically inactive But on introducing a crystal of the bitartrate of the natural alkaloid into a very concentrated solution of the bitartrate of the inactive bases, a gradual separation of the bitartrate of active coniine occurs, the free base from which exhibits the same optical activity as natural coniine The mother-liquid contains a levorotatory isomeric base (Ladenburg, *Ber*, **19,** 2578)

This base, which was the first alkaloid to be synthesised, has the constitution of an α-propylpiperdine

Coniine is the characteristic poisonous alkaloid of hemlock, *Conium maculatum.* It occurs in all parts of the plant, in combination with organic acids, and in association with the following allied bases:

Base	Formula	M p	B p	Sp gr
Ethyl-piperidine	$C_7H_{15}N$ or $C_5H_9(C_2H_5)NH$		142–145°	$\left\{\begin{matrix}0\\-\\x\end{matrix}\right\}=0$ 8674
Coniine (normal-propyl-piperidine)	$C_8H_{17}N$, or $C_5H_9(C_3H_7)NH$	−2 5°	166°	$\left\{\begin{matrix}0\\-\\x\end{matrix}\right\}=0$ 8625
γ-coniceine	$C_8H_{15}N$, or $C_5H_7(C_3H_7)NH$		171–172°	
Methyl-coniine	$C_9H_{19}N$, or $C_5H_9(C_3H_7)$-$N(CH_3)$			$\left\{\begin{matrix}12\ 5\\-\\x\end{matrix}\right\}=0$ 846
Conhydrine	$C_8H_{17}ON$, or $C_5H_9(CHOH$ - $CH_2\ CH_3)NH$	120 6°	240° (225° at 720 mm)	
Pseudo-conhydrine	$C_8H_{17}ON$, or $C_5H_9(CH_3$ - $CH_2OH\ CH)NH$	100–102°	229°–231°	

Coniine is an oily liquid, having a peculiar repulsive odour, suggesting that of a long-used and foul tobacco-pipe. When diluted with water, coniine has a peculiar and characteristic "mousy" odour, perceptible in highly dilute solutions. A few drops of an aqueous solution containing only 1–50,000 of the alkaloid, if enclosed for a short time in a small test-tube, is stated by Wormley to impart a marked mousy odour to the contained air.

Vitali and Stroppa state that this odour is perceptible on warming a 1–100,000 solution, while Dilling considered the odour faint in even a 1–100 dilution.

The b. p. of coniine is variously stated, but is about 166° at normal pressure and is given by Dilling as 163.5° at 739 mm. It may be distilled unchanged in an atmosphere of hydrogen but undergoes slight decomposition at high temperatures in the air. It distils readily with vapour of water or alcohol and volatilises sensibly at ordinary temperatures. A 1% solution of the acetate or tartrate may be evaporated without loss and its other salts are likewise stable. Its sp. rot. is $[\alpha]_D = +16.4°$ at 19°, but its rotatory power is much less in solution in water, alcohol or benzol than when undiluted

Coniine forms an unstable compound with 25% of water, the water being expelled on heating. It is soluble in 50 parts of water and readily soluble in alcohol, ether, ethyl antate, chloroform, petroleum ether, acetone, benzol and amyl alcohol. It is removed with tolerable facility from aqueous or alkaline solutions by all of these solvents which are not miscible with water, and according to Vitali and Stroppa is removed to a slight extent from a faintly acid solution by ether. Melzer found that conine reacts with carbon disulphide and the resulting solution on evaporation gives colourless needle-like crystals of conine coniylthio-carbamic acid.

Coniine dissolves sulphur, but not phosphorus nor calcium chloride.

Coniine is colourless when freshly prepared, but becomes yellow and ultimately resinoid by keeping.[1] It is a strong base, the aqueous solution being powerfully alkaline in reaction, and neutralising acids perfectly. The salts are colourless and odourless, but the peculiar odour of the free base is immediately developed on adding a fixed alkali in excess.

If a beaker moistened with fuming hydrochloric acid be inverted over a watch-glass containing a drop of free coniine, white fumes will be produced, and the alkaloid will be converted after a time into a *crystalline hydrochloride*, $C_8H_{17}N,HCl$. (Nicotine gives an amorphous hydrochloride.) The hydrochloride is also obtained as a brilliant crystalline mass by dissolving conine in anhydrous ether, and passing dry hydrochloric acid gas through the solution. It can be heated to 90° without decomposition or loss of weight. It melts at 218° and is dextrorotatory.

According to Dilling it is soluble at 15° in 4 parts water, 5.2 parts absolute alcohol, 6.6 parts chloroform, 500 parts ether, 500 parts ethyl acetate, 333 parts acetone and insoluble in petroleum ether. The first 3 solvents are much more effective when warm.

Coniine hydriodide is anhydrous. It can only be obtained crystalline by the use of pure hydriodic acid free from any trace of iodine. By slow evaporation the salt is obtainable in large flat needles, which sublime when gently heated *in vacuo*. It melts at 165°.

Coniine also forms a *hydrobromide*, m. p. 211°; a *picrate* in small yellow prisms, m. p. 75°; an *aurichloride* in golden-yellow crystals, m. p. 77°, and a *cadmio-iodide*, m. p. 118°.

On exposing a drop of coniine to the vapours of bromine (avoiding

[1] According to Schorm, pure coniine does not undergo any change by exposure to light (*Pharm. Jour* [iii], 12, 363).

excess), it becomes rapidly converted into a mass of white crystals. This behaviour is regarded by Watts as a proof of the purity of the alkaloid.

By the treatment of coniine with chromic acid mixture, normal butyric acid is produced. The reaction may be employed as a test for coniine, as butyric acid has a highly characteristic odour, and can be readily distilled off and further examined. Butyric acid also results from the oxidation of coniine by bromine water or nitric acid, while permanganate converts it into picolinic acid.

On distillation of coniine hydrochloride with zinc-dust, or the free base with zinc chloride, hydrogen is evolved and α-propylpyridine or conyrine, $C_5H_4(C_3H_7)N$, formed. This base boils at 166–168°, and is reconverted into conine on treatment with hydriodic acid. (By prolonged treatment with hydriodic acid coniine is converted into ammonia and octane, C_8H_{18}.)

The reactions for the detection and identification of coniine are taken up in connection with those of the other conium alkaloids.

Conhydrine has the probable constitution of a piperidyl-ethylalkine, $C_5H_9(CHOH.CH_2.CH_3)NH$. It presents a close resemblance to tropine, $C_8H_{15}ON$, both in composition and chemical behaviour, a fact which suggested to A. W. Hofmann the probability that it was the product of the hydrolysis of a base allied to atropine. From the alkaline liquid left after the distillation of coniine and conhydrine, Hofmann obtained, by acidification and extraction with ether, caffeic acid, $C_9H_8O_4$, a substance having the constitution of a dihydroxy-cinnamic acid.

Conhydrine forms colourless, glittering, double refracting crystals, soluble at 15° in water, 1 in 25 6 parts; in absolute alcohol, 1 in 5.8 parts; in ether, 1 in 66 parts, and in chloroform, 1 in 5.2 parts. Its odour resembles conine and it has a salty taste. It melts at 118°, may be sublimed, and distils without decomposition at 225–226°. It is a secondary base and dextrorotatory. It does not react with nitrous acid, has an alkaline reaction, and is a feeble narcotic poison. According to Wertheim, hemlock contains only 5 to 6 parts of conhydrine for every 100 of coniine.

Conhydrine may be separated from commercial coniine, in which it is not unfrequently present, by cooling the liquid down to 5°, filtering through glass wool, and washing the separated crystals of conhydrine with petroleum ether, in which it is but sparingly soluble.

Pseudoconhydrine is a base isomeric with conhydrine, and probably a stereo-isomer. It crystallises from ether in several different forms: Long thick needles, thin plates with notched edges, fine needles in star-shaped clusters, and long columnar crystals. All of these are double refracting. By heating on a water-bath the various forms are converted into the fine needles. On breathing on the fine needles, they change back into the plate and columnar forms. Pseudoconhydrine melts at 105–106°, b. p. 236–236.5° according to Dilling, and is easily soluble in alcohol and acetone.

Coniceines, $C_8H_{15}N$.—Between coniine, $C_8H_{17}N$, and conyrine, $C_8H_{11}N$, there are known 5 isomers of the formula $C_8H_{15}N$, which have been prepared either by the oxidation of coniine or the dehydration of conhydrine.

α-Coniceine.—When molecular proportions of coniine hydrobromide and bromine are mixed, the bromo-derivative, $C_8H_{17}N.HBr.Br_2$, is obtained. By the regulated action of sodium hydroxide this yields $C_8H_{15}NBr$, which by treatment with sulphuric acid is decomposed into hydrobromic acid and *α-coniceine*, which is a colourless liquid of .893 sp. gr. at 15°, b. p. 158°, and slightly soluble in water. In odour it closely resembles coniine, but is said to be 5 or 6 times as poisonous! It is a tertiary base of strong alkaline reaction, and forms crystallisable salts. The *picrate* forms yellow needles, m. p. 225°, nearly insoluble in cold water, and very slightly soluble in alcohol. α-Coniceïne is partially reduced to coniine by heating under pressure with fuming hydriodic acid and phosphorus.

β-Coniceine is obtained together with α-coniceïne by the action of phosphoric anhydride or fuming hydrochloric acid on conhydrine: $C_8H_{17}ON = C_8H_{15}N.H_2O$. It forms very volatile, colourless needles, melts at 41° and boils at 168°. It is a secondary base of coniine-like odour, and is a less active poison than the α-modification. It is little soluble in water but quite soluble in alcohol and ether.

γ-Coniceine is found naturally associated with coniine and may largely contaminate commercial coniine. It may be obtained by decomposing the bromo-derivatives of coniine, $C_8H_{16}NBr$, with an alkali. It is a colourless liquid, turning dark yellow with age, b. p. 171–172° at 746 mm. pressure and distilling with steam; has a sp. gr. of 0.8825 at 22.5° and is optically inactive. It is slightly soluble in water is strongly alkaline in reaction, possesses a suffocating odour resembling coniine, and a sharp, burning taste. It remains liquid even at −50°.

It is a very powerful poison, being variously stated as from 12 to 17 times more toxic than coniine. γ-Coniceine is a secondary base yielding crystalline, volatile salts with acids, and a characteristic double salt with stannic chloride, $B_2H_2SnCl_6$, which forms large crystals, m. p. 215°. It also forms a hygroscopic, crystalline *hydrochloride*, m. p. 143°; a *hydrobromide*, m. p. 139°; a *hydriodide*, m. p. 102°; a *picrate*, m. p. 62°; and an *auriochloride*, m. p. 69–70°.

δ-Coniceine was obtained by Lellman by treating bromo-coniine with sulphuric acid. It is a tertiary base, not reduced by sodium and alcohol, is lævorotatory and boils at 158°.

ξ-Coniceine may be prepared by the action of alkalies on iodo-coniine It is probably stereo-isomeric with δ-coniceine, it being dextrorotatory, and boils at 150–151°.

Methyl-coniine, while mentioned as early as 1854 as a constituent of Conium, was not isolated in a pure state until 1894 by Wolffenstein. It is a colourless liquid, b. p. 173–174°, having a sp. gr. of 0.8318 at 24° and is lævorotatory. It may be prepared synthetically by heating coniine with an aqueous solution of methyl potassium sulphate.

Poisoning by Coniine and Hemlock.

Coniine is an extremely powerful paralytic poison, which acts on the motor nerves; 1 drop is a distinctly poisonous dose, while 10 drops may be fatal.

The symptoms produced by hemlock and coniine are not uniform, probably due to variable amounts of *γ*-coniceïne as well as of coniine, and cases of poisoning are not numerous. Stupor, coma, and slight convulsions have been noticed, while in other cases the chief effect has been paralysis of the muscular system, especially of the legs. The pupils are somewhat dilated. After death the lungs are found filled with fluid blood and of a dark colour, and the stomach and intestines somewhat congested. The *post-mortem* appearances are not characteristic.

In toxicological inquiries the viscera and contents of the stomach should be treated as described under strychnine, the purified extract being agitated with sodium hydroxide and ether instead of ammonia and chloroform. From ether, the alkaloid may be recovered by allowing the solvent to evaporate spontaneously in a cool place, or extracted as a salt by agitating the ether with dilute hydrochloric acid. From the purified salt of coniine thus obtained, the free base may be again liberated by adding sodium hydroxide and recognised by the

mousy odour of hemlock developed immediately or on warming the liquid. For further tests see the following section.

Coniine may also be isolated from the viscera by the method used for the assay of hemlock. Otto in one case met with a volatile ptomaine, which was very poisonous, but differed from coniine in its reaction with platinic chloride. The seeds of *Lupinus luteus* contain alkaloids somewhat resembling coniine, but which do not yield the characteristic crystalline hydrochloride. Other of the *Umbelliferæ* besides *Coniium* are possessed of poisonous properties, but it does not appear that coniine has been proved to be the active principle.[1]

Colour Reactions and Tests for Conium Alkaloids.

A considerable number of colour reactions for coniine and its allied alkaloids have been published, but none are characteristic and some are fallacious. Dilling (*Pharm. Jour*, 1909 [*iv*], **29**, 34–37, 70–72 and 102–104) has made an exhaustive examination of the colour reactions and other tests for these alkaloids and the following table is adapted from his work.

[1] *Œnanthe crocata*, or hemlock water-dropwort, is described by A S Taylor as one of the most virulent of English vegetable poisons The leading symptoms produced are rapid insensibility, bloated and livid countenance, convulsive movements, stertorous breathing, dilated pupils, and *bloody foam about the mouth and nostrils*

Cicuta virosa, water-hemlock or cowbane, produces symptoms similar to the above, including the foaming at the mouth It is said to contain cicutine.

Sium latifolium and *S. angustifolium* have been mistaken for water-cress, with fatal results

Æthusa Cynapium, the lesser hemlock or fool's parsley, appears to contain an energetic poison, though this has been disputed by Harley (*St. Thomas's Hospital Reports*, new series, **4**, 63, **10**, 257), and also by Tanret, who believes the erroneous statements respecting it to have arisen from a confusion of the plant with *Conium maculatum*, which it closely resemble

TABLE I.—REACTIONS OF CONIINE AND ITS ALLIES.

Reagent	Coniine	Conhydrine	Pseudoconhydrine	Coniceine
Conc sulphuric acid	No reaction	No reaction	No reaction	Orange-red to green
Conc. H_2SO_4 and amm vanadate.	Green in cold, blue-green and brown on heating	On heating, green, blue, then dark green and brown	On heating, green, blue, then dark green and brown	
Conc H_2SO_4 and selenic acid	Green then red on heating	On heating, brown, green, then red, quicker than coniine	Brown, green, red on heating, quicker than coniine	
Dil H_2SO_4 and potassium dichromate	Smell of butyric acid	Smell of butyric acid	Smell of butyric acid	
Conc hydrochloric acid	No reaction, green with old coniine.	No reaction	No reaction	Orange-red to green
Conc nitric acid	No reaction	No reaction	No reaction	Orange to green
Alloxan reaction	Purple colour on heating	Purple colour on heating	Purple colour on heating	
Anhydrous copper sulphate	Indigo-blue colour	No reaction	No reaction	
Antimony trichloride	Green colour	Faint yellow colour	Faint yellow colour	
Hydrogen sulphide	Needle crystals	Thin leaf-like crystals or plates.	Small needles in sheaf form	

TABLE II.—REACTION OF CONIINE AND ITS ALLIES IN SOLUTION.

Reagent	Coniine hydrochloride	Conhydrine hydrochloride	Pseudoconhydrine hydrochloride
Mayer's reagent	1–100 amorphous	1–100 amorphous	1–100 amorphous
Phospho-tungstic acid	1–10,000 crystalline	1–1,000 crystalline	1–1,000 crystalline
Phospho-molybdic acid	1–1,000 crystalline	1–500 amorphous	1–500 crystalline
Iodine in potassium iodide	1–10,000 amorphous	1–1,000 amorphous . .	1–1,000 amorphous
Silico-tungstic acid. .	1–1,000 crystalline	1–500 crystalline	1–100 crystalline
Tannic acid	1–1,000 amorphous	1–1,000 amorphous	1–1,000 amorphous
Trichloracetic acid	1–100 amorphous	1–100 no ppt	1–100 no ppt
Potass ferricyan and ferric chloride	1–100 reduction	1–100 reduction	1–1,000 slow reduct (n
Lead acetate .. .	1–100 no ppt , smell on heating	1–100 no ppt , no smell on heating.	1–100 no ppt , no smell on heating
Gold chloride ..	1–100 no ppt	1–100 no ppt	1–100 no ppt
Platinic chloride	1–100 no ppt	1–100 no ppt	1–100 no ppt
Cadmium potass iodide	1–200 crystalline	1–100 amorphous	1–100 no ppt
Barium mercuric iodide	1–10,000 amorphous	1–500 amorphous	1–1,000 amorphous
Nessler's reagent . .	1–10,000 amorphous	1–10,000 ppt on boiling	1–10,000 ppt on heating

Melzer's observation regarding the reaction of coniine and carbon disulphide was used by him as a test and has been elaborated by Dilling and may be applied as follows as a test for coniine and allied alkaloids:

To 0.5 c c. of a fairly strong alcoholic solution of the alkaloid add a few drops of carbon disulphide, boil and add excess of water. On now adding a few drops of copper sulphate, ferric chloride, ferric sulphate, nickel chloride, cobalt chloride or uranium nitrate, different colours are produced. On shaking this solution with ether the colours dissolve in the ether in some cases and evaporation of the ethereal solution gives different forms of crystals. None of these reactions are given by nicotine.

	Copper sulphate	Nickel chloride	Uranium nitrate
1	**Piperidine:** Vandyke brown colour, soluble in ether, large, opaque brown crystals, like polypodium leaves	Green colour, partly soluble in ether, rhombohedral plates and needle-like crystals	Orange colour, partly soluble in ether; opaque orange, brush-like crystals.
2	**Coniine:** Vandyke brown colour, soluble in ether, long flat, rhomboid plates	Green colour, soluble in ether; thin green rhomboid plates with a corner cut out	Orange colour, soluble in ether, needle-like brown crystals in bushy clumps.
3.	**Conhydrine:** Vandyke brown colour, soluble in ether, long, flat, brown plates of rhomboid form	Green colour, soluble in ether; flat, green rhomboid crystals	Orange colour, slightly soluble in ether, residue amorphous
4	**Pseudoconhydrine:** Vandyke brown colour, soluble in ether, flat, brown rhombic plates	Green colour, soluble in ether, green rhombic crystals	Orange colour, almost insoluble in ether, residue amorphous
5.	**Coniceine:** Brown colour, soluble in ether	Red colour, soluble in ether	Orange colour, partly soluble in ether.

To apply the above reactions the alkaloids must be free and in case of the salts about 0.5 c.c. of the aqueous solution is made alkaline with sodium carbonate, a few drops of alcohol and carbon disulphide added, and the test carried out as above. Toluol may be used to advantage in place of ether, particularly with uranium nitrate.

With aqueous solutions of coniine and nicotine, phenolphthalein gives a red or pink colour which disappears on shaking with chloroform in the case of nicotine but is permanent with coniine. Heut (*Archiv. d. Pharm.*, 1893, 376) uses this reaction for the estimation of

the alkaloids in mixture by titration with $N/10$ acid, using first phenolphthaleïn and then litmus as indicator.

Dilling (*Pharm. Jour.*, 1909 [*iv*], **29**, 103) gives a scheme for the differentiation of sparteine, lobeline, nicotine, coniine, conhydrine, pseudoconhydrine, γ-coniceïne and a coniine isomer, by means of these various reactions.

J. von Braun (*Ber.*, 1905, **38**, 3108) effects the separation of the conium alkaloids as follows: The greater portion of the coniine, which forms the chief constituent, is separated by distillation and the residue then fractionated up to 190°, whereby the conhydrine (m. p. 118°) remains as an undistilled residue. The lower fractions are then benzoylated in alkaline solution, and the resulting oil, after dissolving in ether, is extracted with acid to remove the methyl-coniine. The ethereal solution is then concentrated and treated with petroleum ether to precipitate the benzoyl-amino-butyl-propyl-ketone, which is formed by the action of benzoyl chloride on γ-coniceïne. The mother-liquor is redistilled and yields benzoyl coniine (b. p. 203 to 204° at 16 mm. pressure), and some more of the benzoyl-amino-ketone. Starting with 104 grm. of material, the author was able to obtain 1 grm. of conhydrine, 7 grm. of methyl-coniine, 52 grm. of amino-ketone corresponding with 26 grm. of γ-coniceïne, and 124 grm. of benzoyl-coniine, corresponding with 68 grm of coniine, total 102 grm. The coniine recovered from the benzoyl derivative proved to be a mixture consisting of *i*-coniine and a little *d*-coniine.

Assay of Hemlock and its Preparations.

Coniine exists in all parts of the common or spotted hemlock, *Conium maculatum* (French, *la Cigue;* German, *der Schieling*). It appears to be most abundant in the fruit, the proportion increasing with the maturity of the seeds. In hemlock *leaves*, R. Kordes found 0 24, and in the *fruit* 0.49 % of alkaloid.

For the *extraction* of coniine from hemlock, J. Schorm (*Zeit. Angew. Chem.*, 1894, 266) recommends that the fruit should first be swelled by hot water, and then moistened with a strong solution of sodium carbonate. The coniine is now distilled over with slightly superheated steam until the distillate ceases to be alkaline. The distillate containing coniine and coniine carbonate is neutralised wilh hydrochloric acid and evaporated to dryness. Heat the crude residue on a sand-bath till the resinous and oily impurities are decomposed and the residue is

odourless. Dissolve in water, filter and if coloured treat with hydrogen peroxide, 3%, until colourless. Evaporate and crystallise out the coniine hydrochloride. The mother-liquor retains conhydrine. Hydrogen peroxide will decompose the free alkaloid but not the hydrochloride.

A good product, but somewhat lower yield, is said to be obtained by exhausting the hemlock fruit with acetic acid, and evaporating the solution to a syrup in a vacuum. Magnesia is then added, and the mixture agitated with ether, which extracts the alkaloid.

Various assay processes have been proposed but one of the best is that adopted by the *United States Pharmacopœia*, Eighth Revision. 10 grm. of conium in No. 60 powder is macerated for 4 hours with shaking in 100 c.c. of a mixture of ether 98 parts, alcohol 8 parts, and ammonia water, 10%, 3 parts. Decant 50 c c. of the clear liquid and add $N/1$ sulphuric acid to slightly acid reaction. Evaporate off the ether by gentle heat. Add 15 c.c. of alcohol and after standing in a cool place for 2 hours, filter off the precipitated ammonium sulphate washing the precipitate and filter carefully with alcohol. Neutralise any excess acid with sodium carbonate, leaving the solution faintly acid, concentrate on water-bath to 3 c.c, add 3 c c. water and 2 drops $N/1$ H_2SO_4 and wash with two successive portions of 15 c c ether to remove fat. Make slightly alkaline with sodium carbonate and shake out with successive portions of ether (15 c.c., 15 c.c. and 10 c.c.). To the ethereal solution in a tared beaker add sufficient 5% hydrochloric acid to insure excess and evaporate ether by gentle heat. To residue add 3 c.c. alcohol, evaporate and repeat this operation to remove excess hydrochloric acid. Dry residue thoroughly at temperature not exceeding 60° and weigh. This weight, multiplied by 0.777 gives amount of coniine in 5 grm. of drug.

For the fluid extract or tincture equivalent quantities may be evaporated on sand or washed sawdust and the process carried out as for the drug.

Gordin (*Am. Journ. Pharm.*, 1901, 217) recommends the use of ether, 3 parts, and chloroform, 1 part, with potassium hydroxide as alkali, and extracts the coniine from this solution with oxalic acid. After removing potassium oxalate, the coniine is extracted with petroleum ether and is converted into hydrochloride with solution of hydrogen chloride in absolute ether. After carefully evaporating off the solvent and all excess of hydrogen chloride, the coniine hydrochloride is titrated with $N/40$ silver nitrate.

Squire (Companion to *British Pharmacopœia*, 439) gives the following method:

Five grm. of finely powdered conium or equivalent of fluid extract or tincture, evaporated on sand or sawdust, is extracted with 50 c.c. saturated solution of hydrogen chloride in chloroform. This extraction with a smaller amount of the solvent is repeated until 6 drops of the chloroform extract evaporated to dryness and treated with a few drops of dilute sulphuric acid gives no precipitate with Mayer's reagent. Shake the mixed chloroform extract twice with 25 c.c. of water and this aqueous extract in turn is shaken with 2 portions of 10 c c. of chloroform. The aqueous solution is made alkaline with sodium hydroxide and the coniine extracted by 3 portions of 10 c.c. of chloroform. To the chloroform extract add 10 c.c. of saturated solution of hydrogen chloride in chloroform, evaporate, dry at 90° and weigh. 162.4 parts of anhydrous coniine hydrochloride equals 126.2 parts of coniine.

For other methods of assay see Cripps (*Pharm. Journ.* (*iii*), **18**, 511) and Farr and Wright (*ibid.* [*iii*] **21**, 857)

Conium fruit should yield at least 0 5% of coniine by assay, though it varies considerably.

LOBELINE.

Lobeline, $C_{18}H_{23}O_2N$ (?), is the active principle of *Lobelia inflata*,[1] or Indian tobacco, a plant which has received extensive application and is also official in many pharmacopœias which specify different parts of the plant for use.

It has also been isolated by von Rosen from *L. nicotianæfolia* growing in Madras and Ceylon, and from *L. purpurascens* by Maiden and Hamlet.

Lobeline exists in lobelia in combination with a vegetable acid. In presence of certain other constituents of the plant the alkaloid is extremely unstable, being rapidly decomposed on heating an aqueous, or even an alcoholic infusion of lobelia. In presence of acetic acid the

[1] The root of *Lobelia syphilitica* was employed before *L. inflata* was known to medicine, but the root of the latter species does not appear to have been used. According to J. U. and C. G. Lloyd, all parts of lobelia contain the alkaloid, which, however, is most readily obtained from the seeds.

The dust of the plant produces a painful sensation when inhaled. All parts of the herb and seed produce an acrid, biting sensation on the tongue, and a sharp tobacco-like impression on the throat and fauces. Lobelia contracts the pupil, and acts as an expectorant in small doses and an emetic in larger (10 to 20 grains). In poisonous quantities it acts like nicotine, and kills by paralysing the respiration. Several fatal cases of poisoning by lobelia are on record.

base is more stable, and was obtained by J. U. and C. G. Lloyd (*Pharm. Rundschau*, 1887, 32) as a colourless, odourless, amorphous substance, permanent in the air, only slightly soluble in water, but readily soluble in alcohol, ether, chloroform, benzene, carbon disulphide, etc. In the pure state lobeline is not hygroscopic, and is but slowly changed in exposure to air. Lobeline turns red with sulphuric acid, yellow with nitric acid, and is precipitated by all the general alkaloidal reagents. The salts, which have not been obtained crystallised, are readily soluble in water, alcohol and ether. They are described as most violent emetics, a single drop of a tolerably strong solution producing immediate emesis, without disagreeable after-symptoms. The dust is as irritating as veratrine to the nose and air-passages.

No liquid volatile alkaloid could be obtained by Messrs. Lloyd from lobelia by distilling the herb with water, either with or without the addition of alkali hydroxide, and they considered the supposed volatile base to have been probably a mixture of lobeline, inflatin and volatile oil.

On the other hand, Paschkis and Smita (*Monatsh.*, 1890, **11**, 131) have obtained a volatile alkaloid from *Lobelia inflata*, by extracting the leaves with water acidified with acetic acid, rendering the concentrated solution alkaline, and agitating with ether. On distilling off the solvent, the alkaloid is obtained as a viscous oil, with an odour at once resembling that of honey and tobacco. It is purified by solution in dilute hydrochloric acid, and re-extracted by alkali and ether.[1] After distilling off the ether the base is dried with potassium hydroxide and distilled in a current of hydrogen. On warming the alkaloid so obtained with a 10% solution of potassium hydroxide, and gradually adding a 4% aqueous solution of potassium permanganate, benzoic acid is formed, and can be extracted by filtering off the precipitated oxide of manganese, and agitating the acidified solution with ether.

The sulphate of the above volatile alkaloid, if prepared from lobelia seeds, is obtained in yellow, very hygroscopic granules. When prepared from the leaves, it forms a yellowish-white powder, less hygroscopic than the salt from the former source.

[1] Up to this point the process of Paschkis and Smita is substantially the same as that of the Lloyd Brothers, for the preparation of the non-volatile alkaloid of lobelia Siebert, by the same process, obtained, both from the herb and seeds of lobelia, a pale yellow alkaline syrup, the crystallised hydrochloride and chloroplatinate of which indicated the formula $C_{18}H_{23}O_2N$ for the free alkaloid.

Inflatin was obtained by J. U. and C. G. Lloyd in large colourless, odourless crystals, m. p., 225°, insoluble in water or glycerin, but soluble in alcohol, ether, chloroform, benzene, carbon disulphide, and the oil of lobelia, etc. Inflatin is a neutral principle, and appears to have no therapeutic value. The *lobelacrin* of Enders is considered by the Lloyds to be a mixture of inflatin, resin, lobeline, and the fixed oil which lobelia contains in the proportion of about 30%.

Farr and Wright (*Chem. and Drug.*, 1893, **42**, 454) recommend the following method for the assay of the tincture of lobelia:

Place in a porcelain capsule 50 c.c. of the tincture with 5 drops of 33% acetic acid and 20 or 30 c.c. of water. Evaporate on the water-bath to 25 or 30 c.c., filter through absorbent cotton into a separator, rinsing dish and cotton with a little acidified water. Add ammonia in distinct excess and shake out with 3 successive portions of chloroform —10, 5 and 5 c.c. Evaporate the chloroform by a gentle heat, treat the residue first with 10 c.c., then with 5 c.c. of 1% hydrochloric acid, and filter into a separator. Again render alkaline with ammonia and shake out with 3 successive portions of anhydrous ether—15, 5 and 5 c.c. Evaporate the ether, dry the residue 1 hour at 100° and weigh. The residue when heated by them at 100° for several hours showed a loss of less than 0.001 grm.

For other assay methods as suggested by Patch and by Lyons, see Lyons' "The Assay of Drugs," pp. 189 and 190.

LUPINE ALKALOIDS.

From the different species of lupine several alkaloids have been isolated, some of which belong to the class of volatile alkaloids. From the seeds of the yellow lupine (*Lupinus luteus* L.) *lupinine* and *lupinidine* have been isolated; also from the black lupine (*Lupinus niger*), and lupinidine is reported by Campani and Grimaldi to be present in the white lupine. Two other alkaloids, *dextro-lupanine* and *inactive lupanine*, are found in the white lupine (*L. albus* L.) and the first mentioned is also found in *L. angustifolius* L., *L. perennis* L. and *L. polyphyllus*. K. Gerhard found alkaloids in *L. affinis*, *L. albococcineus*, *L. Cruikshanksi*, *L. Moritzianus*, *L. mutabilis* and *L. pubescens*, but none were extracted in sufficient quantity for identification.

Gerhard (*Arch. Pharm.*, 1897, **235**, 342) gives a quantitative estimation of the alkaloids in various species of lupines as follows:

	Est as lupanine	as lupinine
Yellow	0 4493%	0 6378
Blue	0 7249	
White	1 1115	
Perennial	1 1829	
Black	0 6100	0 8659

Lupinine, $C_{21}H_{40}O_2N_2$ (Baumert, *Ber.*, 1881, 1150, $_9$d14321, 1880 1882; 1882, **15,** 631, 1951) or more probably $C_{10}H_{19}ON$ (Willstätter and Fourneau, *Ber.*, 1902, **35,** 1910), may be prepared from the seeds of the yellow lupine by extracting with alcohol made acid with hydrochloric acid, evaporating off the alcohol, mixing with an equal volume of water, adding sodium hydroxide to strong alkaline reaction and extracting with ether. The mixture of lupinine and lupinidine so obtained may be separated as directed by Baumert (*Annalin,* **225,** 365). Lupinine crystallises in tables from acetone and rhombic crystals from petroleum ether which have a m. p. variously stated at from 67° to 69 2° though the higher figure seems more nearly correct. It boils with some decomposition at 255–261°, but in a stream of hydrogen it distills unchanged at 255–257°, and is also volatile with steam. Lupinine has a pleasant apple-like odour and an extremely bitter taste, the latter characteristic extending to its salts. It has a paralysing effect on the nerve centres. Lupinine is lævorotatory, $[\alpha]_D = -19°$ in aqueous solution of sp. gr. 1.005 and also given as $[\alpha_D] = -20°$ in 0.95% solution. It is soluble in cold water and alcohol, but less soluble in warm water. From its aqueous solution it is separated by excess of alkali hydroxide. Lupinine dissolves readily in ether, chloroform, benzene and acetone. Carbon disulphide dissolves the base while acting chemically upon it. Lupinine is highly caustic, and is a strong base, liberating ammonia from its salts and fuming with hydrochloric acid. $B(HCl)_2$ forms large rhombic crystals readily soluble in water and alcohol, m. p. 212–213° and in 2% solution gives $[\alpha]_D = -14°$. The *platinichloride,* $B\ H_2PtCl_6$, forms yellow crystals, m. p. 163–164°, and the *aurichloride* forms glistening needles, m. p. 196–197°, difficultly soluble in water but readily in alcohol. The *nitrate,* $B(HNO_3)_2$, forms rhombic crystals, very soluble in water and alcohol.

Metallic sodium dissolves in melted lupinine with evolution of hydrogen, forming a sodium-derivative, decomposed by water into

lupinine and sodium hydroxide. When heated with acetic anhydride, lupinine yields $C_{10}H_{17}N.C_2H_3O$, as an oil, insoluble in water and very easily saponified.

Anhydrolupinine, $C_{10}H_{17}N$, originally known as *dianhydrolupinine*, is prepared by heating lupinine with acetic and sulphuric acids at 180° (Willstatter and Fourneau, *Ber.*, 1902, **35**, 1910). It is a colourless oil of heavy unpleasant odour, boiling at 216.5–217.5° at 726 mm. pressure. It forms a *platinichloride*, $(C_{10}H_{17}N)_2$, H_2PtCl_6, which separates from water in reddish-brown crystals, stable in the air, darkens at 170° and decomposes at 216°. The *aurichloride*, $C_{10}H_{17}N$, $H AuCl_4$, forms transparent golden prisms, m. p., 140–141°. The *methiodide* melts at 180° with decomposition.

By oxidising lupinine with chromic acid, *lupinic acid*, $C_9H_{16}N.COOH$, is formed, crystallising from acetone in colourless needles containing $3H_2O$, melting when quickly heated at 255°.

Methyl-lupinine, $C_{10}H_{18}O.NCH_3$, and *dimethyl-lupinine*, $C_{10}H_{17}ON(CH_3)_2$, both oily liquids, boil respectively at 145–146° under 15 mm. pressure, and 169–172° under 28–29 mm. pressure.

Lupinine is probably related to cinchonine in chemical constitution.

Lupinidine, $C_8H_{15}N$ or preferably $C_{15}H_{26}N_2$ (Willstatter and Marx), is a base found by Baumert in the yellow lupine. It forms a volatile, oxidisable, viscous oil, having an odour of hemlock. It is intensely bitter and feebly poisonous, producing symptoms like those of *curare*. The more recent researches of Willstatter and Fourneau (*Ber.*, 1902, **35**, 1910) and Willstatter and Marx (*Ber.*, 1904, **37**, 2351) have shown the great similarity between lupinidine and sparteine, not only in a general way but in numerous specific characteristics, and their identity seems reasonably sure. The last named investigators obtained a yield of 0 23% from the seeds of *Lupinus luteus* after removal of the lupinine with light petroleum. It boils at 311–314° under normal pressure, at 180.5° under 18 mm. pressure, and is volatile with steam. Lupinidine has a sp. gr. of 1.034 at 0° and 1.023 at 20°; $[\alpha]_D = -5.96°$ at 20° when undiluted and -16.41 at 21° in 99% alcohol where $c = 14$ 206. It is soluble in cold water more readily than in hot, readily soluble in alcohol and soluble in ether, difficultly soluble in petroleum ether. The *platinichloride* is stated by Behrend to contain $2H_2O$, and melt at 227°, while Willstatter and Marx pronounce it identical with the same sparteine salt, darkening at 239° and melting with decomposition at 243.5.° Lupinidine is

denser than water. From pyridine piturine differs by giving a precipitate with cupric sulphate insoluble in excess of the base.

When piturine is treated in ethereal solution with iodine (compare sparteine) the liquid becomes brownish-red and turbid, and after a short time deposits yellowish-red needles, leaving a yellow mother-liquor. The crystals melt at about 110°, and dissolve in alcohol with brownish-red colour. This solution leaves indistinct needles and oily drops on evaporation; if treated in the cold with sodium hydroxide, an iodoform-like odour is evolved; whereas the iodine-compound of nicotine is said to reproduce nicotine when similarly treated.

POMEGRANATE ALKALOIDS.

From the bark of *Punica granatum* L., Tanret, in 1877, isolated 4 alkaloids: *Pelletierine, iso-pelletierine, methyl pelletierine* and *pseudo-pelletierine*, named from the French chemist Pelletier, all of which are volatile but not all are liquid at ordinary temperatures According to Fluckiger the first of these predominates in the stem-bark and the third in the root-bark. Stoeder, working on fresh Java bark, obtained from the white-flowered variety 3.75% of mixed hydrochlorides of the alkaloids; from the red-flowered 2 43% and from the black-flowered variety 1.71%. From the root-bark he obtained 1.29% to 1.86%, the plant being chiefly the white-flowered variety.

The alkaloids may be obtained by mixing the powdered bark with milk of lime, exhausting with water, removing from the aqueous solution by extraction with chloroform and again extracting this solution with dilute acid. If this solution is now treated with sodium bicarbonate, methyl and pseudo-pelletierine may be removed by shaking with chloroform while the other two alkaloids remain. For details of further separation see Tanret, (*Compt. Rend.*, 1880, **90**, 695).

Pelletierine, $C_8H_{15}ON$, to which the name *punicine* has also been given, is an oily liquid, colourless when freshly prepared and kept out of contact with oxygen, but oxidising readily in contact with air and forming a resinous mass. It boils with some decomposition at 195° under normal pressure and at 125° at 100 mm. Pelletierine has a sp. gr. of 0.988 at 0° and is dextrorotatory ($[\alpha]_J = +8°$), becoming inactive when heated to 100°. Its salts are lævorotatory rotatory; (sulphate, $[\alpha]_D = -30°$) and on heating, either dry or in solution, lose a portion of the base. The alkaloid is soluble in 20 parts of water and is miscible

in all proportions with alcohol, chloroform and ether. It forms crystalline salts, from aqueous solutions of which alkaline hydroxides liberate the alkaloid but which are not affected by bicarbonates.

Iso-pelletierine, $C_8H_{15}ON$, is apparently a stereoisomer of pelletierine, which it very closely resembles, having the same b. p. and solubility but is optically inactive.

Methyl-pelletierine, $C_9H_{17}ON$, is a liquid, b. p. 215°, soluble in 25 parts of water at 12°, very soluble in alcohol, ether and chloroform, and forming crystalline salts which are very hygroscopic. The *hydrochloride* is dextrorotatory ($[\alpha]_D = +22°$.) The base is liberated from its salts by bicarbonates.

Pseudo-pelletierine, $C_9H_{15}ON$, also referred to as *methyl granatonine* and *granatonine*, is the crystalline alkaloid of pomegranate bark and is the one which has been most carefully studied. It forms prismatic crystals from petroleum ether, m p. 48° (46°, Tanret), b. p. at 246°; is readily soluble in water, alcohol, ether and chloroform; less easily soluble in petroleum ether, and is optically inactive. It has been investigated at some length by Piccinini (*Atti R. Accad dei Lincei Roma* [5] **8,** I, 392; [5] **8,** II, 219. *Gazz. Chim. Ital.*, 1899, **29,** [2] 104, 115; 1901, **31,** [1] 561; 1902, **32,** [1] 260) and by Ciamician and Silber (*Ber.*, 1892, **25,** 1601; 1893, **26,** 156, 2738, 1894, **27,** 2850; 1896, **29,** 481, 490, 2870).

The estimation of the alkaloids in pomegranate bark may be accomplished by the method given by Fromme (*Cæsar and Loretz Report*, Sept., 1905, 81).

Volumetric Method.—To 7 grm. of the air-dried bark in moderately fine powder, add 70 grm. of ether. Shake together thoroughly and then add 5 grm. of 15% solution of sodium hydroxide and macerate for half an hour with agitation. Filter rapidly, taking precautions to avoid all possible evaporation of ether; shake the filtered ethereal solution with 10 or 15 drops of water and set aside for a short time. Into a flask which has been previously washed with hydrochloric acid and then with water until free from acid, weigh 50 grm. of the ethereal solution, representing 5 grm. of the bark; add 30 grm. of distilled water and a few drops of iodeosin solution. Titrate the mixture with thorough shaking with $N/10$ hydrochloric acid solution. Each c c. of $N/10$ acid used $\times 0.01475 \times 20$ gives the percentage of total alkaloids in the bark.

Gravimetric Method.—50 grm. of the extract obtained as above is

shaken out with successive portions of 20, 10 and 10 c.c. of 1% hydrochloric acid. The acid solutions are filtered into another separator, made alkaline with sodium hydroxide and again shaken out with 20, 10 and 10 c.c. of chloroform. The chloroform washings are filtered into a tared flask, treated with 5 drops of hydrochloric acid and the chloroform distilled off. Dry the residue, first at 70–80° and finally in a desiccator over sulphuric acid to constant weight. The weight obtained is that of the hydrochlorides of the total alkaloids, 184 parts of which are equivalent to 147.5 parts of the free bases.

SPARTEINE,[1] $C_{15}H_{26}N_2$.

This alkaloid is obtained by Houdé and Laborde (*Pharm. Jour.* [*iii*], **16**, 543) by exhausting in a displacement-apparatus with proof-spirit the coarsely powdered leaves and branches of broom (*Spartium scoparium*). The product is filtered, distilled under reduced pressure, the residue dissolved in tartaric acid, the liquid filtered to remove a greenish deposit containing chlorophyll and *scoparin*, $C_{21}H_{22}O_{10}$, the filtrate rendered alkaline by potassium carbonate, and agitated several times with ether. The ethereal solution is shaken with tartaric acid, and the acid liquid separated and again rendered alkaline and extracted with ether, which on evaporation leaves the alkaloid; the yield being about 0 3% of the plant used.

Sparteine is a colourless, oily liquid, having a faint, somewhat pyridine-like odour and a bitter taste. Statements regarding its b. p. vary widely, that formerly accepted being 287–288° at normal pressure, but Bamberger gives 311° while Moureu and Valeur (*Compt. Rend.*, 1903, **137**, 194) working on alkaloid prepared from the pure sulphate, found a b. p. of 325° under 754 mm. pressure and 188° under 18.5mm.; and Wackernagel and Wolffenstein record 326° (*Ber.*, 1904, **37**, 3238). It distils readily with steam. Direct distillation must be carried out in the absence of air to avoid browning by oxidation. Exposure of the colourless alkaloid to air causes it to become brown and thick. It is readily soluble in alcohol, ether and chloroform, but in water only to

[1] According to DeRymon, sparteine causes tremor, dilation of the pupils, incoordination of movements, and convulsions alternately tonic and clonic.

Schroff found that a drop of sparteine introduced into a rabbit's mouth occasioned spasms of the muscles of the spine and limbs and paralysis of the latter, slowing of the respiration and heart, and death in 6 minutes

The effects of sparteine have been compared to those of coniine, but they do not explain the value of broom as a diuretic medicine. Solis-Cohen considers that the unsatisfactory medicinal action in many cases is due to insufficient dosage, which should be in some cases up to 0.13 grm. (2 grains).

the extent of 0 3 grm. in 100 c c , insoluble in benzol and petroleum ether. Its sp. gr. is 1.034 at 0° and 1.0196 at 20°; $[\alpha]_D = -16.42°$ in absolute alcohol; $n_D = 1.5293$ at 19° (Moureu and Valeur).

Sparteine is a well-defined base, uniting with acids to form crystallisable salts, and having the constitution of a tertiary diamine. It is monacid in character with litmus and phenolphthaleïn, and diacid to methyl-orange. Commercially available salts of sparteine are the sulphate, hydrochloride, hydriodide and triiodide.

Sparteine sulphate,[1] $C_{15}H_{26}N_2 H_2SO_4 + 5H_2O$, forms large, transparent, very soluble rhombohedra, a solution of which gives with alkali hydroxide and ammonia a white precipitate insoluble in excess.

Cadmium iodide gives a white curdy precipitate, and sodium phosphomolybdate a white precipitate, dissolving on heating the liquid. Platinum chloride yields a yellow precipitate of $BH_2PtCl_6 + 2H_2O$, very insoluble in cold water and alcohol, but crystallising from hydrochloric acid in rhombic prisms. Sparteine gives no colouration with concentrated mineral acids. It crystallises with $5H_2O$ dries to constant weight with $1H_2O$ *in vacuo* over sulphuric acid and when heated to 110° loses this last molecule of water and rapidly turns brown. When anhydrous it melts at 136°. In 3–6% aqueous solution $[\alpha]_D = -22.12°$ at 15–20°. Soluble in 1.1 pts. of water, 2 4 pts. of alcohol at 25°, and insoluble in ether and chloroform. Because of its acid reaction to phenolphthaleïn it may be titrated accurately with $N/10$ alkali.

Sparteine forms also a *hydriodide*, m. p. 226–228°; a *dihydriodide*, m. p. 257–258°, a *platinichloride*, m. p. 244–257°, with decomposition; an *aurichloride*, m. p. 175–184°, with decomposition; a picrate m. p. 199–200°; and oxalate, m. p. 138–140°, crystallising from alcohol with 1 mol. C_2H_5OH. It also forms a *methosulphate*, crystallising with $7H_2O$, $[\alpha]_D = -24.54°$, acid to litmus.

From sparteine excess of methyl iodide readily forms a *methiodide* in white plates, very soluble in alcohol and water, less soluble in acetone, alkaline to methyl orange, melting with decomposition at 240° and having $[\alpha]_D = -22.75°$ in 12% aqueous solution. According

[1] Administered in doses of 0 1 grm , *Sparteine* sulphate is stated (G. See, *Compt. Rend.*, 1885, 101, 1046, *Year-book Pharm.*, 1886, 283) to have a tonic action on the heart more prompt and lasting than that of digitalis of convallamarin, restoring the rhythm of the heart's action better than any known remedy, and resembling belladonna in accelerating the heart-beats in weak and atonic conditions of the heart It does not appear to have any injurious action on the digestion, or on the nervous system generally.

to Bernheimer, on gradually adding 3 parts of iodine dissolved in ether to an ethereal solution of 1 part of sparteine, a black precipitate is formed which, when separated, washed with ether, and dissolved in boiling alcohol, crystallises on cooling in beautiful green needles of formula $C_{15}H_{26}N_2I_3$. This substance is insoluble in cold water or alcohol, but dissolves in either liquid when heated. It is insoluble in ether, permanent in the air, and yields free sparteine when heated with alkali hydroxide. Bromine acts strongly on sparteine at the ordinary temperature, even when largely diluted with ether, forming an undefined resinous mass. The *metho-bromide* melts with decomposition at 219°.

It has been considered that sparteine is reduced by tin and hydrochloric acid to *dihydrosparteine*, $C_{15}H_{28}N_2$, a heavy liquid boiling at 281–284°, but Wackernagel and Wolffenstein (*Ber*, 1904, **37**, 3238) state that it cannot be reduced and is therefore not an unsaturated base.

Sparteine is readily oxidized by various oxidising agents, with the production of a series of related compounds.

Spartyrine (Willstatter and Marx, *Ber.*, 1905, **38**, 1772) or *dehydrosparteine* (Ahrens, *Ber.*, 1893, **26**, 3035), $C_{15}H_{24}N_2$, is recorded by the latter as a liquid boiling at 314–315°, but the former authorities, obtaining it by oxidation of the sulphate with chromic acid, describe it as forming crystals from ethyl acetate, m. p. 153–154°, $[\alpha]_D = -25.96°$ at 18.5°.

Oxysparteine (Willstatter and Marx, *Ber.*, 1905, **38**, 1772) or *hydroxysparteine* (Ahrens, *Ber.*, 1891, **24**, 1095), $C_{15}H_{24}ON_2$, obtained by the former by further oxidation of sparteine sulphate with chromic acid, crystallises in needles, m. p. 87 5° (84°—Ahrens), b. p. 209° under 12.5 mm.; $[\alpha]_D = -10.04°$ at 18°. It is not oxidised further by chromic acid or potassium permanganate. Phosphorus oxychloride causes elimination of 1 mol. of water with the production of a volatile base, $C_{15}H_{22}N_2$. The *platinichloride* of oxysparteine forms short prisms with $2H_2O$, which become anhydrous at 130° and melt with decomposition at 225–227.°

Dioxysparteine, $C_{15}H_{24}O_2N_2$, forms prisms, m. p. 128–129°. By heating with concentrated hydrochloric acid it is converted into *dehydrosparteine* (Ahrens, *Ber*, 1887, **20**, 2218; 1892, **25**, 3607.)

Trioxysparteine, $C_{15}H_{24}O_3N_2$, forms deliquescent crystals and is a monacid base.

Two isomeric, oily, liquid bases of formula $C_{15}H_{26}ON_2$ are also reported by Ahrens as found by oxidation of sparteine.

A number of methyl-, methiodo- and ethiodo-derivatives have also been prepared.

According to Grandval and Valser, when a drop of ammonium sulphydrate is placed on a watch-glass, and a trace of sparteine or one of its salts added to it, a permanent orange-red colouration is immediately produced.

Reichard (*Pharm Centr.*, 1905, **45**, 300) gives the following colour reactions for sparteine:

Ammonium molybdate in sulphuric acid gives no colour with sparteine, but on the addition of ammonium persulphate a yellow colour results.

Mix a few drops of a solution of ferric chloride and potassium thiocyanate; evaporate to dryness and add a drop or two of aqueous solution of sparteine, when a violet-blue to reddish-violet colour appears, permanent on drying.

By substituting potassium ferrocyanide for the thiocyanate in the above test, a pale violet colour is produced. On treating the dried spot from this test with solution of potassium thiocyanate, the colour changes to pale blue and deep blue on drying.

SPIGELINE.

This is said to be the active principle of *Spigelia Marylandica*, or "pink-root." As obtained by W. L. Dudley, by distilling the root with milk of lime, it was volatile, gave with iodine a brownish-red precipitate, and with Mayer's reagent a white crystalline precipitate soluble in alcohol and ether, and differing from most similar precipitates by being soluble in dilute acid. Spigeline is said by Stabler to be bitter, precipitated by tannin, and soluble in water and alcohol, but not in ether (?). Pink-root is often used as a vermifuge, and possesses poisonous properties allied to those of gelsemium, depressing the action of the heart and of respiration, and in large doses causing loss of muscular power (*Practitioner*, July, 1887). It produces strabismus, dilatation of the pupils, and temporary loss of sight, with some drowsiness but not narcotism A fluidextract of spigelia root is official in the *United States Pharmacopœia*.

Other Volatile Alkaloids.

Pictet and Court (*Ber.*, 1907, **40**, 3771) record the isolation of a volatile base of the formula C_5H_9N, or $C_5H_{11}N$, from the fruit of *Piper nigrum L.*, the *aurichloride* of which crystallises from dilute hydrochloric acid in yellow leaflets or needles, m. p. 182°. The *platinichloride* melts at 203° and is insoluble in alcohol.

They obtained from the leaves of the *carrot* two volatile bases, the more easily volatile of which was identified as *pyrrolidine;* the other was a new base to which they gave the name *daucine.* It is a colourless, oily liquid, with an odour resembling nicotine, b. p. 240–250°, soluble in water, alcohol, ether, of distinct alkaline reaction to litmus and having an optical rotation of $[\alpha]_D = +7.74°$. They give for its formula, $C_{11}H_{18}N_2$. From the seeds of the *carrot* they obtained very small quantities of a base which is a *pyrrole* derivative, and giving an insoluble *aurichloride* decomposing at 172–175°.

From *parsley* leaves a small quantily of a volatile base was obtained which received, however, but little investigation.

NICOTINE AND TOBACCO.

By R. W. TONKIN.

Nicotine. $C_{10}H_{14}N_2$; or $C_5H_4N.C_4H_7N.CH_3$.

Nicotine is a liquid alkaloid and is the main poisonous basic principle in tobacco, in which it occurs in very variable proportions. In the plant it exists in combination with organic acids (malic, citric, etc.). Nicotine is β-pyridyl-α-*n*-methylpyrrolidine. Pictet (*Ber.*, 1900, **33**, 2355), starting from β-aminopyridine which was successively converted into β-pyridyl-pyrrole, 1-methyl-2-β-pyridyl-pyrrole, nicotyrin, 1-methyl-2-β-pyridyl-tetraiodo-pyrrole, obtained tetrahydronicotyrine, which is *i*-nicotine. Pure nicotine is a colourless, oily fluid which boils without decomposition at 246.7 under 745 mm.; it does not solidify at $-30°$; the sp. gr. is 1.01 at 20°. It has a sharp burning taste, and if pure but little odour. On exposure to air for any time it acquires the peculiar smell of tobacco, and after prolonged exposure becomes brown in colour, and is eventually changed to a resinous mass.

Nicotine is powerfully lævorotary. $[\alpha]_D^{20} = -166.33°$, but its salts, on the contrary, are dextrorotary. Landolt gives $[\alpha]_D^{20} = -161.55°$.

The following values are given by Landolt (*Optische-Drehungsvermogen*) for the specific rotation of nicotine in aqueous solution.

% Nicotine	% Water	Sp gr at 20°/4°	*l* in dm	α_D^{20}	$[\alpha]_D^{20}$
89 92	10 08	1 0267	0 9992	−123 47°	−133 85
78.39	21 61	1 0353	0 9992	− 88 82	−109 53
65.90	34.10	1 0401	0 9992	− 64 54	− 94.24
53 48	46.52	1 0365	0 9992	− 47 95	− 86.58
34.29	65.71	1.0228	0 4982	− 14 11	− 80.78
17.68	82 32	1 0116	0 4982	− 6.85	− 76 94
16.34	83 66	1 0096	0 4982	− 6.32	− 76 88
8.97	91.03	1 0047	0 9992	− 6.80	− 75 53

For the values of specific rotatory power of nicotine salts in aqueous solution at different concentrations consult Landolt (*loc. cit.*).

Nicotine is very hygroscopic; on dissolving the alkaloid in water heat is developed, and a contraction in volume occurs.

C. S. Hudson (*Zeit. Physikal. Chem.*, 1904, **47**, 113) has investigated the solubility of nicotine in water, and finds that 5% of nicotine is soluble at any temperature in water and 15% of water in nicotine. The solubility curve is a closed figure and shows the formation of a definite hydrate which between the temperatures of 60° and 210° will separate out from the excess of either water or nicotine; the density of the hydrate varies according to the temperature more rapidly than that of water or nicotine, so that the upper or lower layer may be the hydrate according to the temperature. The solubility of the hydrate in water varies according to the temperature.

The aqueous solution of nicotine is powerfully alkaline in reaction. The nicotine is partially separated by addition of excess of potassium or sodium hydroxide (compare pyridine). Nicotine in aqueous solution, and in the absence of any other free base, can be estimated by titration with standard acid and methyl-orange.

Nicotine forms 2 classes of salts. The monacid salts are stable and neutral to litmus and methyl-orange, but the diacid salts have an acid reaction Most of the salts of nicotine crystallise with difficulty. The *acid tartrate*, $C_{10}H_{14}N_2(C_4H_6O_6)_2+2H_2O$, is an exception, and forms handsome tufts when ether is added to its alcoholic solution.

Detection of Nicotine.

Although of high b. p., nicotine, like coniine, is readily volatile with steam and if an aqueous alkaline solution be distilled the nicotine passes over in the earlier distillates.

The usual reagents for alkaloids precipitate nicotine from more dilute solutions than coniine, which is the most likely alkaloid to distil with nicotine; thus the platinichloride of nicotine is precipitated from a 1:5,000 solution (coniine only in 1:1000).

Gold chloride precipitates nicotine from 1:10,000; mercury-potassium iodide solution from 1.15,000; bismuth-potassium iodide solution from 1·40,000; iodine in potassium iodide solution from 1.250,000.

If a small quantity of nicotine dissolved in dry ether with a little iodine is allowed to stand in a sealed tube a resinous precipitate is gradually formed, while in the liquid ruby-red crystals, which look dark blue by reflected light, are formed (Roussin's crystals).

Kippernberger (*Zeitsch. Anal. Chem.*, 1903, **42**, 274) states that the formation is most certain when 1 mol. nicotine is acted on by 2 mols.

iodine; but that the colour and formation of the crystals may vary considerably, and does not regard it as a very decisive test.

Schindelmeiser's Reaction (*Pharm. Zentr-h.*, 1899, **40**, 703).—If nicotine, free from resin, is treated with a drop of formaldehyde (which should not contain any formic acid) and then with a drop of concentrated nitric acid the mixture is coloured deep rose-red. If the mixture of nicotine and formaldehyde is allowed to stand for some hours a solid compound is formed which will give a more pronounced colouration with the acid; but in this case excess of formaldehyde must be avoided as otherwise the mass formed is greenish coloured and rapidly decomposes. Trimethylamine, piperidine, pyridine, picoline, quinoline and aniline give no reaction in this way

Extracts of decomposing horseflesh or the entrails of other animals, prepared by Stas-Otto's method, also give no reaction.

Melzer (*Zeitsch. Anal. Chem*, 1908, **37**, 357) find that nicotine reacts energetically with epichlorhydrin when heated to 120°, giving a deep red coloured product; in concentrated solutions the reaction becomes violent. 0.5 c.c of a 1:500 sol. of nicotine in alcohol boiled with 2 c.c. epichlorhydrin gives a distinct red colour; and lesser quantities may be detected by boiling the mixture for some time. About 0.0005 grm. nicotine may be detected in this way Strong solutions of lobeline give a somewhat similar colour, but solutions of coniine give only a feeble yellowish colouration.

Aniline, dimethylaniline, cadaverine and ammonia give no reaction.

Picric acid, if added *in excess* to solution of nicotine, throws down *nicotine picrate* as an amorphous yellow precipitate, which rapidly changes to a mass of crystalline tufts, even in presence of foreign organic matter.

Nicotine is precipitated by Mayer's reagent from very dilute solutions; and, by operating in strongly acid liquids, Zinoffsky obtained very good quantitative results. The formula of the precipitate is $C_{10}H_{16}N_2HgI_4$, and 1 c.c. of the reagent represents 0.00202 grm. of nicotine.

On adding mercuric chloride to a solution of nicotine a white *crystalline* precipitate is produced, soluble in dilute hydrochloric or acetic acid. This is the most characteristic reaction of nicotine. Strychnine produces a similar precipitate, nearly insoluble in acetic acid. Many other alkaloids are precipitated, but the compounds are almost invariably amorphous. This is the case with the precipitate produced

by coniine, which is almost the only alkaloid which will distil over with nicotine on boiling the solution with a slight excess of sodium hydroxide. Ammonia, however, behaves like nicotine, and must, if necessary, be separated before applying the test. In addition to the above chemical tests the physiological action of nicotine may be used to detect small quantities of the alkaloid. Frogs show very characteristic symptoms of nicotine poisoning; at first excitation, and afterward paralysis of the brain and respiratory muscles, with tetanic convulsions similar to those produced by curare. The intermittent diastolic stopping of the heart is extremely characteristic of nicotine.

Estimation of Nicotine.

A variety of methods have been devised for the estimation of nicotine in tobacco, insecticides, and similar preparations. The majority of the earlier ones employed distillation of the nicotine to separate the alkaloid, but have now been superseded by methods involving extraction with some suitable solvent.

E. Algrain (*Mem. des Manufactures de l'Etat*, 1908, 256) gives the following process: 20 grm. of the finely powdered tobacco are triturated with 20–50 c.c. of a liquor composed of 90% saturated salt solution and 10% sodium hydroxide solution of 45° Bé. in a wide-mouthed bottle. 100 c.c. petroleum are added and the mixture shaken well for 1/4 hour. An aliquot part of the petroleum is filtered off and after adding to it 50 c.c. water and a drop or two of indicator (litmus is named) the nicotine is titrated with standard sulphuric acid. The results should be increased by one-tenth. A quick method is that of Kissling's (*Zeitsch. Anal. Chem.*, 1895, **34**, 345) which is described in detail on page 250.

C. C. Keller (*Ber. d. Deut. Pharm. Ges.*, 1898, **8**, 155) gives the following: Take 6 grm. of the powdered tobacco and shake up with 10 c.c. of 20% potassium hydroxide solution and 60 c.c. each of ether and petroleum ether for 15 minutes in a closed vessel. Then allow to rest for 3 hours. Filter off 100 c.c. into a glass and remove the ammonia by blowing a strong current of air through it for 2 minutes. To the residue add 10 c.c. alcohol and 10 c.c. water with a drop of iodeosin solution as indicator, and shake well. Add an excess of $N/10$ sulphuric acid, and titrate back with $N/10$ potassium hydroxide.

Tóth's Method (*Chem. Zeit.*, 1901, **25**, 610).—6 grm. of dry pow-

dered tobacco are triturated in a mortar with 10 c.c. of 20% sodium hydroxide solution and into this paste enough burned gypsum (plaster of Paris) is worked to produce a dry mass which will not "bind" when pressed into a lump. This mixture is put into a well stoppered bottle and to it is added 60 c.c. of ether and 60 c.c. of petroleum ether. The bottle is shaken at frequent intervals for an hour; 20 c.c. of the ether mixture is drawn off and titrated after adding to it 50 c.c. water and a drop of iodeosin solution. It is best to add excess of acid and shake well, and then titrate back with $N/10$ potassium hydroxide solution. It is of interest to note that Kippenberger (*Zeitsch. Anal. Chemie*, 1900, **39**, 210) investigated the action of the following indicators when titrating various alkaloidal solutions; iodeosin, ethyl orange, azolitmin, uranin, cochineal, hæmatoxylin, phenolphthalein, lacmoid and alkanin. He found that lacmoid worked best for nicotine, followed by iodeosin, uranin, and cochineal in the order given.

Of the methods just given, that of Tóth is most used in England at present, and is applicable, with slight variations, to many estimations of alkaloids. The plaster of Paris removes any ammonia which may be produced from the tobacco, and the only drawback to it is that the titration may take a long time, owing to the ether and aqueous layers separating slowly if much resinous matter has been extracted from the tobacco material employed. It is the most satisfactory to use. Keller's and Kissling's methods are liable to give results too high owing to the presence of ammonia in the extracts, but have been much used on the continent, and Kissling's method was adopted by the A. O. A. C. in 1904.

Poisoning by Nicotine and Tobacco.

Nicotine is one of the most violent poisons known. Only a few instances are on record of poisoning of the human subject by the pure alkaloid, but the effects of *tobacco*, which owes its poisonous properties entirely to nicotine, are well known. Impure solutions of nicotine and infusions of tobacco are employed as insecticides.

"The usual effects of a poisonous dose of tobacco, when taken into the stomach, are confusion in the head, paleness of the countenance, vertigo, nausea, severe retching and vomiting, heat in the stomach, great anxiety, a sense of sinking at the pit of the stomach with extreme prostration, trembling of the limbs, and sometimes violent purging.

The pulse is small, feeble, and almost imperceptible; the respiration difficult, and the skin cold and clammy; the pupils are generally dilated, but sometimes contracted, and the vision is usually more or less impaired. Death is often preceded by convulsions and paralysis" (T. G. Wormley, *Microchemistry of Poisons*).

In toxicological investigations, nicotine may be isolated from the viscera in the same manner as coniine. An alternative method is to digest the suspected matters with water acidified with acetic acid, and treat the filtered liquid with excess of lead acetate. The liquid is again filtered, the lead removed from the filtrate by passing hydrogen sulphide, and the clear solution treated with sodium hydroxide, separated from any precipitate, and distilled, when a fluid having the odour and exhibiting the reactions of nicotine will be obtained.

Tobacco.

Tobacco is the dried leaf of *Nicotianum Tabacum*, or *N. Rustica*, the latter furnishing East Indian and Turkish tobacco. Persian tobacco which has been regarded as a separate species is a variety of *N. Tabacum*.

The tobacco plant is very susceptible to variations of climate and soil, which have in time a very marked effect on the texture and properties of the leaves, no less than 50 varieties of tobacco plants being recognised in the United States.

The plant owes its use to the volatile alkaloid nicotine which it contains in very variable proportions. In addition nicotinine, nicoteine, nicotelline, pyrrolidine and methyl-pyrroline have been separated in small quantities from the crude alkaloid extracted from tobacco.

T. E. Thorpe (*Trans.*, 1901, **79**, 982) finds that tobacco leaf contains 0.1% of a paraffin, and 0.2% of waxy matter.

The leaves of tobacco when gathered must be cured and the manner in which the process is carried out has a most important effect on the quality, colour and texture of the resulting tobacco.

The curing process does not seem to be simply a matter of drying out moisture, but involves the action of life.

J. Behrens (*Landw. Versuchs-Stat.*, 1894, **40**, 248) finds that if leaves are subjected to the vapour of chloroform before curing, they dry up, and contain much more starchy matter, more sugar and more albumin-

oid nitrogen than leaves normally cured. In addition the heavier and darker tobaccos are subjected to a process of fermentation. The result of this is to modify the composition of the leaf and alter the flavour by producing "ferment oils."

The loss on fermentation varies from 8 to 16%, but the amount lost is no indication of a good or bad fermentation, the success of the operation depending on the flavour imparted to the tobacco.

Schizomycetes occur in fermented tobacco in large numbers, but the number of species is very limited. Trial experiments by E. Suchsland, with foreign ferments on German tobacco-leaves, yielded a tobacco not recognisable as of German origin.

In the curing of Louisiana "Perique" tobacco most of the organic acids present in the leaf are converted into butyric acid, to which the peculiar flavour of this tobacco is due.

E. Quajat (*Bied. Centr.*, 1880, 345) found the ash of 14 samples of dry tobacco (including both superior and common kinds) to range from 31.03% in a Bassano sample to 17.11 in Virginian and 16.78% in Turkish. He considers that the quality of tobacco varies inversely with the ash, but Nessler recognises no relation between the two. The proportion and composition of the ash of English tobacco has been investigated by A. Wingham (*J. Soc. Chem. Ind.*, 1887, **6**, 76, 400), of Indian and Burmese tobaccos by R Romanis (*Chem. News*, 1881, **46**, 248), and of various kinds of tobacco grown in Japan by J. Takayama (*Chem. News*, 1885, **49**, 301), and Fesca and Imai (*J. Soc. Chem. Ind.*, 1888, **7**, 759).

The combustibility of tobacco is profoundly affected by the proportion and nature of the universal constituents, especially the calcium and potassium, and the forms of combination in which these metals occur. The ash of the more combustible tobaccos is comparatively rich in potassium carbonate, showing the presence of a large proportion of organic salts of potassium in the original tobacco, while the ash of tobacco of inferior burning quality contains a larger proportion of sulphates or chlorides, and hence proportionately less alkaline carbonates. According to Schloesing and Nessler tobacco burns best when it contains a considerable proportion of potassium malate, which is a natural constituent of the leaf; but the effect may be imitated, and a slow burning tobacco improved, by the addition of potassium acetate or other organic salt of potassium.

The mode of existence of the *nitrogen* in tobacco has been investi-

gated by Fesca and Imai (*J. Soc. Chem. Ind.*, 1888, **7**, 759), who have published the following among other interesting analytical data:[1]

	Highest percentage	Lowest percentage	Average of 8 samples
In air-dried tobacco:			
Sand	1.91	1 02	1 48
Moisture	12 21	8 39	10 46
In dry, sand-free tobacco:			
Pure ash	14 64	10 68	12 82
Containing soluble CO_2	0 57	0 34	0.44
Containing insoluble CO_2	4 19	3 05	3 54
Containing K_2O	4 73	3.14	3 97
Crude fat	14 44	10 34	12 12
Crude fibre	15 50	13 17	14 10
Total nitrogen	1 69	1 29	1 44
Amino-nitrogen	0 67	0 32	0 48
Albuminoids	3 62	0 69	2 58
Nicotine	4 09	2 63	3 16
Per 100 parts of total nitrogen:			
N as amino-compounds	41 3	23 2	32 7
N as albuminoids	40 0	9 6	29 2
N as nicotine	48 6	29 7	38.1

The influence of the soil and fertilizers used on the quantity and quality of tobacco produced have been investigated by many experimental agricultural stations, and the following tables show the composition of tobacco grown with various fertilizers at the Connecticut Agric. Station in 1896, with the deductions of the Board; the experiments were carried on for 6 years, 1891 to 1896.

[1] Fesca and Imai deduce the following conclusions from their researches The quantity of nicotine may be considered as bearing the same relation to tobacco as the percentage of alcohol does to spirituous liquors; but as yet a high percentage of nicotine has not been shown to be an indication of the good quality of tobacco. Nitric acid should not be found in well-fermented tobaccos Ammonia estimations are frequently too high, as they include some amino-nitrogen 0 1% or so of ammonia does not seem to lower the quality of the tobacco The proteins in a tobacco afford no indication of quality unless the proportion of amines is simultaneously considered The amino-nitrogen represents for the most part harmless, or, perhaps, even beneficial, nitrogenous compounds It is possible that a further study of these substances and their decompositions will reveal the presence of substances exercising a direct influence on the quality of tobacco Anyway, the conversion of proteins into amines is one of the most important results of the fermentation Ordinary fat determinations, or rather extracts, are of no use in tobacco analysis Carbohydrates should not be present in well-fermented tobacco, but a study of the changes they undergo would doubtless be of great value in connection with tobacco. Only considerable differences in the amount of the various constituents of tobacco can give any conclusive indication of the quality of a tobacco Very bad tobaccos always contain much protein matter, sulphuric acid, chlorine, and large quantities of mineral acids, with small proportions of amino-nitrogen, potassium salt, etc By the present methods of analysis it is easier to recognise a bad tobacco than one of good quality. Bases, particularly potash and lime, in medium quantity, are favourable to the good quality, and especially the combustibility, of tobacco An excess of either of these bases over a liberal mean percentage is neither a sign of good quality nor combustibility, and only an exceptionally low percentage of either of them can be regarded with certainty as a bad sign Very high magnesia is prejudicial to the combustibility. Mineral acids in large quantities indicate both bad combustibility and quality, but only a very high proportion of an individual acid can be safely considered a decidedly bad indication The combustibility is influenced to the greatest extent by the quantity of sulpuhric acid present, and in a diminishing degree by the percentage of chlorine, phosphoric acid, and silica in the tobacco The percentage of soluble carbonates appears to have no important influence on the quality and combustibility of tobacco; the influence of the total quantity of carbonates in the ash is much greater, but even in this there is a maximum beyond which the percentage of carbonic anhydride in the ash cannot be regarded as indicating increase of combustibility The relation of carbonates to the mineral acids is a much more important factor, a large preponderance of the former being a favourable sign High basicity of ash is an excellent indication of good combustibility, especially when not due either entirely, or to a great extent, to magnesia or iron.

Plot	Manure used	Quantity of ingredients supplied in lbs. per acre			Tobacco in 1896, lbs. per acre		
		Nitrogen	Phosp. acid	Potash	Total	Long wrapper	Short wrapper
P....	Cotton seed meal, double carbonate of potassium and magnesium and bone.	105	157	340	1255	525	265
D . .	Cotton seed meal, cotton hull ashes	210	195	340	1730	945	275
F . . .	Linseed meal, cotton hull ashes, bone.	105	143	150	1210	400	215
Y ..	Cotton seed meal, wood-ashes.	105	150	340	1390	635	200
AA .	Horse manure .. .	111	71	149	1375	650	160
O . . .	Cotton seed meal, potassium carbonate, bone	105	157	340	1440	680	275
H ...	Castor pomace, cotton hull ashes	210	188	340	1775	940	275
L....	Cotton seed meal, double sulphate of potassium and magnesium, bone.	105	157	340	1515	645	225
BB.	Tobacco stems	111	36	486	1585	830	295
M . .	Cotton seed meal, high grade potassium sulphate, bone.	105	157	340	1540	645	250

TOBACCO GROWN AT CONN. AGRIC. STATION IN 1896. COMPOSITION OF LEAF FROM VARIOUS PLOTS.

Long Wrapper Leaves.

	P %	D %	F %	Y %	AA %	O %	H %	L %	BB %	M %
Ash . ..	15 84	18 04	15 60	17 35	18 26	16.10	17 51	18 33	16 90	18.34
Ether extract .	5 28	4 66	5.38	4 55	5 78	5 29	4 85	4 90	5.26	4.90
Fibre	13 84	12.73	13 91	13 30	14 77	13 75	12 90	13 81	13.62	14.13
Nicotine . .	3 66	3.12	3 35	2 97	2 67	3 19	3 29	2 89	3 66	2.81
Nitric acid	1 48	4.16	0 62	1.43	1 00	1 81	3.76	1.43	3.44	0.61
Proteids.. . . .	19.49	18.46	17.84	17.64	17 07	19.03	18 91	18.12	18.96	18.09
Nitrogen-free extract.. ..	40.41	38.83	43.30	42 76	40.45	40 83	38 78	40.52	38.16	41.12
Total nitrogen. . . .	4.14	4.58	3 59	3.71	3 45	4 06	4.57	3.77	4.55	3.53
Nitrogen as nicotine..	0 64	0.55	0 58	0.52	0.46	0.55	0.57	0.50	0.63	0.48
Nitrogen as nitric acid . .	0.39	1.08	0.16	0.37	0 26	0 47	0.97	0.37	0.89	0.16
Nitrogen as proteid ...	3.11	2 95	2.85	2.82	2.73	3.04	3.03	2.90	3.03	2.89

Short Wrapper Leaves.

	P %	D %	F %	Y %	AA %	O %	H %	L %	BB %	M %
Ash	17 47	19 88	17 80	19 38	20 61	18 20	20 16	21 13	20.13	21.21
Ether extract	5 80	5 29	6 13	5 28	6 01	5 65	5 19	5 10	5 82	5 46
Fibre	12 78	12 03	14 40	13 14	14 15	12 82	12 16	12 58	12 93	12.98
Nicotine	3 50	2 69	2 71	2 31	2 36	2 80	2 58	2 53	2 52	2.24
Nitric acid	0 67	2 47	0 27	0 71	0 41	0 83	3 47	0 54	3 38	0 32
Proteids	13 00	13 67	13 16	13 12	12 99	13 54	14 84	12 80	14 27	13 77
Nitrogen-free extract	46 78	43 59	45 53	46 07	43 47	46 16	41 60	45 32	40 95	44 02
Total nitrogen	2 87	3 29	2 64	2 68	2 59	2 86	3 72	2 62	3 60	2 68
Nitrogen as nicotine	0 61	0 46	0 47	0 40	0 40	0 48	0 44	0 44	0 44	0 39
Nitrogen as nitric acid	0 18	0 64	0 07	0 18	0 11	0 22	0 90	0 14	0 87	0 08
Nitrogen as proteids	2 08	2 19	2 10	2 10	2 08	2.16	2 38	2 04	2 29	2 21

ASH ANALYSIS.

Long Wrappers.

	P %	D %	F %	Y %	AA %	O %	H %	L %	BB %	M %
Silica.	1 64	1 67	1 47	1 17	1 04	1 43	1 52	1.29	1 09	1 32
Potash.....	45 54	44 40	41 88	42 14	47 91	46 56	44 46	38 62	53 76	38.61
Soda .	1 07	0 73	0 85	0 68	0 40	0 59	0 81	0 49	0 48	0 71
Lime..	23 89	30 50	50 95	34 76	19 80	35 85	28 20	32.17	27 35	38 98
Magnesia	16 43	12 18	12 10	9 44	12.16	5 12	13 13	8 87	6 84	3 49
Iron and alumina	2.06	2.03	1 47	1 72	1.14	2 01	1 84	1 78	1 24	2 08
Phoshphoric acid	3 80	3 38	3 38	3 26	3.41	3 57	3 40	3 95	2 98	4 76
Sulphuric acid	4 92	4 40	6 80	5 32	5.41	4 14	5 35	11 11	4.58	9.31
Chlorine	0.84	0 91	0 65	1.95	11 26	0 94	1 85	2 20	2 14	0.96

SHORT WRAPPERS.

	P %	D %	F %	Y %	AA %	O %	H %	L %	BB %	M %
Silica	2 41	2 77	2 41	1 70	1 80	2 69	3 24	1 89	1 95	2 36
Potash	46 34	40 29	39 88	41 43	42 70	44.92	44 48	35.82	51 41	36 62
Soda	76	1 18	1 34	1 24	0.65	0 77	1 03	0.72	1 09	0 90
Lime	18 76	34 07	31 04	36 39	23 45	38 11	25 59	33 68	29 90	40 41
Magnesia	22 37	12 58	14 44	9 19	13 68	5 16	16 12	10 00	6 99	4 71
Iron and alumina	2 57	2 55	2 22	2 37	2 79	2 66	2 06	2 73	2 32	2 10
Phosphoric acid	2 84	2 90	2 71	2 43	2 97	2 62	2 61	3 00	1 91	3 77
Sulphuric acid	3 57	3 27	5 63	4 47	4 51	2 62	4 06	11 23	3 45	8 72
Chlorine	0 49	0 48	0 41	1.00	9 61	0 56	1.03	1 18	1 25	0 52

FIRE-HOLDING TESTS.

	P	D	F	Y	AA	O	H	L	BB	M
Long leaves	4	3	6	1	8	5	9	7	2	10
Short leaves	5	4	2	1	8	3	7	10	6	9

On fermentation the tobacco lost 10%, and was submitted to a tobacco expert who arranged the samples in the following order of commercial merit.

P. Y. AA. H. F. O. D. BB. L. M.

The main influence of the fertilizer is seen in the composition of the ash. There is but little difference in the amount of ether extract, fibre and nitrogen-free extract traceable to the fertilizer used.

Where a large quantity of nitrogen was supplied the nitrates in the leaf increased, also the percentage of nicotine and proteids.

The plot dressed with high grade potassium sulphate contained least potash in the ash, and the burning properties were very unsatisfactory, which agrees with the generally accepted views on the action of sulphates in tobacco.

The plot treated with stable manure contained 5 times as much chlorine as any other. The short leaves were riper when gathered and contained slightly more nicotine, and somewhat less nitrates than the long wrappers.

It appears to be essential to the growing of good crops of tobacco to

manure heavily, as otherwise the ground soon becomes exhausted. As the main growth takes place only in the summer for a period of 2 or 3 months, a larger proportion of available plant food must be present than would be required for crops of a longer period of growth. This has the result that in northern districts more manure is required than in those where the summer heat lasts longer, in order that the tobacco may reach maturity.

Also as good tobacco land is of light texture, it suffers more loss from drainage, decomposition, etc., than soils of a more compact nature.

The quality of smoking tobacco depends largely on its burning properties; if it burns well, less narcotic products are produced than if it burns badly, and so it is free from "rankness," which is due to the presence of large quantities of protein matter in the leaf. Too large a quantity of nitrogen in the fertilizer should be avoided to prevent excessive formation of proteid matter.

From experiments in North Carolina (N. C. Agric. Exp. Stat. Bulletin No. 122) it has been found that nicotine is derived from protein matter, the nitrogen of which is absorbed from the soil as nitrates. When the plant is fully ripened the percentage of nicotine is highest, and only traces of nitrates are present in any part of the plant.

Besides cellulose, protein compounds, pectic acid, gum-resins, and other ordinary plant-constituents, the leaf of tobacco contains a peculiar volatile, crystalline principle called nicotianin or tobacco-camphor, to which the formula $C_{23}H_{32}O_3N_2$ has been attributed. Tobacco also contains the volatile alkaloid nicotine, which is apparently peculiar to the genus. This base exists in combination with malic acid, but the presence of citrates, acetates, and oxalates has also been established.[1]

[1] From 100 grm. of dried tobacco leaves, Goupel obtained from 3 to 4 grm. of ammonium hydrogen malate. J. Takayama (*Chem. News*, 50, 300) obtained the following percentage results by the analysis of Japanese tobacco.

	Nagato	Shimozuki	Settzu	Osumi
Water	6.41	10 01	7.63	13.18
Ash	15 76	8 45	20.71	9.80
Nicotine	2.45	3.02	3.92	1.89
Acetic acid	0.05	0 04	0 01	0 08
Oxalic acid	trace	0 27	0.25	trace
Mallic acid	0.79	1 02	1.83	2 98
Citric acid	0.52	0 59	0.92	0.89
Pectic acid	1.24	5 84	7.42	2.35

Estimation of Non-volatile Acids in Tobacco.—(Kissling, *Chem. Zeit.*, 1904, **28**, 775) 10 grm. of tobacco powder, 10 grm. powdered pumice stone and 10 grm. 20% sulphuric acid are mixed and extracted with ether for 20 hours. The residue obtained by distilling off the ether is dissolved in water and oxalic acid estimated in an aliquot part.

In a similar part the acids are titrated with barium hydroxide solution and barium salts separated by adding alcohol until the solution contains 20%. The precipitate, which is almost pure barium citrate, is quickly filtered and washed with 20% alcohol. On adding sufficient alcohol to the filtrate to bring the alcohol up to 70%, barium malate is precipitated. In 6 samples of tobacco the oxalic acid was 1.84 to 2.77%; malic acid 1.53 to 3.95%; citric acid 3.25 to 7.33%.

It is impossible to determine the actual commercial value of a tobacco by any chemical standard; the utmost that can be done is to point out what substances have, in general, a deleterious effect on the quality; but so many considerations of colour, texture, aroma, weight, freedom from holes and spots, etc., affect the value, that, commercially, tobacco analysis is confined to estimation of moisture, ash and oil for revenue purposes, and the estimation of nicotine in material for insecticides.

Manufactured Tobacco in England.

The excise regulations in England prohibit the use of any materials in the manufacture of tobacco but water, oil, acetic acid and volatile essential oils for flavouring purposes. Any addition of sugary material, liquorice, glycerin, must be made before the tobacco leaves a bonded factory, and such tobacco pays extra duty.

The amount of moisture (which includes water, acetic acid, and other volatile matters) must not exceed 32% (in year 1910), and is defined as the "loss on drying at 212° F. for a period of 18 hours." The oil is estimated by extraction with petroleum ether and saponification of the extract with potassium hydroxide, the result being calculated as olive oil. Oil is only allowed to be added to tobacco which is to be spun into roll or twist.

Snuff is manufactured from heavy "snuff leaf" and from refuse-tobacco, such as stems, tobacco-smalls, and sweepings. These are moistened with water, subjected to a process of fermentation during 6 or 8 weeks, then ground, mixed with alkaline salts as preserva-

tives, and flavoured as desired.[1] In the United Kingdom, nothing is allowed to be added to snuff but the chlorides, sulphates, and carbonates of potassium and sodium, and ammonium carbonate; and any snuff which contains a greater proportion of these salts than 26% on the dry snuff, including the salts natural to the tobacco, is liable to forfeiture and a penalty of £50. As the proportion of alkali salts in tobacco-ash varies considerably, it is important that the manufacturer should know the amount present, in order that he may compound a snuff of uniform composition, and not exceed the legal limit. Of the *salts* allowed to be added to snuff, common salt and potassium and ammonium carbonates are those most commonly used. In addition, most snuff contains from 25 to 45% of *water*, and sometimes a considerable quantity of *sand*, the proportion, according to J. Clark (*J. Soc. Chem. Ind.*, 1884, **3**, 554), averaging 5% on the dry snuff; but ranging from 0.5 to over 10%, and in one case exceeding 30%. A large number of gross and more or less apocryphal adulterants of snuff have been recorded. Among these the sulphides of arsenic, mercury and antimony, lead chromate, potassium dichromate, sulphates of copper and iron, alum, lamp-black, ivory-black, cream of tartar, red ochre, brick-dust, and various organic matters find a place. As snuff is neither a "drug" nor an article of food, it is not liable to examination under the Adulteration Acts, and the Excise systematically ignore sophistications which do not affect the revenue. Hence, authentic information respecting the present adulterations of snuff is very limited.

Insecticides and Germicides.

The A. O. A. C. method for estimating nicotine in insecticides is that of Kissling, and is prescribed as follows in Bulletin 107 (1907):

(1) Solutions Required.

(*a*) *Alcoholic Sodium Hydroxide.*—Dissolve 6 grm. of sodium hydroxide in 40 c.c. of water and 60 c.c. of 90% alcohol.

(*b*) *Sodium Hydroxide.*—Dissolve 4 grm. of sodium hydroxide in 1.000 c.c. of water.

(*c*) *Sulphuric Acid.*—A standard solution.

[1] For flavouring purposes up to 3% of Tonquin Beans may be ground in with the snuff.

(2) Estimation.

Weigh from 5 to 6 grm. of tobacco extract or 20 grm. of finely powdered tobacco, which has been previously dried at 60° so as to allow it to be powdered, into a small beaker. Add 10 c.c. of the alcoholic sodium hydroxide solution and follow, in the case of the tobacco extract, with enough chemically pure powdered calcium carbonate to form a moist but not lumpy mass. Mix the whole thoroughly. Transfer this to a Soxhlet extractor and exhaust for about 5 hours with ether. Evaporate the ether at a low temperature by holding over the steam bath, and take up the residue with 50 c.c. of the dilute sodium hydroxide solution. Transfer this residue by means of water to a Kjeldahl flask, capable of holding about 500 c.c. and distil in a current of steam, using a condenser through which water is flowing rapidly. Use a three-bend outflow tube, a few pieces of pumice, and a small piece of paraffin, to prevent bumping and frothing. Continue the distillation till all the nicotine has passed over, the distillate usually varying from 400 to 500 c.c. When the distillation is complete, only about 15 c.c. of the liquid should remain in the distillation flask. Titrate the distillate with standard sulphuric acid, using phenacetolin or cochineal as indicator. 1 molecule of sulphuric acid is equivalent to 2 mols. of nicotine.

In Kissling's method rosolic acid or iodeosin can also be used as indicator and $N/10$ oxalic acid can be substituted for sulphuric acid.

Tobacco smoke varies in character according to the proportion of air admitted during combustion, oxidation being necessarily more perfect in the case of a cigar than when the tobacco is smoked in a pipe. In the latter case, a portion of the condensible products is deposited in the liquid state. Tobacco smoke consists in part of permanent gases, the proportions of carbon dioxide and carbon monoxide in which have been determined by G. Krause. Vohl found hydrogen sulphide and hydrocyanic acid, and from 0.7 to 2.8 grm. of ammonia per 100 of tobacco smoked.

When tobacco is smoked, the greater part of the nicotine is converted into pyridine and other pyrogenous compounds, and the entire decomposition of the nicotine is sometimes asserted; but Melsens appears to have fully proved the presence of unchanged nicotine in tobacco smoke in a proportion equal to about one-seventh of that present in the original tobacco. Melsens' conclusion has been

endorsed by R. Kissling (*Ding. Polyt. Jour.*, 1882, **244**, 64), who has collected and reviewed the observations of previous investigators. He considers Vohl's conclusion as to the non-existence of nicotine in tobacco-smoke to be due to that chemist having overlooked the fact that the alkaloid is decomposed by warm potassium hydroxide, a reaction which, if a fact, has certainly not met with general recognition

Kissling suggests in his book on tobacco that the nicotine in smoke is present as a salt of an organic acid, but Tóth (*Chem. Zeit.*, 1909, **33**, 866) says that 93% of the organic bases in tobacco smoke are in the free state. Tóth also finds thiocyanates present in the smoke of Hungarian cigars to the amount of 0.027% of the tobacco burnt. He also finds cyanides present to the extent of 0.094% of the tobacco (*Chem. Zeit.*, 1910, **34**, 298).

Lehmann (*Münchner medizin. Wochenscrift*, 1908, **50**, 723) finds sulphur compounds to the extent of 0.02% of the weight of cigars smoked. . He draws attention to the fact that many cigars considered very mild contain as much, and sometimes more, nicotine than others thought to be very strong; and shows that the amount of nitrogenous matter in the leaf seems to bear a close relation to the quantity of nicotine present in the smoke. A similar conclusion is reached by A. Fouquet (*Mem. des Manuf. de l'Etat*, 1908, **81**), who also points out the influence of good combustibility in cigar leaf. By reducing iodic acid Fouquet finds that 6.5 c.c. carbon monoxide are formed for every grm. of tobacco smoked.

By passing the vapour of nicotine through a red-hot tube Cahours and Etard obtained pyridine, β-propylpyridine, picoline and other pyridine bases. The physiological effect of cigar smoke is largely due to the collidine, which is formed in quantity when cigars are smoked. The toxic action of the pyridine compounds increases as the series is ascended.

ACONITE ALKALOIDS.

By FRANCIS H. CARR.

There are many different species of *Aconitum*. Over 50 European,[1] and 24 Indian[2] species have been admitted, and a number of these have been shown to contain alkaloids. The most important group of aconite alkaloids is that of which aconitine, the alkaloid of *Aconitum Napellus*, is the type. Six closely allied members of this group are already known. They possess very similar physiological properties, characterised by excitation of nerve endings, slowing of heart beat and extreme toxicity.

All parts of the plant contain the alkaloid, but the root is richest. If any part of a poisonous aconite plant be chewed, it will be found to have a taste which may be at first bitterish sweet, but after a time becomes acrid and burning, causing a persistent sense of tingling and numbness of the gums and tongues; this effect lasts for some time and is highly characteristic.

For medicinal use, the British, French, German, United States and most other pharmacopœias admit the tuberous roots of *Aconitum Napellus* (Monkshood) and in the French, Russian, Spanish, Portuguese and Mexican the fresh leaves of the same species are also employed.

The root of *A. Napellus* is tuberous, and of an irregular conical form, from 2–3½ inches long and ⅔ to over 1 inch in diameter at the widest portion. It is somewhat wrinkled longitudinally and is more or less covered with scars and bases of broken rootlets.

In commerce it is usually met with dried whole, but occasionally sliced. Externally it is brownish, and white or light brown and starchy within; the transverse section exhibits a 6 to 8 rayed pith,

[1] Gayer (*Vorarbeiten z. e. Monogr. d. Europ. Aconitum Arten*, in *Ungar. Bot Blätt*, Vol. 8, Nos 5, 12).
[2] Stapf ("*The Aconites of India*," *Ann. Roy Bot. Gard*, Calcutta, Vol. 10, Part II).

a small group of vessels at each angle, and a well-marked dark cambium line. The root is biennial and normally paired, 1 tuber of each pair when mature bearing a flowering stem, the other being crowned with a bud. The daughter tubers alone are accepted in the *British Pharmacopœia*. The parent tubers may be distinguished by the scar of the stem and the spongy or hollow condition of the root. The freshly cut section of the undried root rapidly acquires a reddish tint—a character distinguishing it from horse-radish, which it remotely resembles and for which it has been fatally mistaken.

The various natural alkaloids of the aconites are characteristic of the species from which they are derived. Thus aconitine is the peculiar alkaloid of *A. Napellus*, japaconitine of *A. Fischeri* (of most authors) and so on. Besides the eminently poisonous alkaloids of the aconitine group, other species contain alkaloids, which appear in some cases to be poisonous—as the alkaloids of *A. Vulparia* and *A Lycoctonum*—and in other cases are harmless bitter tonics. Thus the alkaloid of *A. paniculatum* (which was the official aconite of the *London and Dublin Pharmacopœias* of 1836) is an inert bitter principle, as are also the alkaloids of *A. heterophyllum* and *A. palmatum*.

Apart from the importance of distinguishing the roots of the poisonous aconites from the non-poisonous roots, the researches of Cash and Dunstan on the physiological action of the various members of the aconitine group indicate that it is extremely important to distinguish poisonous roots from each other; thus aconitine has been shown to possess but two-fifths the activity of pseudaconitine. The amount of alkaloid present in the root also varies from one species to another.[1]

There are at least 12 varieties of aconite indigenous to the United States of America; but these, although probably in many cases active physiologically, are not employed in medicine. One of them—*A. uncinatum*—has been described as poisonous; but according to V. Coblentz the root, although it contains an alkaloid, is entirely devoid of the tingling and numbing taste of *A. Napellus*.

In the following tables are enumerated the chief members of the aconite family which have been submitted to chemical examination and the principal alkaloids obtained from them. The root has been, in every case, employed as the source of the alkaloid.

[1] The root of *Imperatoria Ostruthium* or masterwort has been met with as an adulterant of aconite It resembles aconite tubers in shape but has an aromatic odour and pungent taste, and exhibits in transverse section numerous oil cells arranged in several circles. (Holmes, *Pharm Journ*, [iii], 7, 49).

ACONITE ALKALOIDS.

ACONITINE GROUP.

Species	Habitat	Principal alkaloid	Toxicity
Aconitum Napellus Monkshood	Europe	Aconitine	Very poisonous
Aconitum Sp, *A Fischeri* of authors	Japan (Hondo)	Japaconitine	Very poisonous
Aconitum chasmanthum Stapf	India	Indaconitine	Very poisonous
Aconitum deinorrhizum Stapf *A ferox* of some authors	India	Pseudaconitine	Very poisonous
Aconitum spicatum Stapf *A ferox* of some authors	India	Bikhaconitine	Very poisonous
Aconitum Fischeri of authors Bushi. *A japonicum*	Japan (Hokkaido)	Jesaconitine	Very poisonous

LYCACONITINE GROUP.

Species	Habitat	Principal alkaloid	Toxicity
Aconitum Lycoctonum[1] L	Europe	Lycaconitine . Myoctonine	Poisonous
Aconitum septentrionale (Koelle)	Europe	Lapaconitine Septentrionaline	Poisonous

ATISINE GROUP

Species	Habitat	Principal alkaloid	Toxicity
Aconitum heterophyllum	India	Atisine .	Non-poisonous
Aconitum palmatum	India	Palmatisine	Non-poisonous
Aconitum paniculatum Lam	Europe	(Bitter unnamed alkaloid)	Non-poisonous
Aconitum Anthora L	Europe	(Bitter unnamed alkaloid)	Non-poisonous
Aconitum uncinatum of authors	Japan	(Bitter unnamed alkaloid)	Non-poisonous

[1] In a private communication Dr O Stapf informs the writer that the plant from which lycaconitine and myoctonine were isolated could not have been *A Lycoctonum*, but was probably *A Vulparia*, Reichb At the same time the plant known as *A Septentrionale* Koelle, from which lapaconitine and septentrionaline were prepared, was probably the true *A. Lycoctonum* L.

Constitution and Characters of the Aconite Bases.

Much of the earlier work on the alkaloids of the aconites is of little value. This may be attributed in a large measure to insufficient botanical knowledge and to failure on the part of the investigators to differentiate the roots which they employed; also to the readiness with which the bases undergo hydrolysis, rendering it difficult to obtain them in a pure condition.

The researches of the late C. R. Alder Wright did much to elucidate the matter, and those who have followed him in this field may claim to have settled in a large degree the composition and properties of the principal members of the most important group, namely, the aconitines proper. Of greatest importance are the chemical researches of W. R. Dunstan and his collaborators, the physiological researches of J. Theodore Cash, and the botanical researches of Otto Stapf, but many other workers—notably Freund, Schmidt and Schulze—have contributed results of importance.

The following table shows the leading properties of the known members of the aconitine group and the principal bases derived from them:

	M p	$[\alpha]_D$	Derived bases
Aconitine $C_{34}H_{47}O_{11}N$	197°	+ 12.3 in alcohol	Benzaconine Pyraconitine Aconine Pyraconine
Japaconitine. $C_{34}H_{49}O_{11}N$	204°	+ 23.6° in alcohol	Japbenzaconine Pyrojapaconitine Japaconine
Indaconitine $C_{34}H_{47}O_{10}N$	202–203°	+ 18.2° in alcohol	Indbenzaconine Pyroindaconitine Indaconine
Pseudaconitine. $C_{36}H_{51}O_{12}N$	211–212°	+ 18.6° in alcohol	Veratrylpseudaconine Pyropseudaconine Pseudaconine.
Bikhaconitine. $C_{36}H_{51}O_{11}N$	113–116°	+ 12.2° in alcohol	Veratrylbikhaconine Pyrobikhaconitine Bikhaconine
Jesaconitine $C_{40}H_{51}O_{12}N$	?	?	Aconine

These aconitines possess remarkable similarity as regards both the physiological action which they exert and their chemical constitution. All of them are extremely toxic and contain 4 *methoxyl* groups and 2 *acetyl* groups, which may be successively removed by hydrolysis. The

relationship between them so far as it is known is given in the following table:

Name	Nature	Formula
Aconitine	Acetylbenzoylaconine	$C_{21}H_{27}O_3N$—(OAc, (OMe)$_4$, OBz)
Japaconitine	Acetylbenzoyljapaconine	$C_{21}H_{29}O_3N$—(OAc, (OMe)$_4$, OBz)
Indaconitine.	Acetylbenzoylpseudaconine	$C_{21}H_{27}O_2N$—(OAc, (OMe)$_4$, OBz)
Pseudaconitine	Acetylveratrylpseudaconine	$C_{21}H_{27}O_2N$—(OAc, (OMe)$_4$, $OCOC_6H_3(OMe)_2$)
Bikhaconitine.	Acetylveratrylbikhaconine.	$C_{21}H_{27}ON$—(OAc, (OMe)$_4$, $OCOC_6H_3(OMe)_2$)
Jesaconitine	Benzoylanisylaconine	$C_{21}H_{27}ON$—($OCOC_6H_4(OMe)$, (OMe)$_4$, OBz)

The 6 aconitines are thus seen to be derived from 4 different "aconines"—namely, aconine, pseudaconine, japaconine and bikhaconine. Aconitine and jesaconitine are *diacyl* esters of the same base, viz., aconine. Pseudaconitine and indaconitine are similarly *diacyl* derivatives of pseudaconine. The relation between these aconines has not been established, and further investigation may show—what indeed their composition and properties lead one to expect—that they are quite simply related. Aconitine and japaconitine have been shown to yield triacetyl derivatives, they therefore contain 3 hydroxyl groups, thus accounting for all the oxygen in the molecule. Aconitine has also been shown, by the method of Herzig and Meyer, to contain a methyl group attached to the nitrogen.

Aconitine may be written therefore:

$C_{20}H_{21}N$—(CH_3, $(OH)_3$, OAc, OBz, $(OMe)_4$)

and the members of the series may be regarded as derivatives of a hypothetical base, $C_{20}H_{31}N$.

Aconitine. $C_{34}H_{47}O_{11}N$, *Acetylbenzoylaconine.*

Aconitine is the only crystalline alkaloid of the root of *Aconitum Napellus* L, monkshood (French, *Coqueluchon;* German, *Eisenhut, Sturmhut*). It occurs naturally in combination with aconitic acid, $C_6H_6O_6$ (Vol. I).

Aconitine is difficult to obtain in a state of purity owing to its readily undergoing hydrolysis in the presence of alkalies or acids. The following is the best method of preparing it; success depending largely upon the rapidity with which the whole operation is conducted.

The powdered root is extracted by percolation with a mixture of 5 parts of methyl alcohol with 1 part of amyl alcohol. The extract is concentrated by distillation *in vacuo*, until the temperature of the liquid reaches 60–65°. The oily extract is transferred to a separating funnel and repeatedly agitated with dilute hydrochloric acid which after separation is thoroughly washed with ether to remove amyl alcohol and other extractable matter. The acid solution of the alkaloids is rendered alkaline with a small excess of ammonia and extracted with ether, the ether is then, without concentration, extracted with successive small quantities of 1% hydrochloric acid until the acid is no longer neutralised. A slight excess of ammonia is next added to the combined extract and a little ether is also added from which, after a gentle agitation, the aconitine will crystallise out in white hexagonal crystals. A proportion of it will remain in the ether; this is most readily separated by crystallising as hydrobromide from water.

The purification of aconitine is best achieved by recrystallisation of its salts and finally by crystallising the base itself from methyl or ethyl alcohol. The associated alkaloids being amorphous (although they yield crystalline salts) are entirely eliminated by a final crystallisation of the base.

The composition of aconitine has been the subject of much controversy. The formula originally assigned to it by Wright,[1] $C_{33}H_{43}O_{12}N$, was practically confirmed by Jurgens,[2] who gave it the formula $C_{33}H_{47}O_{12}N$, and by Dunstan and Ince,[3] who adopted the mean formula $C_{33}H_{45}O_{12}N$, but later Freund and Beck[4] proposed

[1] *Trans.*, 1877, 31, 143.
[2] *Inaug. Diss* Dorpat, 1885.
[3] *Trans*, 1891, 59, 271.
[4] *Ber.*, 1894, 27, 433.

the formula $C_{34}H_{47}O_{11}N$, and Schulze, $C_{34}H_{45}O_{11}N$.[1] These two last mentioned formulæ differing by H_2 only have been practically accepted by Dunstan and Henry,[2] for the crystalline aconitine of commerce. They suggest as an explanation of the discrepancies that the alkaloid which the earlier workers employed was derived from a different variety of *A. Napellus*, a conclusion which is by no means improbable in the present state of our botanical knowledge of the species and in view of the close chemical resemblance which exists between the various alkaloids of the aconitine group. Another likely explanation is that incomplete combustion occurred in the determination of carbon and hydrogen. Dunstan and Carr[3] have pointed out that methane is generated during the combustion of aconitine, and that when diluted with nitrogen, methane will pass over heated copper oxide without being completely burnt. Whatever view may be taken of these explanations, the formula $C_{34}H_{47}O_{11}N$ may be regarded as representing our present knowledge of the composition of the aconitine of commerce.

A determination of the crystallographic characters of aconitine has been made by Tutton.[4] The crystals belong to the orthorhombic system, are prismatic and tabular, and may generally be seen as colourless hexagonal transparent plates.

Aconitine is almost insoluble in water, light petroleum and carbon disulphide, readily soluble in benzene and chloroform and less readily in ether, or in methyl and ethyl alcohols. It may be precipitated by adding alkali to solutions of its salts in water as a curdy amorphous white precipitate, which becomes crystalline on the addition of a little ether.

When extremely dilute solutions of its salts are placed upon the tongue a characteristic tingling and numbing sensation is produced, spreading to the lips and pharynx and lasting several hours. This completely masks any taste which it may possess. The alkaloid should never be employed for this purpose, and great caution is required in tasting a solution of a salt In working with aconitine care must be taken to avoid absorption of it through the skin or otherwise. A minute particle of the dust, too small to be seen, if accidentally blown into the eye sets up the most painful irritation and lachrymation,

[1] *Apoth Zeit*, 1904, 19, 782.
[2] *Trans*, 1905, 87, 1650
[3] *Proc*, 1896, 12, 48
[4] *Trans*, 1891, 59, 288.

lasting many hours and accompanied by disturbance of the heart's action, while if inhaled a like amount will produce great bronchial irritation or profuse sneezing and considerable catarrh.[1]

The m. p. of aconitine has been variously stated by different workers, but there is most common agreement for the point 197°. Decomposition occurs at this temperature and is accompanied by ebullition and escape of acetic acid; this change will take place at 188° if the substance is kept at that temperature long enough, hence it will readily be seen that the m. p. will vary according to the length of time taken in heating the bath. The limit of variation recorded by recent workers is 188–203°. It is best to heat the bath to 160° before introducing the substance.

Aconitine is dextrorotatory, $[\alpha]_D + 12.3°$ in alcohol and $+15°$ in dry chloroform; on the other hand its salts are lævorotatory.

Salts of Aconitine.—Aconitine has well marked basic properties and forms a series of crystalline salts. The salts with mineral acids are neutral to the usual indicators but may be titrated with alkali hydroxide and phenolphthalein just as if the acid existed in a free state.

Aconitine nitrate, $\overline{B}$,HNO_3,$5\frac{1}{2}H_2O$, crystallises well from water; it has m. p. 198–199°. A sesquinitrate, $B_2(HNO_3)_3$, has also been described as separating in large crystals from strongly acid solutions.

Aconitine hydrobromide, $\overline{B}$,HBr, crystallises from water in lustrous rhombic plates with $2\frac{1}{2}H_2O$; also from alcohol, or a mixture of alcohol and ether, with $\frac{1}{2}H_2O$. The m. p. is somewhat indefinite, it sinters at 164° but does not melt entirely below 175–180°. Specific rotation in water, $[\alpha]_D - 30.5°$.

Aconitine hydrochloride, $\overline{B}$,HCl, crystallises from water in rhombic plates with $3H_2O$, also from alcohol and ether, m. p. 149–153°, according to the rate of heating; $[\alpha]_D - 35.1°$, in water.

Aconitine hydriodide, $\overline{B}$,HI,$3\frac{1}{2}H_2O$, m. p. 226°, is sparingly soluble in water from which it separates on adding a solution of potassium iodide to a solution of aconitine hydrochloride.

Aconitine periodide,[2] $\overline{B}$,HI,I_2, is prepared by acting on aconitine with iodine in various solvents. It crystallises from alcohol in brown-red crystals, m. p. 211–212°, and is an unstable compound from which

[1] Further details of its toxicity are given on page 283.
[2] Dunstan and Jowett, *Proc*, 1894, 10, 96.

iodine is removed by recrystallisation. By the same reaction there is also produced iodoaconitine, $C_{34}H_{46}IO_{11}N$, a compound devoid of basic properties and non-crystalline, melting vaguely near 208°.

Aconitine thiocyanate is prepared by adding an aqueous solution of ammonium thiocyanate to a saturated solution of the hydrochloride; the crystalline salt which is precipitated, when crystallised from alcohol and ether, melts at 193–195°.

Aconitine aurichloride, $B,\overline{H}AuCl_4$, is thrown down as a yellow amorphous precipitate on adding gold chloride solution to a solution of aconitine hydrochloride or another salt to which sodium chloride or hydrochloric acid has been added. The precipitate is formed in very dilute solutions and is only very sparingly soluble in dilute hydrochloric acid. When washed or dried *in vacuo* the aurichloride may be obtained in 3 modifications.[1]

Aconitine α-aurichloride, m. p. 135°, is obtained by crystallising from acetone, aqueous alcohol or chloroform and ether.

Aconitine β-aurichloride, m. p. 152°, is formed by crystallising from absolute alcohol.

Aconitine γ-aurichloride, m. p. 176°, is prepared by recrystallising the β-modification from a mixture of chloroform and ether.

Chemical Reactions of Aconitine.

The following table gives the reactions of aconitine salts with the common alkaloidal precipitants in aqueous solution.

Iodine solution	Red-brown ppt	1 20,000
Mercury potassium iodide	Yellowish-white ppt	1 10,000
Gold chloride	Yellow ppt	1 5,000
Phospho-molybdic acid	White ppt	1 5,000
Phospho-tungstic acid	White ppt	1 5,000
Picric acid	Yellow ppt	1 4,000
Potassium permanganate	Red crystalline ppt	1 4,000

Mercuric chloride, platinic chloride, potassium chromate, iodide, ferrocyanide and ferricyanide fail to precipitate aconitine salts unless the solution be very concentrated.

The most characteristic of the above mentioned tests is that with potassium permanganate[2] which is best applied by faintly acidifying with acetic acid and adding a few drops of $N/5$ potassium permanganate solution; the precipitate as seen under the microscope consists of rosettes of red prismatic crystals. Cocaine, hydrastine and papaverine yield similar precipitates in concentrated solution, but not at

[1] Dunstan and Jowett, *Trans.*, 1893, 63, 994; *Proc.*, 1895, 11, 27.
[2] Dunstan and Carr, *Pharm. Journ.*, 1896 [iv], 2, 122.

dilutions of 1 in 500. The presence of other alkaloids, including those associated with aconitine in the root and in the amorphous aconitine of commerce, considerably modifies its sensitiveness.

Various colour tests for aconitine have been proposed but they may be relied upon only as supplementary tests to aid identification.

When dropped upon nitric acid (1.43) no colour is produced; sulphuric acid (1.75) also produces no colour, but on adding a crystal of ammonium vanadate an orange colour is formed. If 0.0002 grm. of aconitine be gently warmed on a water-bath with 4 drops of sulphuric acid (1.75) a smell of benzoic acid is observed. If at the end of 5 minutes a few crystals of resorcinol be added and the heating continued, a reddish-yellow colour changing to intense red is produced. Other colour tests which have been proposed[1] are of doubtful value.

When heated in a tube at 190° aconitine decomposes with effervescence and acetic acid is volatilised. This test may be made use of to assist identification, but by far the most characteristic property of the alkaloid is the tingling and numbing sensation produced when a very dilute solution is tasted (see page 282).

When hydrolysed with boiling alcoholic sodium hydroxide aconitine yields 9.3% of acetic acid and 18.8% of benzoic acid. This change, together with the specific rotatory power and m. p. of the various salts given in the text, assists in distinguishing between the different members of the aconitine group.

Derivatives of Aconitine.

Diacetyl aconitine,[2] $C_{34}H_{45}(Ac_2)O_{11}N$, is obtained when acetyl chloride is allowed to react with aconitine in dry chloroform.

It is a crystalline base melting at 158° and forming stable salts. Its physiological action, though weaker than that of aconitine, closely resembles it.

Triacetyl aconitine,[1] $C_{34}H_{44}(Ac_3)O_{11}N$, results when an excess of acetyl chloride acts on aconitine. It crystallises in fine needles, m. p. 207°, is feebly basic, and forms unstable salts. Like the *diacetyl* derivatives it possesses the characteristic physiological action of aconitine.

Acetic anhydride does not act on aconitine.

[1] Reichard, *Pharm. Centralh*, 1905, 46, 476. Alvarez, *Chem. News*, 1905, 91, 179.
[2] Dunstan and Carr, *Trans*, 1895, 67, 459.

Methyl aconitine has not been purified, but evidence that it is produced, when methyl iodide acts upon aconitine, was given by Dunstan and Jowett[1] who were unable. however, to isolate aconitine methiodide. They consider that the action takes place according to the equation:

$$2C_{34}H_{47}O_{11}N + MeI = C_{34}H_{47}O_{11}N,HI + C_{34}H_{46}(Me)O_{11}N.$$

Hydrolysis of Aconitine.

Whether the hydrolysis of aconitine be effected by acids, alkalies, or by heating the base in a sealed tube with water alone, 1 molecular proportion of acetic and benzoic acids may be ultimately split off and the base aconine remains. If, however, a neutral aconitine salt, preferably the sulphate or hydrochloride, be heated in 5% aqueous solution in a sealed tube to 120–130° for about 3 hours, acetic acid only is split off and benzaconine results.[2]

$$C_{34}H_{47}O_{11}N + H_2O = C_{32}H_{45}O_{10}N + CH_3COOH.$$

In the second stage of hydrolysis benzaconine undergoes change into aconine and benzoic acid, according to the equation:

$$C_{32}H_{45}O_{10}N + H_2O = C_{25}H_{41}O_9N + C_6H_5COOH.$$

Benzaconine (picraconitine, napelline, benzoyl-aconine), $C_{32}H_{45}O_{10}N$, is an amorphous alkaloid which occurs in the roots of *A. Napellus*. It is slightly soluble in water, giving a bitter tasting solution, which does not, however, produce the tingling sensation so characteristic of aconitine. The removal of the *acetyl* group renders it no longer poisonous in the ordinary acceptation of the term and its action on the heart is to some extent antagonistic to that of aconitine. Benzaconine is readily soluble in alcohol, chloroform and ether. $[\alpha]_D$ in alcohol + 5.6°. It forms well crystallised salts.

Benzaconine hydrochloride, $\overline{B},HCl,H_2O$, crystallises in two forms: (1) in small crystals, m. p. 217°, (2) in long needle-shaped prisms, m. p. 268°. $[\alpha]_D$ in water −28.7°.

Benzaconine hydrobromide, $\overline{B},HBr$, crystallises in long needles, m. p. 282°.

Benzaconine hydriodide, $\overline{B},HI$, forming granular crystals, sparingly soluble in water, may be prepared by adding potassium

[1] Dunstan and Jowett *Proc*, 1894, 10, 96
[2] Dunstan and Carr, *Trans*, 1894, 65, 290, and Freund and Beck, *Ber.*, 1894, 27, 433.

iodide solution to a solution of benzaconine hydrochloride. It crystallises in 2 forms, m. p. 204° and 246°.

Benzaconine aurichloride, $\overline{B}$,$HAuCl_4$, may be precipitated from water by adding a solution of gold chloride to an aqueous solution of benzaconine hydrochloride and it may be crystallised from alcohol. It has m. p. 135°. It readily parts with 2 molecular proportions of hydrogen chloride, forming an aurichlor-derivative; the best method for inducing this change is to dissolve the dry aurichloride in absolute alcohol and add light petroleum when

Aurichlorbenzaconine,[1] $C_{31}H_{44}O_{10}NAuCl_2$, slowly separates in colourless crystals, m. p. 204°.

Benzaconine forms *diacetyl*, *triacetyl* and *tetracetyl* derivatives.[2] Triacetylbenzaconine and tetracetylbenzaconine are not identical with the respective isomers, diacetylaconitine and triacetylaconitine and are unlike them in being comparatively non-toxic.

Aconine results from the second stage of the hydrolysis of aconitine, and is best prepared by heating aconitine with steam in an autoclave at a pressure of 6 or 7 atmospheres and extracting the resulting liquid after the addition of alkali, with chloroform.

Aconine, $C_{25}H_{41}O_9N$, is a colourless amorphous resin-like, hygroscopic alkaloid. It probably occurs in aconite root associated with other amorphous alkaloids. It is soluble in water, chloroform, alcohol and acetone, only very sparingly soluble in ether and petroleum, has m. p. 132° and $[\alpha]_D$ in water +23°.

Aconine is not poisonous but is a cardiac tonic and acts antagonistically to aconitine to a greater extent even than benzaconine.

Aconine hydrochloride, $\overline{B}$,HCl,$2H_2O$, crystallises in colourless lamellar plates, m. p. 175–176°, extremely soluble in water. $[\alpha]_D - 7.7°$ in water.

Aconine hydrobromide, $\overline{B}$,HBr,$1\frac{1}{2}H_2O$, colourless crystals, m. p. 225°.

Dibenzoylaconine and **tetracetylaconine**,[3] m. p. 196°, have been prepared. Unlike aconine, they are both crystalline, insoluble in water and soluble in ether.

When aconine is oxidised with alkaline permanganate, acetaldehyde

[1] Dunstan and Harrison, *Trans.*, 1893, 63, 443.
[2] Dunstan and Carr, *Trans*, 1895, 67, 459.
[3] Dunstan and Carr, *Proc.*, 1895, 11, 178.

s produced and also a new base which forms a crystalline *hydrochloride*, $C_{24}H_{37}O_8N,HCl,3H_2O$,[1] m. p. 213°, is produced.

When aconitine is heated with methyl or ethyl alcohol in a sealed ;ube to 120–130° the negative *acetyl* group is replaced by the positive *nethyl* or *ethyl* group. Since it is the *acetyl* group which determines :he intense physiological activity of aconitine, it is of great interest that when a *methyl* group occupies the same position, the toxicity is reduced o $\frac{1}{80}$–$\frac{1}{100}$ that of the parent base. Methyl-benzaconine is, however, more toxic than benzaconine in which hydrogen occupies the position of the *acetyl* group. It exerts a curare-like action upon motor nerves.

Methyl-benzaconine,[2] $C_{33}H_{47}O_{10}N$, crystallises in colourless rectangular plates, m. p. 210–211°, and forms crystalline salts.

Ethyl-benzaconine,[3] $C_{34}H_{49}O_{10}N$, crystallises in colourless glistening crystals, m. p 188°.

When heated at 190° aconitine melts, giving off 1 mol. of acetic acid, and a new residual base, *pyraconitine*,[4] is formed.

Pyraconitine, $C_{32}H_{43}O_9N$, is sparingly soluble in water, readily soluble in ether, chloroform and alcohol and crystallises from ether in white slender needles, m. p. 167–168°; it is optically inactive though its salts are lævorotatory, it has a bitter taste and though devoid of the characteristic physiological action of aconitine it slows the heart and is considerably more toxic than benzaconine.

Pyraconitine hydrobromide crystallises in two forms, m. p. 204° and 280°. $[\alpha]_D$ in water $-46.8°$.

Pyraconitine hydrochloride forms acicular prisms, m. p. 249°.

Pyraconitine and its salts readily undergo hydrolysis when heated with water, yielding benzoic acid and pyraconine.

Pyraconine, $C_{25}H_{37}O_9N$, is amorphous, readily soluble in water and also in ether, $[\alpha]_D$ in water $-91°$. It forms crystalline salts.

Pyraconine hydrochloride, $\overline{B},HCl,H_2O$, crystallises in colourless cubes with 1 mol. H_2O, which is lost at 100°, m. p. 154°, $[\alpha]_D$ in water $-102°$.

Pyraconitine forms a crystalline *triacetyl*-derivative which is not toxic; m. p. 204°.

[1] Schulze, *Arch. Pharm.*, 1906, 244, 165.
[2] Dunstan, Tickle and Jackson, *Proc.*, 1896, 12, 159.
[3] Schulze, *Arch Pharm*, 1906, 244, 165
[4] Dunstan and Carr, *Trans*, 1894, 65, 178.

Amorphous Alkaloids of A. Napellus.

In addition to aconitine, benzaconine and aconine, indications of the presence of other uncrystallisable alkaloids in *A. Napellus* have been met with by several observers; but since these alkaloids are stated to be non-toxic, and have not been isolated or purified, no great importance can be attached to them.

Japaconitine, $C_{34}H_{49}O_{11}N$, acetylbenzoyljapaconine, is the characteristic toxic alkaloid of the roots of a species of *Aconitum* from Hondo, usually but erroneously referred to as *A. Fischeri*,[1] a Kamtchatka species. It is indigenous in Japan, where its tubers are known as "Kusa-uzu" or "Bushi." These names are applied to two species but it is that grown in Hondo which affords this alkaloid. It may best be prepared by the method given (page 258) for aconitine, and purified in the same manner.

Wright regarded japaconitine as chemically different from aconitine, but later Lubbe, and Freund and Beck[2] pronounced them to be identical. More recently, however, the composition and properties of japaconitine have been fully investigated by Dunstan and Read,[3] who show that although aconitine and japaconitine are remarkably alike in their characters yet they are distinct and separate chemical individuals. This conclusion has since been confirmed by Makoshi.[4] Dunstan and Read assigned to it the formula $C_{34}H_{49}O_{11}N$. Makoshi, however, gives preference to $C_{34}H_{47}O_{11}N$, but the accuracy of the method of analysis is hardly such as to permit of a decision between these two formulæ.

The crystallographic characters of japaconitine have been described by Pope,[5] who states that no points of similarity with aconitine or pseudaconitine are traceable. The crystals are very small, transparent, colourless needles, their sides being made up of two parallel prism faces.

Japaconitine resembles aconitine in its solubility in various solvents, the pure base melts at 204–205° with effervescence and evolution of acetic acid. In nearly all respects it shows a remarkable similarity to aconitine, yet it is chemically a distinct individual. Japaconitine

[1] It was formerly supposed that the plant which yields japaconitine was *A. Fischeri* Reichb, but Prof Miyabe, a Japanese authority, finds that it is not the true Reichenbachian species, but a distinct variety which he names *A. Fischeri*, var.
[2] *Ber*, 1894, 27, 723
[3] *Trans*, 1900, 77, 45
[4] *Arch Pharm*, 1909, 247, 270.
[5] *Trans*, 1900, 77, 49.

undergoes a similar change on hydrolysis in two stages with the formation of acetic and benzoic acids, it possesses 4 methyl groups, and 3 hydroxyl groups—since it forms a *triacetyl* derivative. When heated at its melting-point it is split up into pyrojapaconine and acetic acid. It differs from aconitine in yielding a crystalline methiodide and methyl derivative, in the m. p of the hydriodide, and the specific rotation of both the base and its salts.

The physiological action of japaconitine differs from that of aconitine in degree only and not in kind, being slightly more toxic than the latter alkaloid.

The following table gives the properties of the principal salts and derivatives of japaconitine:

Substance	Formula	M p	$[\alpha]_D$	Remarks
Japaconitine	$C_{34}H_{49}O_{11}N$.	204°	+20 2° in chloroform, +23 6° in alcohol	Prismatic needles
-hydrobromide	$C_{34}H_{49}O_{11}N,HBr,4H_2O$	172–173°		Rosettes of hexagonal plates
-hydrochloride	$C_{34}H_{49}O_{11}N,HCl,3H_2O$	149–150°	−23 8° in water	Rosettes of hexagonal plates
-hydriodide	$C_{34}H_{49}O_{11}N,HI$	208–210°		Small rosettes
-nitrate	$C_{34}H_{49}O_{11}N,HNO_3$	173–177°		Minute rosettes
-aurichloride	$C_{34}H_{49}O_{11}N,HAuCl_4$	α 153° β 231°		Yellow prisms Opaque canary-yellow needles
Triacetyljapaconitine	$C_{34}H_{46}Ac_3O_{11}N$.	189°		Colourless rosettes
Japbenzaconine	$C_{32}H_{47}O_{10}N$.	182–183°	+40 2° in ethyl alcohol	Rectangular plates
-hydrobromide	$C_{32}H_{47}O_{10}N,HBr$	205–217°		Small prisms
-hydrochloride	$C_{32}H_{47}O_{10}N,HCl,H_2O$	253°	−19 7° in water	Rosettes of needles
Aurichlorjapbenzaconine.	$C_{32}H_{46}O_{10}N,AuCl_2$	178°		Colourless lustrous octahedra
Japaconine . . .	$C_{25}H_{43}O_9N$.	97–100°	+10 9° in water	Amorphous hygroscopic varnish

Substance	Formula	M p	$[\alpha]_D$	Remarks
-hydrobromide .	$C_{25}H_{43}O_9N,HBr$.	221°	. ..	Triangular plates.
Tetracetyljapaconine.	$C_{25}H_{39}Ac_4O_9N$.	231–232°	.. ..	Compact colourless crystals.
Pyrojapaconitine .. .	$C_{32}H_{45}O_9N$.	165–168°	−65.9 in ethyl alcohol	Colourless needles
Pyrojapaconine.. ..	$C_{25}H_{41}O_8N$	128°	−74° in water	Amorphous hygroscopic varnish.

For the description of other salts and further details of the properties of those mentioned, the reader is referred to the original communications of Dunstan and Read, and Makoshi (*loc. cit.*).

Indaconitine, $C_{34}H_{47}O_{10}N$, acetylbenzoylpseudaconine, is the characteristic alkaloid of *Aconitum chasmanthum*, Stapf, which species is indigenous in India and is known to the natives as "Mohri." The plant was formerly considered to be identical with the European *A. Napellus*, to which it is allied.

Indaconitine has been investigated by Dunstan and Andrews.[1] The method of preparation is similar in all respects to that given for aconitine, which alkaloid, like japaconitine, it closely resembles in general properties. It melts with decomposition at 202–203°, is soluble in alcohol, chloroform, acetone and ether and insoluble in water and benzin. By rapid crystallisation from ether the base is deposited in rosettes of needles, but if crystallised slowly it is obtained in transparent hexagonal prisms or large irregular masses. This property distinguishes it from aconitine, with which, however, it is apparently isomorphous.

It resembles aconitine and japaconitine in undergoing hydrolysis in 2 stages. In the first, it forms acetic acid and indbenzaconine and in the second, benzoic acid and pseudaconine, the latter being the ultimate hydrolytic product of pseudaconitine.

When heated at its m. p. acetic acid splits off and pyroindaconitine is formed. Like the other members of this group of alkaloids it contains 4 methoxyl groups.

[1] *Trans.*, 1905, 87, 1620.

The physiological action[1] of indaconitine closely resembles that of aconitine and differs from it very slightly in degree. It produces the tingling sensation on the tongue which is characteristic of the entire group.

Indaconitine may be distinguished from aconitine by the rotatory power of its salts, which is much lower than that of aconitine salts.

The properties of indaconitine and its principal salts and derivatives are given in the following table:

Substance	Formulæ	M p	$[\alpha]_D$	Remarks
Indaconitine .	$C_{34}H_{47}O_{10}N$	202–203°	+18 2° in ethyl alcohol	Rosettes of needles and hexagonal prisms
-hydrobromide .	$C_{34}H_{47}O_{10}N,HBr$	α183–187° β217–218°	−17.3° in water.	Large hexagonal prisms from water.
-hydrochloride .	$C_{34}H_{47}O_{10}N,HCl,3H_2O.$	166–171°	−15 8° in water.	Glistening scales or rosettes of needles from alcohol and ether Hygroscopic
-nitrate . .	$C_{34}H_{47}O_{10}N,HNO_3$	202–203°		Small masses of prismatic crystals
-aurichloride .	$C_{34}H_{47}O_{10}N,HAuCl_4$	147–152°		Rosettes of needles from chloroform and ether
Indbenzaconine .	$C_{32}H_{45}O_9N$	130–133°	+33 6° in ethyl alcohol	Amorphous
-hydrobromide .	$C_{32}H_{45}O_9N,HBr,2H_2O.$	247°	.	Large irregular rosettes efflorescent
-hydrochloride .	$C_{32}H_{45}O_9N,HCl$	242–244°	−8 0° in water	Crystalline.
Aurichlorindbenzaconine.	$C_{32}H_{44}O_9N,AuCl_2$	234–235°	.	Minute colourless crystals
Indaconine	$C_{25}H_{41}O_8N$	94–95°	+38 2° in water	Crystallises with 1 mol of alcohol
Pyroindaconitine . . .	$C_{32}H_{43}O_8N.$		+91 9° in ethyl alcohol	Amorphous
-hydrobromide .	$C_{32}H_{43}O_8N,HBr.$	194–198°	+54 7° in water.	Crystalline

[1] Cash and Dunstan, *Proc Roy. Soc.*, 1905, 76, 468.

Pseudaconitine, $C_{36}H_{49}O_{12}N$, acetyl-veratrylpseudaconine, is the chief alkaloidal constituent of *Aconitum deinorrhizum* Stapf, indigenous in Bashahr and of *A. Balfourii* Stapf, a native of Garhwal, Kumaon and Nepal. Both were formerly included under *A. ferox*—a species only found once by Wallich in Northern Central Nepal and both yield "bikh" or "bish" poisons.[1]

Pseudaconitine is best prepared in the manner described for aconitine (page 258), and may be recrystallised from a mixture of chloroform, ether and petroleum.

Its crystallographic characters have been described by W. J. Pope;[2] the crystals are colourless and transparent rhomboidal in shape, and belong to the monosymmetric system.

Pseudaconitine resembles aconitine in its solubility in various solvents, but is rather less soluble in ether; it melts with decomposition at 211–212°, giving off acetic acid. It undergoes hydrolysis in two stages with the formation of acetic acid and veratrylpseudaconine in the first and veratic acid and pseudaconine in the second. When heated at its m p. acetic acid is split off and pyropseudaconitine is produced. It possesses 6 *methoxyl* groups, two of which form part of the *veratryl* residue.

The physiological action of pseudaconitine resembles that of the other members of the group, but is more intense than any of them; it becomes therefore important to distinguish it from aconitine. This may best be done by the m. p. of its aurichloride and by the fact that it yields veratric acid, m. p. 178°, when hydrolysed with alcoholic potassium hydroxide.

The following table gives the properties of the principal salts and derivatives of pseudaconitine:

Substance	Formulæ	M p	$[\alpha]_D$	Remarks
Pseudaconitine	$C_{36}H_{49}O_{12}N$.	211–212°	+18 6° in ethyl alcohol	Rhomboidal crystals.
-hydrobromide	$C_{36}H_{49}O_{12}N,HBr,2H_2O$	191°	−19 5° in water	Rosettes of cubical crystals
-hydrochloride .	$C_{36}H_{49}O_{12}N,HCl$	. .	.	Not crystallised

[1] See "The Aconites of India," Otto Stapf, *Ann Roy Bot Gard*, Calcutta, 1905, vol x, part 2; also "The Constituents of Some India Aconites," Dunstan, *Agricultural Ledger*, 1897, No. 19, 373

[2] Dunstan and Carr, *Trans*, 1897, 71, 350.

Substance	Formulæ	M p	$[\alpha]_D$	Remarks
-hydriodide	$C_{36}H_{49}O_{12}N,HI$	215–217°		Crystallises in prisms
-nitrate	$C_{36}H_{49}O_{12}N,HNO_3,H_2O$	192°		Thin prisms
-aurichloride .	$C_{36}H_{49}O_{12}N,HAuCl_4$	236°		Crystalline
Veratrylpseudaconine.	$C_{34}H_{47}O_{11}N,H_2O$	199°	−38 3° in ethyl alcohol	Crystalline
-hydrobromide	$C_{34}H_{47}O_{11}N,HBr,3H_2O$			Large prismatic crystals
-hydriodide	$C_{34}H_{47}O_{11}N,HI$	205–207°		Prisms
-nitrate	$C_{34}H_{47}O_{11}N,HNO_3$	222–232°		Rosettes of rhombic prisms
Pseudaconine .	$C_{25}H_{39}O_8N$	94–95° (from alcohol)	+30 1° in water	Crystallises combined with alcohol or actone Its salts are not crystalline
Pyropseudaconitine	$C_{34}H_{45}O_{10}N$	86–87° (from acetone)		Amorphous

For further particulars of the properties of the salts and derivatives of pseudaconitine the reader is referred to the papers of Dunstan and Carr,[1] and Freund and Niederhofheim.[2]

Bikhaconitine, $C_{36}H_{51}O_{11}N$, acetyl-veratryl-bikhaconine, has been fully investigated by Dunstan and Andrews;[3] it is the highly toxic crystalline alkaloid of *Aconitum spicatum* Stapf and *A. laciniatum* Stapf, both natives of Sikkim and probably extending into Sikkim and Bhutan. These plants were formerly like *A. dionorrhizum* Stapf, and *A. Balfourii* Stapf included in *A. ferox*, and in common with them are known commercially as "bikh" or "bish." The alkaloid is extracted by the method employed for the other members of the group but it does not crystallise so readily, the best method of causing it to do so being the gradual addition of water to an alcoholic solution. The manner in which it crystallises, especially from ether, serves to distinguish it from the other aconitines; from this solvent it separates as white button-shaped masses composed of concentric rings of the substance. When crystallised from ether it melts without decomposition at 118–123°. Its solubility in various solvents corresponds with that of the other members of this group.

[1] *Trans.*, 1897, 71, 350.
[2] *Ber.*, 1896, 29, 852.
[3] *Trans.*, 1905, 87, 1636.

Bikhaconitine undergoes hydrolysis in 2 stages, forming acetic acid and veratryl-bikhaconine in the first and veratric acid and bikhaconine in the second. When heated to 180° it undergoes decomposition, forming acetic acid and pyrobikhaconitine. It contains 6 *methoxyl* groups, two of which are contained in the *veratryl* residue.

The physiological action of bikhaconitine differs in degree and not in kind from that of the other aconitines, this member of the group being intermediate in toxicity between japaconitine and pseudaconitine. It may be readily distinguished from the other aconitines by the m. p. and crystalline character of the base. The chief properties of the principal salts of bikhaconitine and of its derivatives are given in the following table:

Substance	Formulæ	M p	$[\alpha]_D$	Remarks
Bikhaconitine.....	$C_{36}H_{51}O_{11}N,H_2O$.	113–116°	+12 2° in ethyl alcohol.	Crystalline button shaped glomerates
-hydrobromide.....	$C_{36}H_{51}O_{11}N,HBr,5H_2O$	173–175°	−12 4° in water	Crystalline
-hydrochloride....	$C_{36}H_{51}O_{11}N,HCl,5H_2O$.	159–161°	−8 8° in water	Crystalline
-hydriodide.....	$C_{36}H_{51}O_{11}N,HI,2\frac{1}{2}H_2O$	193–194°		Crystalline.
-nitrate.....	$C_{36}H_{51}O_{11}N,HNO_3$	178–180°	. . .	White needles
-aurichloride...	$C_{36}H_{51}O_{11}N,HAuCl_4$	232–233°	.	Canary yellow needles
Veratrylbikhaconine.	$C_{34}H_{49}O_{10}N$.	.	+29 9° in ethyl alcohol	Uncrystallised
-hydriodide.....	$C_{34}H_{49}O_{10}N,HI$.	189–190°	.....	Rosettes of needles.
-nitrate.....	$C_{34}H_{49}O_{11}N,HNO_3$.	175–178°	.....	Rosettes of acicular prismatic hexagons
Bikhaconine.....	$C_{25}H_{41}O_7N$.	..... .	+33 8° in ethyl alcohol.	Uncrystallised, soluble in ether.
-hydrobromide.....	$C_{25}H_{41}O_7N,HBr$.	125–130°	....	Tetragonal prisms.
-nitrate.....	$C_{25}H_{41}O_7N,HNO_3,2H_2O$.	125–128°	+15.4° in water.	Tetragonal prisms.
Pyrobikhaconitine...	$C_{34}H_{47}O_9N$.	... .	.....	Neither the base nor its salts crystallise.

For fuller description of the salts and derivatives of Bikhaconitine the reader is referred to the paper by Dunstan and Andrews.[1]

Jesaconitine, $C_{40}H_{51}O_{12}N$, anisylbenzoylaconine, is an amorphous alkaloid contained in the roots of an aconite from Hokkaido (Yeso) which is identical with *A. japonicum*, Thunb, but usually mistaken for *A. Fischeri*, Reichb. It has also been confused with an aconite from Hondo yielding japaconitine as the tubers of both species are known as "Kusa-uzu" and "Bushi" (see under Japaconitine).

Jesaconitine has been investigated by Makoshi,[2] who was unable to crystallise it or its salts. It possesses the typical physiological action of aconitine and is the only member of the series which does not contain an acetyl group.

On hydrolysis it yields anisic and benzoic acids and the base aconine, identical with that obtained from aconitine of *A. Napellus*. The presence of the *anisyl* radicle distinguishes it from all the other aconite alkaloids which have been investigated.

Although it does not yield crystalline salts, jesaconitine yielded a crystalline *triacetyl* derivative when treated with acetyl chloride. Triacetyljesaconitine, $C_{40}H_{48}O_{12}NAc_3,2H_2O$, crystallises in small needles, m. p. 213°; it is readily soluble in alcohol and less readily in ether.

LYCACONITINE AND MYOCTONINE.

The root of *Aconitum Vulparia* (*A. Lycoctonum of authors*),[3] a species of aconite growing in the Alps and bearing yellow flowers, has been found to contain 2 alkaloids which differ from the bases isolated from other aconites. So far, the products of hydrolysis of these bases have not been fully studied, and some obscurity rests on other of their characters.

For the extraction of the bases of *A. Valparia*, Dragendorff and Spohn[4] exhausted the roots with alcohol acidified with tartaric acid. The extract was concentrated, mixed with water, filtered, and repeatedly agitated with ether while still acid. The aqueous liquor was then treated with sodium hydrogen carbonate and extracted with ether, which removed lycaconitine (1.13%). Subsequent agitation with

[1] *Trans.*, 1905, 87, 1636.
[2] *Arch. Pharm.*, 1909, 247, 251.
[3] See footnote to page 271
[4] *Pharm. Zeitsch. für Russland*, 1884, 23, 313.

chloroform removed the remainder of the lycaconitine, together with myoctonine (0.8%). The successive treatment with ether and chloroform removed all but traces of alkaloid from the solution. Neither base could be obtained crystalline.

Lycaconitine, $C_{27}H_{34}O_6N_2,2H_2O$, was obtained, after further purification by ether of the base extracted as above, in the form of a pale yellow resinous substance.

After drying *in vacuo*, the base begins to melt at 112°, and at 115° is completely fused with decomposition. It is sparingly soluble in water; very readily in absolute alcohol, chloroform, carbon disulphide and benzene; less readily in ether; and practically insoluble in petroleum spirit. A 10% solution of the base in alcohol has $[\alpha]_D + 31\ 5°$. An aqueous solution of the nitrate shows $[\alpha]_D + 19.4°$.

None of the salts of lycaconitine has been crystallised. The *nitrate* can be obtained and purified by dissolving the base in ether and cautiously adding nitric acid mixed with ether. The nitrate is precipitated, the first fraction carrying down any colouring matter contained in the solution.

With strong sulphuric acid, lycaconitine gives a reddish-brown colouration, and with syrupy phosphoric acid a violet colouration on warming. When treated with a mixture of 8 c.c. of water, 6 c.c. of strong sulphuric acid and 0.3 grm. of sodium selenate, lycaconitine is coloured a rose or pale reddish-violet—a reaction which is not exhibited by the bases from other species of aconite.

Lycaconitine is incompletely precipitated by potassium hydroxide, alkali carbonates and ammonia. Alkali hydroxides slowly decompose it, however. Thus, when warmed for a few minutes to a temperature of 35° with a 4% solution of sodium hydroxide it dissolves, and crystalline lyaconine separates from the liquid and may be extracted by ether. By agitation with chloroform a second base can be extracted, while the salts of lycoctonic acid and of another acid, probably a dihydroxybenzoic acid, remain in the solution.

Lyaconine, $C_{27}H_{46}O_7N_2,1\frac{1}{2}H_2O$, is apparently identical with the base described by Hubschmann under the name of *lycoctonine.*[1]

[1] A specimen of lycoctonine from *A. Lycoctonum* (*A Vulparia*), presented by Hübschmann to Flückiger, is described by the latter chemist (*Year-book Pharm.*, 1870, 96) as being crystallised in perfectly white and distinct prisms and needles melting at 98–104° without decomposition. The base was soluble in alcohol, ether, chloroform, amyl alcohol, petroleum spirit and carbon disulphide

The aqueous solution of the base had an alkaline reaction and intensely bitter taste The physiological effects of lycoctonine were found to differ from those of the other aconite bases both in degree and kind As a poison, lycoctonine was found much less energetic than aconitine. Mercuric chloride, platinic chloride, phosphomolybdic acid and potassium iodide

It melts at 90–92°, has an alkaline reaction, and an optical activity of $[\alpha]_D + 46.4°$. It is very soluble in alcohol and chloroform, less readily in ether and benzene, and dissolves in about 250 parts of water. Its solution has an alkaline reaction, exhibits a fine blue fluorescence, is coloured purple by chlorine water, and is precipitated by the ordinary reagents for alkaloids.

The *aurichloride*, *platinichloride* and *nitrate* of the base have been prepared.

Acolyctine, a base described by Hubschmann, is probably identical with the second base extracted by Dragendorff and Spohn from the product of the action of alkali hydroxide on lycaconitine. It is probably a product of the further action of the alkali on lyaconine (lycoctonine). It is described as a white powder, soluble in water, alcohol and chloroform, but insoluble in ether. It forms white precipitates with tannic acid and lead acetate, and a yellow one with gold chloride. Its sulphate forms a white precipitate with ammonium molybdate. Acolyctine produces physiological effects similar to those of myoctonine, but less powerful.

Lycoctonic acid, $C_{27}H_{18}O_7N_2$, produced by the action of alkalies on lycaconitine (or by heating the base with water or dilute acid in a sealed tube), is crystallisable and melts at 146–148°. It is sparingly soluble in water, moderately in ether, and readily in alcohol and chloroform.

Myoctonine, according to Dragendorff and Spohn, has the formula $C_{27}H_{30}O_8N_2,5H_2O$, while Einberg regards it as $C_{40}H_{56}O_{12}N_2,5H_2O$, the water being lost on drying in a current of air at 60°. It is amorphous, has a bitter but not pungent or tingling taste, melts at 143–144°, and is dextrorotatory· $[\alpha]_D$ of the alkaloid in 10% solution in alcohol = +29.5°; of the nitrate in aqueous solution, +21.2°. It is difficultly soluble in water, but very soluble in ethyl and amyl alcohols, ethyl acetate, chloroform, benzene, and carbon disulphide. Ether and petroleum spirit dissolve only traces of it. The salts refuse to crystal-

produced no precipitate in solutions of lycoctonine salts; but the base was thrown down by tannin, iodised potassium iodide and bromine water (which last gave a precipitate of microscopic needles) Potassium mercuro-iodide threw down a precipitate which crystallised on standing. In solution of 1 in 8,000 no immediate effect was produced, but in about 15 minutes beautiful crystals made their appearance, and in a dilution of 1 in 20,000, they were formed in 24 hours The precipitate was readily soluble in alcohol, and crystallised very beautifully from the solution

Sulphuric, nitric and phosphoric acids produced no colour reactions. The nitrate of lycoctonine crystallised in tables, the sulphate in prisms Solutions of the salts were not precipitated by alkali hydroxides or carbonates, though the base itself was not notably soluble in alkalies.

lise. Myoctonine is precipitated by most of the general reagents for alkaloids in solutions not too dilute, and may be titrated by Mayer's solution (1 c.c. = 0.0176 of alkaloid).

An aqueous solution of myoctonine hydrochloride gives with excess of bromine-water an amorphous, very sparingly soluble precipitate, said to contain a dibromo-compound, $C_{40}H_{54}Br_2O_{12}N_2$.

If a fragment of myoctonine be moistened with fuming nitric acid and dried, the residue acquires a reddish-brown colour on adding a drop of alcoholic potassium hydroxide (compare atropine.)

On heating to 100° with a 4% solution of sodium hydroxide myoctonine is stated by Dragendorff and Spohn to behave similarly to lycaconitine, yielding lycoctonic acid, lyaconine, a base resembling acolyctine, and a fourth product of indefinite nature. The behaviour of myoctonine with alkali hydroxide has also been studied by F. Einberg (*Inaugural Dissertation*, Dorpat, 1887). When myoctonine was heated on the water-bath with 4% sodium hydroxide solution a sparingly soluble basic decomposition product separated in crystals, which, when filtered off and purified, amounted to 24% of the myoctonine taken.[1] The filtrate was brownish, and had a peculiar pungent smell. When acidified and shaken with ether, a substance exhibiting a blue fluorescence was extracted; and on evaporation 30.45% of a brownish semi-crystalline residue was obtained, of which Einberg recognised benzoic acid as the main constituent. The acid liquid, when rendered alkaline with sodium carbonate, yielded 11.84% to ether and an additional 8.89% to chloroform, both solvents leaving amorphous yellowish-brown residues on evaporation.

According to Salmonowitz, myoctonine is a powerful poison resembling curare in its action, and acting most energetically when introduced directly into the circulation. The subcutaneous injection of 0.075 grm. of the nitrate produced distinct toxic symptoms in cats, and the injection of 0.100 grm. always caused death in about half an hour. Mice were killed in 3 minutes by a dose of 0.001 grm.

[1] To this base, after drying at 80°, Einberg ascribed the formula $C_{24}H_{38}O_6N$. He considered it identical with Hübschmann's lycoctonine. It melted at 94°, and had a specific rotation in absolute alcohol of $[\alpha]_D + 38.9°$. It became amorphous when melted, reassuming the crystalline form on contact with steam. It dissolved in about 250 parts of water, 4 of absolute alcohol, 3 4 of chloroform, 55 of ether, and 63 of benzene, which characters agree with those ascribed by Hübschmann to lycoctonine. The base formed a crystalline nitrate, very hygroscopic and easily soluble in water. Strong sulphuric acid coloured the base bright yellow, changed to a fine orange on warming.

LAPACONITINE, SEPTENTRIONALINE AND CYNOCTONINE.

The root of *Aconitum septentrionale*[1] (Koelle) contains 3 alkaloids which have not hitherto been thoroughly investigated. Rosendahl has, however, described their properties in a general way.

Lapaconitine,[2] $C_{34}H_{48}O_8N_2$, forms bulky hexagonal crystals, m. p. 205°, is soluble in alcohol (1 in 126), ether (1 in 330), and sparingly soluble in water. Its solutions have a reddish-violet fluorescence. No salts have been described, but it forms a *tribromo*-substitution product. Lapaconitine has a bitter taste, is not extremely toxic but exercises a depressant action upon the heart. When heated with alkalies it yields an ether-soluble alkaloid melting at 98°, an alkaloid insoluble in ether melting at 106°, and a crystalline acid (m. p. 114°) which gives a blue colour with ferric chloride.

Septentrionaline, $C_{31}H_{48}O_9N_2$, also occurs in *Aconitum septentrionale*. It is obtained as a white or yellowish-white powder, m. p. 129° soluble in alcohol (1 in 1.7), ether (1 in 2.1) and water (1 in 58). Unlike lapaconitine, it does not give fluorescent solutions. The nitrate is precipitated by the addition of nitric acid to a solution in ether as a white hygroscopic powder. It forms a *tribromo*-derivative.

Septentrionaline has a bitter taste and a local anæsthetic action, but it is not toxic. When heated with alkalies, it yields an ether-soluble alkaloid m. p. 88°, an alkaloid m. p. 105° difficultly soluble in ether, and the same unknown acid as is obtained by hydrolysis of lapaconitine.

Cynoctonine, $C_{36}H_{54}O_{13}N_2$, is also obtained from *A. septentrionale*. It is an amorphous light grey powder, m p 137°, readily soluble in alcohol and water and difficultly soluble in ether (1 in 1,373) It does not form fluorescent solutions. No salts have been described but it forms a *tribromo*-derivative It has a bitter taste and resembles septentrionaline in its physiological action

Atisine, $C_{22}H_{31}O_2N$, is the characteristic alkaloid of *Aconitum heterophyllum*, a species of aconite which grows in the Himalayas at altitudes from 6,000–15,000 feet. The root is very commonly employed throughout India as a mild and bitter tonic, and is sold in the bazaars under the name "Atis" or "Atees."

A complete investigation of the properties of atisine has been made

[1] See footnote to page 255
[2] Rosendahl, *J Pharm. Chim.*, 1896 [*vi*], 4, 262.

by Jowett,[1] who was unable to find evidence of the existence in the root of any other alkaloid. Atisine occurs in the root in combination with aconitic acid. The method of isolating it is similar to that described for aconitine and the final purification may be accomplished by crystallising as hydrochloride or hydriodide. The alkaloid is a colourless uncrystallisable varnish, readily soluble in water and insoluble in petroleum ether. It melts at about 85° and readily decomposes when heated. $[\alpha]_D$ in alcohol $-19.6°$.

According to Wasowicz,[2] strong sulphuric acid colours atisine a faint violet which changes to red and dirty brown. Nitric acid produces a brown, sulphuric acid a red, and potassium dichromate a green colouration with a distinct reddish-violet zone.

Shimoyama[3] obtained with some of the alkaloid prepared by Wasowicz a yellowish solution in concentrated sulphuric acid, gradually changing to a purple-red and becoming momentarily violet on adding a drop of water. No colouration was produced by nitric or hydrochloric acid.

Ammonia precipitates atisine in white flocks from solutions of its salts. Tannic acid gives a yellowish-brown precipitate, and Mayer's reagent a white precipitate which readily crystallises from alcohol.

The following table gives the properties of Atisine and its chief salts:

Substance	Formulæ	M p	$[\alpha]_D$	Remarks
Atisine . .	$C_{22}H_{31}O_2N$	85°	−19 6° in ethyl alcohol	Uncrystallised
-hydrobromide	$C_{22}H_{31}O_2N,HBr.$	273°	+24 3° in water	Long acidular crystals
-hydrochloride .	$C_{22}H_{31}O_2N,HCl$	296°	+18 5° in water	Long prismatic crystals.
-hydriodide .	$C_{22}H_{31}O_2N,HI$	279–280°	+27 4° in water	Transparent tables sparingly soluble in water
-nitrate . . .	$C_{22}H_{31}O_2N,HNO_3.$	252°	+28 3° in water	Hexagonal plates

Atisine does not undergo hydrolysis on treatment with acids or alkalies, but forms a new base—atisine monohydrate—which does not crystallise or yield crystalline salts; nor does atisine contain any methoxyl

[1] *Trans.*, 1896, 69, 1518.
[2] *Arch. Pharm*, 1879, 11, 193.
[3] *Arch. Pharm.*, 1885, 23, 495.

groups. Physiologically atisine is non-toxic, it has a bitter taste, and is employed medicinally in India as an antiperiodic, aphrodisiac and tonic.

Palmatisine is a crystalline alkaloid contained in the root of *A. palmatum*, a bitter tasting root, known in India as bikhma or bishma, which grows in the Alpine Himalayas of Nepal and Sikkim.

Palmatisine, which may be crystallised from a mixture of alcohol, ether and light petroleum, melts at 285° and the *hydrobromide* at 245°.

Palmatisine has not been fully characterised and no formula has been given to it. It resembles atisine in its physiological properties so far as they are known.

Assay of Aconite Root and its Preparations.

The chemical assay of aconite and of its various pharmaceutical preparations yields very unsatisfactory results—not so much on account of any difficulty in isolating and identifying the total alkaloid as from the difficulty of ascertaining what proportion of aconitine is contained in it. The extremely high toxicity of aconitine as compared with the other alkaloids makes any estimation of the total alkaloid of small value, not only because the proportion of aconitine contained in the total alkaloid of the original plant is subject to variation but also because the base is liable to become hydrolysed from various causes, both during the drying and storing of the crude drug and in the process of making the particular preparation.

The chemical methods of assaying aconite and its preparations are based upon:

1. The use of alkaloidal precipitants.
2. The estimation of total ether-soluble alkaloid.
3. The estimation of the crystallisable alkaloid.
4. The estimation of the acetic or benzoic acids split off on hydrolysis.

As already premised none of these methods can be regarded as satisfactory.

1. **Alkaloidal Precipitants.**—The use of iodine was proposed by Prescott and Gordin,[1] and of silico-tungstic acid by Ecalle;[2] but since both methods fail to effect any separation of the alkaloids or allied substances they may be dismissed.

2. **Total Alkaloids.**—Methods based upon the weighing and titra-

[1] *J. Amer. Chem. Soc.*, 1898, 20, 706.
[2] *J. Pharm. Chim.*, 1901 [vi], 14, 97.

tion of the alkaloid extracted by ether have found practical acceptance, and the pharmacopœias of the United States and Germany adopt such a method for standardising the preparations of aconite.

In dealing with the dried root or stem it is best to follow a process substantially identical with that employed in preparing aconitine. 25 grm. of the drug in coarse powder is exhausted by cold percolation with a mixture of 5 parts of methyl alcohol and 1 part of amyl alcohol. (Fusel oil frequently contains pyridine from which it must be purified if employed for this purpose.) The percolate is concentrated to a small volume by distillation *in vacuo* over a water-bath, at a temperature which should not exceed 70°. It is next shaken with 3 successive portions of 10 c.c. of 1% sulphuric acid and subsequently twice with water, taking care to neutralise the separated acid with ammonia as quickly as possible. The aqueous extract is then rendered slightly acid and extracted with ether 3 times (or as often as necessary) to remove colouring matter and amyl alcohol, and the aqueous portions are rendered alkaline with dilute ammonia and completely extracted with ether. With good roots there is a tendency for crystalline aconitine to separate at this stage, and care must be taken to employ enough ether to redissolve it. The ethereal extract is evaporated to dryness and weighed.

In dealing with a tincture or extract the equivalent of about 25 grm. of root is employed, and the alcohol is first removed by evaporation. 25 c.c. of 1% sulphuric acid is added, the insoluble matter is filtered off and washed and the filtrate and washings are extracted with ether while acid, then rendered alkaline and extracted with ether and weighed as above.

The weighed alkaloid may be titrated using hæmatoxylin as indicator —1 c c. $N/10$ acid is equivalent to 0.0645 grm. aconitine. The large proportion of alkaloids of lower molecular weight than aconitine which are commonly present render titration inadvisable, however, the titration figure sometimes being greater than the weight. If the extraction of the acid liquid with ether is thorough, there is not much non-alkaloidal matter weighed in the final residue.[1]

The amount of ether-soluble alkaloid yielded by *A. Napellus* of good quality is 0.4 to 0.5%.

[1] Stevens (*Pharm Arch*, 1903, 6, 49) and Taylor (*J Ind and Eng Chem*, 1909, 1, 557) point out that rapid loss of aconitine occurs when aconite extracts are heated to 100°, though the amount of total alkaloid may remain practically unchanged This fact further emphasises the unsatisfactory nature of methods which depend upon the estimation of total alkaloid.

The *British Pharmacopœia* does not adopt any method of standardising either aconite or its preparations. The *United States Pharmacopœia* (8th rev.) adopts the following:

To 10 grm. of powdered root 75 c c. of 66% alcohol are added in a 200 c.c. Erlenmeyer's flask and the contents stoppered up and agitated at intervals for 4 hours; they are then transferred to a percolator 25 mm. in diameter and the percolation continued with 66% alcohol until 150 c.c. is obtained. The percolate is evaporated in a basin at a temperature not exceeding 60°, and 5 c.c. of $N/10$ sulphuric acid and 10 c.c. of water are added, the solution is filtered and the filter paper and basin are washed with about 40 c.c. of water. The filtrate and washings are transferred to a separating funnel, 25 c.c of ether and 2 c.c of ammonia solution are added, and after shaking and separating they are further extracted 3 times with ether using 15 c.c., 10 c.c., and 10 c.c., successively. The combined ether solutions are evaporated to dryness and the residue is dissolved in 3 c.c., $N/10$ sulphuric acid, 5 drops of hæmatoxylin are added to the solution which is then titrated back with $N/50$ potassium hydroxide and the amount of aconitine is calculated, using the factor 1 c c. = 0 06 grm. of aconitine.

Reference to communications on this subject are given below:

Ecalle (*J. Pharm. Chim.*, 1901 [*vi*], **14**, 97).

Beckurts (*Ap. Zeit.*, 1903, **18**, 67).

Caesar and Loretz (*Pharm. Centralh.*, 1905, **46**, 860).

Gordin (*Proc. Am. Pharm. Ass.*, 1906, 379).

Lyons (*Am. Drug.*, 1908, 89).

Bernegau (*Am. J. Pharm.*, 1909, 122).

Panchaud (*Pharm. J.*, 1904, **72**, 716).

Dohme (*Proc. Am. Pharm. Ass.*, 1895, 206).

3. **Estimation of the Crystallisable Alkaloid.**—Some good roots contain only a relatively small amount of amorphous alkaloid. In such cases it is possible to crystallise a large proportion of the alkaloid and so obtain a minimum figure for the quantity of aconitine present but hitherto no method has been proposed for quantitative separation. The total alkaloid is dissolved in 10 c.c. of dilute hydrochloric acid and filtered, 5 c.c. of ether is added and then a slight excess of dilute ammonia, the solution being stirred during the latter process. Crystallisation may be promoted, if necessary, by the addition of a minute crystal of aconitine, and should be complete in 15 minutes. The whole is then filtered under pressure, the crystals are washed with a

little ether and the ether is extracted by shaking twice with a slight excess of dilute hydrobromic acid and once with water. The acid extract is neutralised with ammonia and evaporated on a water-bath until crystallisation commences. As much crystalline salt as possible being thus obtained, it is dissolved and regenerated in the presence of ether as above; the crystals are then added to those already obtained, and weighed. This process is tedious and from its nature incomplete. It has been found to give better results with *A. deinorrhizum* than with *A. Napellus*, owing to the larger proportion of crystalline alkaloid contained in the former species.

4. **Estimation by Hydrolysis.**—Alder Wright and A. H. Allen have advocated a method of estimating aconitine based upon the estimation of the benzoic acid produced on hydrolysis; but later workers have shown that this method is unsuitable owing to the fact that benzaconine, equally with aconitine, yields benzoic acid on hydrolysis. Since aconitine is the only alkaloid present in *A. Napellus* which is known to yield acetic acid, better results were to be expected from determinations of the volatile acid obtained by hydrolysing the total alkaloid, the benzoic acid being removed by extraction with benzene. Dunstan and Tickle[1] and Dohme,[2] however, after repeated unsuccessful attempts to utilise this principle, abandoned it because the results afforded by it were too high and uncertain.

The following proportions of total and isolated alkaloids have been obtained from the various species, where many results are available an average figure has been taken. Good quality roots of the various species of aconite have been shown to contain the following percentages of alkaloid:

Species	Total ether-soluble alkaloid	Essential alkaloid
A. Napellus	0.5%	0.15% aconitine.
A. deinorrhizum	0.7%	0.4 % pseudaconitine.
A. Fischeri Var Hondo	0.5%	japaconitine.
A. japonicum Hokkaido	0.5%	jesaconitine.
A. Spicatum	0.6%	0.4 % bikhaconitine.

Physiological Methods of Assay.—A simple physiological method of judging the quality of aconite preparations was proposed by E. R. Squibb.[3] This method is based upon the peculiar property of aconitine

[1] *Pharm J*, 1896 [*iv*], 2, 121.
[2] *Proc. Am. Pharm Assn*, 1895, 206.
[3] *Ephemeris*, 1, 125.

already referred to—of producing a tingling and numbing sensation upon the tongue and mouth. The principle followed is to ascertain the limit of dilution of an extract or tincture which will produce this characteristic effect within 10 minutes after 1 dram of the diluted mixture is held in the mouth for 1 minute. According to Squibb $\frac{1}{10}$ of a minim of a 1 in 1 extract, or the corresponding quantity of another preparation diluted with 1 dram of water, should produce the aconite effect within this limit of time. The mouth should be first rinsed with water, and the liquid held in the front of the mouth and expelled at the expiration of exactly 1 minute. Aconitine should respond to this test at a dilution of 1 in 500,000, the liquid extract of the root at 1 in 600, the liniment 1 in 400, the liquid extract of the leaves 1 in 100, the *British Pharmacopœia* tincture 1 in 30, and the *United States Pharmacopœia* tincture 1 in 60. The Squibb test cannot be looked upon as a satisfactory quantitative method, the results varying to a certain extent according to individual sensitiveness; nevertheless certain workers regard it as of great value and superior to all the chemical methods hitherto proposed. In a valuable contribution, Taylor[1] compares this test with other chemical methods of assay and draws the conclusion that it is the most reliable method of ascertaining the activity of aconite preparations.

Another physiological test based upon a determination of the minimal lethal dose for frogs has been proposed,[2] but the results obtained were erratic.

Cash and Dunstan found that the toxicity toward frogs was variable according to the time of year and the size of the frog, but from the work of these authors there appears to be no reason why the minimal lethal dose method cannot be successfully applied to warm-blooded animals.

Toxicology of Aconite.

The members of the aconitine group of alkaloids are among the most violent poisons known. The lethal dose of aconitine, determined by Cash and Dunstan on cats and rabbits, is approximately 0.13 mgrm. per kilo of body weight for warm-blooded animals. Calculated from this figure the fatal dose for an adult man would be 9 mgrm. (⅛ grain), but there is reason to believe that human beings are comparatively more sensitive. Recorded cases of poisoning show

[1] *J. Ind and Eng Chem*, 1909, 1, 557.
[2] Stevens, *Pharm. Arch*, 1903, 6, 49.

a much greater toxicity than this. The fatal dose is difficult to fix, as in most of the cases in which such a dose of the pure alkaloid has been administered, the quantity taken has not been known. Headland considers 6 mgrm. ($\frac{1}{10}$ grain) an ordinary fatal dose for an adult. Death appears to have been caused in 1 hour by 0.5 mgrm. ($\frac{1}{130}$ grain).[1] Winter Blyth considers 2 mgrm. ($\frac{1}{30}$ grain) the minimum fatal dose for an adult when the poison is taken by the mouth; but if given hypodermically 0.15 mgrm. ($\frac{1}{45}$ grain) would probably kill, since the whole of the poison is then thrown into the circulation at one time and there is no chance of its elimination by vomiting. Pereira relates a case in which $\frac{1}{50}$ grain nearly proved fatal to an elderly lady. Recovery has occurred after taking 2 5 grains; in this case, however, there was violent vomiting immediately, but most dangerous symptoms persisted for 30 hours.[2] In the Lamson case,[3] which proved fatal, the victim probably received about 2 grains. Other important cases are recorded by Dr. Busscher[4], one in which recovery occurred after a patient had taken a total of 9.2 mgrm. of aconitine nitrate in 7 doses over a period of 50 hours, and another in which 4 mgrm. of aconitine nitrate taken in 1 dose proved fatal.

The symptoms of aconite poisoning usually begin to manifest themselves a few minutes after the poison is taken, and are, in some respects, quite peculiar and characteristic. They usually, but not invariably, commence with tingling and numbness of the lips, tongue, gums and throat, accompanied with a burning sensation in the stomach. These effects are succeeded by tingling and creeping sensations in various parts of the body, pains in the abdomen, headache, vertigo, and nausea, frequently accompanied by vomiting and sometimes by purging. There is also diminished sensibility of the skin, constriction in the throat, frothing at the mouth, partial or entire loss of voice, impaired vision, ringing in the ears, and a feeling of tightness in various parts of the body accompanied by muscular tremors, cold perspirations, loss

[1] *Pharm J*, 1890 [iii], 20, 734

[2] In a case of poisoning by aconite an emetic should at once be given or the stomach-pump promptly used Stimulants may be given with advantage Animal charcoal, to be afterward removed by the stomach-pump, has been recommended Strychnine and digitalis have been used successfully as antidotes, and a solution of iodised iodide of potassium has been suggested

[3] In 1881 a medical man named Lamson gave his brother-in-law, P M John, a youth of 19, who was paralysed below the pelvis, a dose of Morson's aconitine contained in a gelatin capsule Some 20 or 30 minutes after, John was seized with pain in the stomach, which he at first called heart-burn He then vomited, and suffered great pain, complained of the skin of his face being drawn, of a sense of constriction in the throat, and of being unable to swallow. He retched violently, and vomited a small quantity of dark brown fluid Injections of morphine gave some relief, but the symptoms returned, and he was with difficulty kept down by two men Death occurred 4 hours after administration of the poison, the victim remaining conscious almost to the last (*Guy's Hospital Reports*, 1883, 307)

[4] *Berl. klinische Wochenschrift*, 1880, 24, 338.

of muscular power, and great prostration generally. Smoetimes there is alternate contraction and dilatation of the pupil.

The most constant symptoms of aconite poisoning are difficulty in breathing, progressive muscular weakness, a weak intermittent pulse, and, in most cases, vomiting, especially when the poison has been taken by the mouth instead of subcutaneously. Death usually occurs from syncope, preceded in some cases by delirium and convulsions. Convulsions occurred in 10 cases out of 94 collected by Drs. Tucker and Reichert,[1] and opisthotonos happens occasionally. Death from aconite poisoning commonly ensues in from 2 to 6 hours, though there is considerable variation in this respect.[2]

The postmortem appearances from aconite poisoning are by no means characteristic. They are congestion of the lungs and liver, with an injected condition of the brain and its membranes. There is more or less redness of the stomach and intestines, which organs are frequently found empty. Great redness of the stomach and intestines is sometimes the only abnormal appearance after aconite poisoning, and this does not occur when the poison has been given hypodermically. The right side of the heart usually contains more or less blood, and the blood throughout the body is generally fluid and dark in colour.[3]

Toxicological Detection of Aconite.

In any case of suspected poisoning by aconite or its preparations, the symptoms presented before and after death are of the utmost importance.[4] The poison is so violent, so readily decomposed, and so wanting in delicate and characteristic chemical reactions, that there is great difficulty in detecting it in the body by chemical analysis.

The aconite alkaloids have been recovered from the urine, the blood, and the liver, and have been detected in the stomach several months after death; but the poison has been destroyed in cases where the

[1] These symptoms probably depend largely on the dose taken With large doses, the heart's action is arrested before the poison has had time materially to affect the excitability of the motor nerves, and, the heart once stopped, further absorption is diminished or arrested

[2] In 5 cases of aconite poisoning recorded by J W. Mallett, death ensued respectively in 8, 10, 15, 75 and 135 minutes, while in the sixth case it did not occur until 4 days after the poison was taken.

[3] In the Lamson case, 64 hours after death there was great redness and inflammation of the cardiac end of the stomach, which had a blistered appearance, the mucous membrane showing in places small, slightly raised, yellowish-grey patches.

The duodenum was greatly congested, and there were congested patches in other parts of the small intestine. The brain and its membranes were slightly congested, and the lungs much so, especially toward the posterior parts. The heart was very flaccid, nearly empty, and stained with blood-pigment. The pupils were dilated and the lips and tongue pale. The bladder contained 3 or 4 oz. of urine

[4] It is for this reason that the symptoms of aconite poisoning are described in the text at greater length than might appear necessary in a work treating of the chemist's duties rather than those of the medical practitioner.

viscera have become and remained alkaline for some time from putrefactive decomposition.

In cases of supposed poisoning by aconite, the stomach and intestines have been carefully examined for portions of the leaves or other parts of the plant. These, if found, may be identified by comparison of their botanical characters with those of real aconite. The fragments may be washed with a little distilled water and masticated with the front teeth, when the persistent tingling and numbness so characteristic of aconite will be distinctly recognisable.

For the isolation of aconite bases in cases of poisoning, the suspected matters should be finely divided and treated at the ordinary temperature with strong alcohol, which should be slightly acidified with tartaric acid, unless already distinctly acid. The liquid is strained and evaporated to a low bulk *in vacuo* at a temperature not exceeding 40°. The residual liquid is filtered cold, acidified with tartaric acid, if requisite, shaken with ether to remove fats, etc., separated, and rendered alkaline with sodium carbonate. The alkaloids are then extracted by shaking with ether, and the solution is washed by agitation with water and evaporated at a gentle heat.

The alkaloid residue having been obtained, it should be tested according to the method given on page 261.

The Squibb test given on page 282 is of great value in identification and confirmation may be obtained by administering a fatal dose to mice or guinea-pigs.

The relative toxic doses of aconitine and its allies are as follows:

Aconitine,	1
Indaconitine,	1
Japaconitine,	0.85—0.9
Bikhaconitine,	0.75
Pseudaconitine,	0.4—0.45

When administered therapeutically in the above proportions it may be considered that they exert identical physiological action, such differences as exist being very small and unimportant. It must not be forgotten, however, that the action is more or less modified by the other alkaloids present in galenical preparations and in the amorphous aconitine of commerce.

Benzaconine, which has a toxicity of about 1/250 that of aconitine, and aconine, which has a toxicity of about 1/2,000 that of aconitine,

both exert an action antagonistic to that of aconitine and in sufficient doses may effectively act as antidotes to it.

Pharmacology of Aconite.[1]

Aconitine is employed in medicine to reduce the temperature in fevers; even in minute therapeutic doses it has a decided slowing effect upon the heart. It is excreted mainly by the kidneys and has been found in the stomach after administration by hypodermic injection.

The drug is readily absorbed by the skin, especially when applied in the form of an ointment, producing a tingling sensation followed by local anæsthesia. It is therefore employed in this way to remove the pain of rheumatism, neuralgia, etc.

It will readily be understood that the amorphous aconitines[2] of commerce are undesirable; it has been shown that the physiological activity of some is about 50 times as great as that of others, while the chemical examination of a number of samples has proved similar variation.

The commonly accepted medicinal dose of crystalline aconitine is $\frac{1}{600}$ grain per dose or $\frac{1}{130}$ grain per day.

The ointment of aconitine has an official strength of 2 parts. of aconitine in 100 parts and is employed only on unabrased surfaces.

The relative strengths and doses of the principal preparations of aconite root are given in the following table·

Preparation	Proportion of root in preparation	Dose
Aconite root, pulv . . .		0 03–0 06 grm 0 5–1 grain
Fluidextract, *United States Pharmacopœia*	1 in 1	0 03–0 06 c c 0 5–1 min
Liniment, *British Pharmacopœia*	1 in 1½	
Tincture, *British Pharmacopœia*	1 in 20	0 3–0 9 c c 5–15 min
Tincture, *United States Pharmacopœia*	1 in 10	0 2–0 6 c c 3–9 min
Tincture, Dr Fleming's	1 in 1 43	0 06–0 3 c c 1–5 min

[1] A very full and detailed account of the pharmacology of the aconite bases is given in the papers of Cash and Dunstan (*Phil Trans*, 1898, B, 190, 239 and 1903, B, 195, 39, *Proc Roy. Soc*, 1898, 62, 338, 1901, 68, 378, 1905, B, 76, 468), and Heinz (*Arch Pharm*, 1909, 247, 266).

[2] For reports upon commercial aconitine see papers by Duquesnel (*Y B Pharm*, 1872, 241); Plugge (*Arch Pharm*, Jan, 1882), Squibb (*Ephemeris*, 1, 135), Buntzen and Madsen (*Pharm. J.*, 1885 [*iii*], 16, 366), Dunstan and Carr (*Trans*, 1893, 63, 491).

ATROPINE AND ITS ALLIES. TROPEINES AND SCOPOLEINES.

By FRANCIS H. CARR.

There occurs in many of the plants of the family *Solanaceæ* a remarkable series of alkaloids distinguished by the property of dilating the pupil of the eye. The series is, on this account, often termed that of the "mydriatic alkaloids"; the different members of it have been given names derived from the plants which contain them; and as the same alkaloids occur in different species, some confusion has arisen through identical substances obtained from different plants being differently named. Thus the names "duboisine" and "daturine" have been given to mixtures of atropine and hyoscyamine which have been obtained respectively from *Duboisia* and *Datura*.

The plants which are known to yield mydriatic alkaloids are:[1]

Anisodus luridus[2] (*Scopolia lurida*)	Hyoscyamine.
Atropa Belladonna (deadly nightshade)	Hyoscyamine. Belladonnine.
Datura arborea	Hyoscyamine. Hyoscine.
Datura fastuosa	Hyoscyamine. Hyoscine.
Datura metel	Hyoscyamine. Hyoscine.
Datura meteloides	Hyoscyamine. Hyoscine. Meteloidine.

[1] A full account of the quantities of alkaloids present in these plants will be found on page 311

[2] Siebert (*Arch. Pharm*, 1890, 28, 145) found that *Anisodus luridus*, a Himalayan solanaceous plant, contains hyoscyamine.

Datura quercifolia	Hyoscyamine. Hyoscine.
Datura stramonium (thorn apple)	Hyoscyamine. Hyoscine.
Duboisia myoporoides	Hyoscyamine. Pseudohyoscyamine.
Hyoscyamus niger (henbane)	Hyoscyamine.
Hyoscyamus albus	Hyoscine.
Hyoscyamus muticus	Hyoscyamine.
Mandragora vernalis[1] (mandrake)	Hyoscyamine. Pseudohyoscyamine. Mandragorine.
Scopolia carniolica (*Scopolina atropoides*)	Hyoscyamine. Hyoscine. *i*-Scopolamine.
Scopolia japonica	Hyoscyamine.
Solandra lævis[2]	Mydriatic alkaloid.

Where hyoscyamine has been found its racemic modification atropine has been observed also, but it is uncertain as to whether it exists in the living plant or is formed in the process of separation.

The three best known natural alkaloids of this group, viz., atropine, hyoscyamine and hyoscine, have wide application in pharmacology, not only on account of their valuable mydriatic property, but also for their effect on muscles, secretory glands, and the central nervous system. The others are not of practical importance. All these alkaloids are readily saponifiable and traces of the products of hydrolysis are therefore liable to pre-exist with them in the plant or to be formed during the production of the alkaloids or of their galenical preparations. The following table exhibits the leading properties of these alkaloids:

[1] Hesse, *J. Pr. Chem*, 1901 [*ii*], 64, 274

[2] Petrie communicated to the Linnean Society of New South Wales an account of a new mydriatic alkaloid contained in *Solandra lævis*. The alkaloid resembles hyoscine, but differs from it in not reddening phenolphthalein; it yields atropic acid upon hydrolysis (*Chemist and Druggist*, 1908, 72, 14).

Base	Formulæ	M p.	$[\alpha]_D$	Form	Products of hydrolysis	
					Acid	Base
Atropine (*i*-hyoscyamine)	$C_{17}H_{23}O_3N$	117–118°	Inactive	Lustrous prisms	Tropic acid	Tropine.
Hyoscyamine (*l*-hyoscyamine)	$C_{17}H_{23}O_3N$	107°	−22 0° (50% alcohol)	Slender needles	Tropic acid	Tropine.
Hyoscine ...	$C_{17}H_{21}O_4N$	56–57°	−28 0° (water)	Amorphous Crystalline hydrate	Tropic acid	Scopoline (oscine).
i-Scopolamine (atroscine)	$C_{17}H_{21}O_4N$	82–83°	Inactive	Crystalline.	Tropic acid	Scopoline (oscine)
Pseudohyoscyamine	$C_{17}H_{23}O_3N$	133–134°	−21 1° (abs alcohol)	Needles.	Tropic acid	Base $C_8H_{15}NO$.
Atropamine .	$C_{17}H_{21}O_2N$	60–62°	Inactive	Prisms	Atropic acid	Tropine.
Belladonnine .	$C_{17}H_{21}O_2N$			Amorphous	Atropic acid	Tropine.
Mandragorine	$C_{15}H_{19}O_2N$			Amorphous	Atropic acid	New base
Meteloidine	$C_{13}H_{21}O_4N$	141–142°	Inactive	Tabular needles	Tiglic acid	Teloidine

The pre-existence of atropamine and belladonnine in the plant is not absolutely established.

Atropine and hyoscyamine are respectively the inactive and lævo-*tropyl* esters of tropine. These and other esters of tropine have been produced synthetically, thus giving rise to a large class of substances to which the name tropeines has been given. Similarly, *i*-scopolamine and hyoscine are the inactive and lævo-*tropyl* esters of scopoline, and the esters of this base have been termed scopoleines.

The natural tropeines and scopoleines are all easily hydrolysed by treatment with acids or alkalies. Thus the hydrolysis of atropine or hyoscyamine by the latter results in the formation of tropic acid and tropine in accordance with the equation:

$$\underset{\text{Atropine}}{C_{17}H_{23}O_3N} + H_2O = \underset{\text{Tropic acid.}}{C_9H_{10}O_3} + \underset{\text{Tropine.}}{C_8H_{15}ON}.$$

When the hydrolysis is effected by an acid, especially concentrated hydrochloric acid, the tropic acid loses the elements of water; atropic acid, $C_9H_8O_2$, results, and at a high temperature this is more or less changed into its polymers α- and β-isatropic acid, $C_{18}H_{16}O_2$. Such

products also result from the hydrolysis of the anhydro-bases belladonnine and atropamine by barium hydroxide.

The preferable method of effecting the hydrolysis of the tropeines is to heat the alkaloid with saturated barium hydroxide solution to 60 or 80° for a few hours. Dilute sulphuric acid is next added to the liquid till a drop ceases to give a pink colouration with phenolphthaleïn The liquid is then filtered, and the filtrate acidified with hydrochloric acid and twice shaken with ether. The ether is separated and when evaporated yields the acid product of the hydrolysis; on treating the aqueous layer with alkali hydroxide in excess and agitating with chloroform, the basic product is extracted, and may be recovered by separating and evaporating the chloroform.

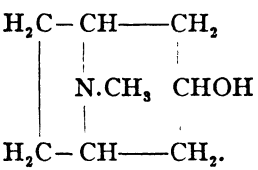
Tropic acid, $C_6H_5.CH(CH_2.OH)CO.OH$, is α-phenyl-β-hydroxypropionic acid. It crystallises from hot water in needles or slender prisms, and, on the spontaneous evaporation of its aqueous solution, in tablets, m. p. 125–126°. It is optically inactive, as obtained from atropine or from hyoscyamine by alkaline hydrolysis but from the latter alkaloid by acid hydrolysis the lævo acid of $[\alpha]_D - 71.8°$ results. Tropic acid is not volatile without decomposition. It has a slightly sour taste, dissolves in 40 parts of cold water, and is soluble in alcohol and ether. When heated with a dilute solution of potassium permanganate, tropic acid gives an odour of bitter-almond oil, and on further treatment benzoic acid is produced. Tropic acid has been prepared synthetically (*Ber.*, 1880, **13**, 2041).

Atropic acid, $C_6H_5.C(:CH_2).CO.OH$, is α-phenylacrylic acid. It is isomeric with cinnamic acid (Vol. 3), from which it differs by its solubility in water (1 in 692 at 19°), its lower m. p., and in the failure of manganous salts to precipitate it from its neutral solutions. Atropic acid has been prepared synthetically, and may also be obtained by heating tropic acid with hydrochloric acid, or by the direct action of fuming hydrochloric acid at 120° or boiling concentrated barium hydroxide solution on atropine. It crystallises from hot water in needles, and from alcohol in tablets or monoclinic prisms, which melt at 106–107°, are volatile with steam and boil with decomposition at about 267°. Atropic acid is very soluble in carbon disulphide. It is oxidised to benzoic acid by chromic acid mixture, and yields formic and phenylacetic acids when fused with potassium hydroxide. Sodium-amalgam reduces it to α-phenylpropionic acid. Bromine-water converts it into α-β-dibromo-α-phenylpropionic acid.

Isatropic acid, $C_{18}H_{16}O_4$, is polymeric with atropic acid, $C_9H_8O_2$, and is always formed, together with that acid and tropic acid, when atropine is heated with hydrochloric acid. Isatropic acid is also formed in small quantity when atropic acid is recrystallised from hot water, and in larger amounts if the solution be boiled for some time.

Several isomeric modifications of isatropic acid exist; α-isatropic acid is almost exclusively formed when atropic acid is heated for many hours at 140–160° in a closed flask. It forms small warty crystals, which melt at 237°, are very slightly soluble in water, and nearly insoluble in ether. It is not affected by sodium-amalgam or cold bromine-water. β-isatropic acid is formed, together with much of the α-modification, when the aqueous solution of atropic acid is boiled. It crystallises on cooling, in small quadratic tablets, m p. 206°, and is converted at 220–225° into the α-acid. γ- and δ-isatropic acids were obtained by Liebermann by the hydrolysis of truxilline (cocamine), a base contained in some varieties of coca leaves. From their source he subsequently named them α- and β-truxillic acids.

Tropine, $C_7H_{11}(OH)N.CH_3$, has the constitution of a 7-atom carbon ring with an N-bridge having a methyl attached:



It crystallises from absolute ether in rhombic tablets, m. p. 63°, b. p. 233° without decomposition. It is hygroscopic and very easily soluble in water and alcohol, remaining as an oil on the evaporation of these solutions. Tropine is optically inactive, and cannot be resolved into its active constituents; it is a strong tertiary base and forms salts which crystallise well. The *plantinichloride*, B_2,H_2PtCl_6, forms large, orange-red monoclinic prisms, easily soluble in warm water, insoluble in alcohol, m. p. (decomp.) 198–200°. The *aurichloride* $B,HAuCl_4$ forms large yellow plates, m p. (decomp.) 210–212°. The *picrate* is formed as a yellow precipitate, crystallising from hot water in yellow needles. On distilling with soda-lime or barium hydroxide, tropine yields methylamine, water and tropilidene—$C_8H_{15}ON = CH_3NH_2 + H_2O + C_7H_8$. When heated with fuming hydrochloric acid to 180°, or with glacial acetic and strong sulphuric acid, it loses the

elements of water and is converted into *tropidine*, $C_5H_6(C_2H_4)N.CH_3$, a liquid base boiling at 162°, smelling like coniine. Tropidine is of interest from its relation to anhydro-ecgonine (compare page 338).

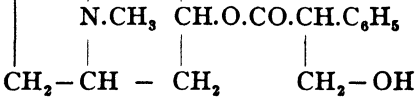
Pseudotropine,[1] $C_8H_{15}ON$, is a stereo-isomer of tropine, and results from the hydrolysis of tropacocaine—an alkaloid obtained by Liebermann from Java coca leaves. It may also be readily prepared from tropine by treating the latter with sodium amyl oxide. It forms prismatic crystals, m. p. 108°, b. p. 241° without decomposition, and is soluble in water, benzene, chloroform, alcohol and less readily in ether.

Pseudotropine *hydrochloride* crystallises in prisms, m. p. 280–282°, the *aurichloride* in golden plates, m. p. 225°, and the *platinichloride* in rhombohedra, m. p. 205–206°.

Like tropine it yields tropidine when treated with strong acids; the tropyl ester and other esters of pseudotropine, unlike the corresponding tropeines, are not mydriatic.

Scopoline (oscine), $C_8H_{13}O_2N$, results from the hydrolysis of *i*-scopolamine and hyoscine. It crystallises in prisms from light petroleum or chloroform, m. p. 109–110°, b. p. 241–243°. It is easily soluble in water and alcohol and less readily in ether. It forms a crystalline *hydrochloride*, *hydrobromide*, *hydriodide*, *sulphate*, etc. It has been suggested that its constitution differs from that of tropine in the substitution of a *CO* group for a CH_2, but E. Schmidt[2] has failed to obtain any direct evidence to support the view that scopoline contains a *CO* group. Scopoline is optically inactive, but unlike tropine it may be resolved into its *d*- and *l*-constituents. (Tutin, *Trans.*, 1910, **97**, 1793.)

Atropine (*dl*-hyoscyamine), $C_{17}H_{23}O_3N$,



[1] According to Ladenburg, an isomer of tropine results from the hydrolysis of hyoscine; this also is named pseudotropine but is not identical with Liebermann's base. It has m. p. 106° and b. p. 241–243°, and forms rhombohedral crystals. It is less hygroscopic than tropine, but very soluble in water and chloroform and somewhat sparingly in ether. B_2,H_2PtCl_6 forms small orange-red rhombic prisms, easily soluble in water, m. p. 205–206°. $BHAuCl_4$ forms small crystals, m. p. 198°. Later workers, however, have stated that the basic hydrolytic product of hyoscine is scopoline, which, though of different composition, has similar properties to these recorded by Ladenburg for pseudotropine (*Ber.*, 1884, **17**, 151).

[2] *Apoth. Zeit.*, 1905, **20**, 669, and *Arch. Pharm.*, 1906, **243**, 559.

is commonly regarded as the characteristic alkaloid of *Atropa belladonna* or deadly nightshade, though in the living plant it is often largely, or perhaps entirely, replaced by *l*-hyoscyamine, the latter alkaloid being readily converted to atropine, which is its racemic form. Atropine is also obtained from *Scopolia carniolica*, *S. japonica*, *Datura Stramonium*, *D. metel*, *D. meteloides*, *D. fastuosa* and *Duboisia myoporoides*. Atropine has been completely synthesised, and by crystallisation as the salt of an optically active acid, its resolution[1] into *d*- and *l*-hyoscyamine has been effected.

Pure atropine[2] forms tufts or groups of colourless or white lustrous needles or prisms. It melts at 118° but the commercial alkaloid often begins to melt at a lower temperature owing to the presence of hyoscyamine. At a higher temperature atropine decomposes into atropic acid and tropine, which latter shows signs of volatility.

Atropine is odourless but has a disagreeable bitter and acrid taste. It is a powerful poison, producing delirium and convulsions (page 309). From 0.05 to 0.2 grm. is commonly fatal, and 0 001 grm. (and 0.004 grm. per day) the maximum medical dose for an adult. Much smaller amounts than this produce marked mydriasis or dilation of the pupil when applied to the eye (page 310).

Atropine is soluble in about 450 parts of cold or 35 of boiling water. It dissolves in glycerin and in olive oil. It is soluble in alcohol (3 parts), ether (60 parts), chloroform (2 parts), amyl alcohol, and benzene (42 parts), but is only slightly soluble in cold petroleum spirit or carbon disulphide. The solutions are optically inactive.

The aqueous solution of atropine exhibits a distinctly alkaline reaction to litmus, hæmatoxylin and phenolphthaleïn, the latter character distinguishing it and its isomers from almost all other known alkaloids. Atropine is a tertiary base with a methyl group attached to the nitrogen, and forms salts which are well crystallised.

Atropine is readily produced from hyoscyamine by treating a solution of the latter with dilute alcoholic alkali hydroxide, or even by allowing a plain solution to stand for a long time. When heated to 110° hyo-

[1] Barrowcliff and Tutin, *Trans*, 1909, 95, 1966

[2] For the preparation of atropine from belladonna the dried leaves should be macerated for several days in cold water, and the liquid concentrated by evaporation, treated with sodium carbonate and agitated with benzene The benzene solution is separated and agitated with dilute sulphuric acid and the acid liquid again rendered alkaline with sodium carbonate The liberated alkaloid is then extracted with chloroform, the solution in which, when mixed with petroleum spirit and allowed to evaporate spontaneously, deposits the atropine first, while the associated alkaloids remain in the mother-liquor It is perhaps more easy to prepare atropine from belladonna root Chloroform is the best solvent for the extraction of atropine from an alkaline liquid but ether is preferable for its subsequent purification and crystallisation (A W. Gerrard).

scyamine is also converted into atropine. At about 130° atropine loses the elements of water, and belladonnine is produced. It is readily hydrolysed into tropine and tropic acids (see page 291). Concentrated sulphuric or nitric acids and acetic or phosphoric anhydrides convert atropine into atropamine. Other reactions of atropine are described on page 303, *et seq.*

Atropine sulphate, $\overline{B}_2,H_2SO_4$, prepared by neutralising atropine with dilute sulphuric acid and evaporating the solution to dryness at 100°, is a colourless and odourless crystalline powder, neutral or faintly alkaline, easily soluble in water and alcohol. The commercial salt is often faintly alkaline and keeps better when so made. It should be anhydrous or contain not more than 4% of water. When dried at 100° it melts at 194°, but the presence of moisture considerably lowers this point. According to E. Schmidt, the more hyoscyamine a sample of commercial atropine sulphate contains the finer is its crystalline appearance, the pure salt occurring as granular white masses. The absence of hyoscyamine in a sample is shown by the optical inactivity of the solution.

Atropine hydrobromide, $\overline{B}$,HBr, crystallises in fine needles, m. p. 163–164°.

Atropine hydrochloride, $\overline{B}$,HCl, forms needles, m. p. 162°.

Atropine picrate forms rectangular plates, m. p. 175–176°.

Atropine oxalate, $\overline{B}_2,C_2H_2O_4$, crystallises readily in prisms, m. p. 198°.

Atropine aurichloride, $\overline{B}$,HAuCl$_4$, shows a tendency to separate as an oil which subsequently crystallises; it forms opaque yellow glomerates, m. p. 137–139°.

Atropine auribromide, $\overline{B}$,HAuBr$_4$H$_2$O, forms chocolate-coloured prisms, m. p. 120°.

Atropine methobromide (mydriasine), B,MeBr, results from the interaction of atropine and methyl bromide in chloroform. It forms glistening scaly crystals, m. p. 222–223°, readily soluble (1 in 1) in water.

Atropine salicylate and valerate are occasionally employed in ophthalmic surgery.

Commercial atropine and its salts should be free from yellow colour, and should not become coloured on treatment with strong sulphuric acid or excess of ammonia. A solution should be entirely

without effect on polarised light. This substance should leave no appreciable residue on gentle ignition. A solution in water—1 in 1,000—should have an acrid and bitter taste and on the addition of a drop of gold chloride solution yield a non-lustrous golden-yellow precipitate, which melts under boiling water. 1 drop of a solution of atropine or its salts in 45,000 parts of water (or less than 2 grains per gallon), when placed in the human eye, should cause dilation of the pupil in from 40 to 60 minutes.

Hyoscyamine, *l-hyoscyamine, daturine, duboisine,* $C_{17}H_{23}O_3N$.—This base is characteristic of the various species of *Hyoscyamus*, but also occurs in *Belladonna*, *Stramonium*, and other solanaceous plants in association with atropine, into which alkaloid it readily passes by simple racemisation. If hyoscyamine be kept at a temperature slightly above its m. p., the optical activity gradually falls and the product is found to consist of atropine. Conversion of hyoscyamine into atropine also occurs when its cold alcoholic solution is allowed to stand after a slight addition of potassium, sodium or ammonium hydroxide.

Hyoscyamine crystallises from alcohol or hot petroleum in slender needles with a peculiar tendency to come out in a jelly-like mass. It melts at 107°. In its solubilities it closely resembles atropine. The specific rotatory power of the pure base, taken in 50% alcohol, is $[\alpha]_D - 22°$; but as met with in commerce it generally contains a small proportion of atropine and rarely has a higher rotation than $[\alpha]_D - 21°$. The rotation of the basic ion contained in its salts is $[\alpha]_D - 32.5°$ (Carr and Reynolds, *Trans.*, 1910, **97**, 1328). It resembles atropine in taste and physiological action, though its activity is much greater.

On hydrolysis hyoscyamine yields tropic acid and tropine. If hydrolysed by heating with water, lævo-tropic acid and inactive tropine are produced; but if alkali is employed, both products are optically inactive. The difference between hyoscyamine and atropine, therefore, lies in the activity in the one case and inactivity in the other of the tropic acid residue.

As in the case of atropine, atropamine and belladonnine result from the action of strong acids and acid anhydrides upon hyoscyamine.

A table showing the principal distinctions between the properties of hyoscyamine and atropine and those of their salts will be found on page 291.

Hyoscyamine sulphate, $\overline{B}_2,H_2SO_4$, crystallises more readily than atropine sulphate, and melts at 205–209°. It is very soluble in water. A 4% aqueous solution gives $[\alpha]-27\ 8°$.

Hyoscyamine hydrobromide forms fine needles, m. p. 151°.

Hyoscyamine picrate crystallises in plates, m. p. 165°.

Hyoscyamine oxalate, $\overline{B}_2,C_2H_2O_4$, forms very long prisms, m. p. 176°.

Hyoscyamine methobromide crystallises in colourless crystals, m. p. 210–212°.

Hyoscyamine aurichloride, $\overline{B},HAuCl_4$, crystallises from alcohol in anhydrous golden-yellow hexagonal plates, m. p. 165°.

Hyoscyamine auribromide, $\overline{B},HAuBr_4H_2O$, crystallises in deep red coloured and highly lustrous needles having 1 molecule of water; the anhydrous salt sinters at 155° and melts sharply at 160°.

d-**Hyoscyamine** resembles its optical antipode in all its chemical properties and those of its salts, but though its physiological activity is similar in kind it is much less in degree,[1] the mydriatic action being about 1/100 and the paralysis of the vagus nerve 1/25 of that of *l*-hyoscyamine.

Pseudohyoscyamine, $C_{17}H_{23}O_3N$, was obtained by Merck from *Duboisia myoporoides*,[2] in which it is accompanied by hyoscyamine and atropine. It also occurs in *Mandragora vernalis*.[3] It crystallises in needles from ether and chloroform, m. p. 133–134°, and is readily soluble in chloroform and alcohol, less readily in ether and water. It forms a crystalline *aurichloride*, m. p. 176°, and *picrate*, m. p. 220°. It is lævorotatory, mydriatic, and only slightly poisonous. On hydrolysis it yields tropic acid and a *base*, $C_8H_{15}ON$, isomeric with tropine.

Atropamine.—Apo-atropine, $C_{17}H_{21}O_2N$, is the *anhydro*-derivative of atropine. It is reputed to occur naturally associated with atropine and hyoscyamine in the solanaceous plants, but is best prepared from either of the two latter alkaloids by adding the substance gradually to strong nitric acid or sulphuric acid, or by heating it with acetic or phosphoric anhydrides.

[1] Cushny (*J. Physiol*, 1904, 30, 176) and Laidlaw (*Trans.*, 1909, 95, 1969).
[2] *Arch. Pharm*, 1893, 231, 117.
[3] Hesse, *J. Pr Chem*, 1901 [*ii*], 64, 274.

Atropamine crystallises from ether in prisms, m. p. 60–62°, which are very slightly soluble in water and petroleum ether, readily in alcohol, chloroform, benzene and carbon bisulphide. It forms salts which crystallise well from water. The sparing solubility of the hydrochloride and hydrobromide affords a ready means of separating atropamine from the other alkaloids of this group. The aurichloride crystallises from water in fine yellow needles, m. p. 110–112°.

On hydrolysis atropamine yields atropic acid and tropine,

$$C_{17}H_{21}O_2N + H_2O = C_9H_8O_2 + C_8H_{15}ON.$$

Atropamine is optically inactive, does not redden phenolphthaleïn (being thus unlike atropine and hyoscyamine), but colours red litmus blue. It has a bitter taste, does not possess mydriatic properties, but produces a burning sensation when dropped into the eye.

Belladonnine, $C_{17}H_{21}O_2N$, is isomeric with atropamine, and is formed by the treatment of the latter alkaloid with moderately concentrated hydrochloric acid at 80° or with other acids or barium hydroxide. It is stated to be present in belladonna root in small amount and is generally found in the mother liquors of hyoscyamine and atropine. Belladonnine forms a varnish-like mass, very sparingly soluble in water, but readily in alcohol, ether, chloroform and benzene.

The salts are amorphous; B_2,H_2PtCl_6 and $B,HAuCl_4$ are yellow pulverulent precipitates quite insoluble in cold water.

On hydrolysis it is decomposed into atropic acid and tropine in the same manner as its isomer atropamine.

Hyoscine[1] (*l*-scopolamine), $C_{17}H_{21}O_4N,H_2O$, occurs in small amounts, together with hyoscyamine and other alkaloids, in many solanaceous plants (see page 291). Schmidt and Kircher[2] have shown that the various species of *Datura* are peculiar in producing larger proportions of it than is the case with most other solanaceous plants.

[1] Much confusion and controversy have arisen as to the relationship between, and the identity of, the alkaloids hyoscine, scopolamine and atroscine, but there appears good reason to believe that these names have been variously applied to the lævorotatory and racemic forms of the same base, though recent work has shown that several optical isomers are possible

In Germany, where this subject has been chiefly investigated, the names lævo- and inactive-scopolamine have been more generally employed In England and the United States the term hyoscine is used in the respective pharmacopœias to designate the lævo-base, it is therefore adopted in this article but the name *i*-scopolamine has been retained for the inactive base A more general adoption of the name atroscine for the latter would draw a parallel between hyoscyamine-atropine and hyoscine-atroscine.

[2] *Arch. Pharm*, 1905, 243, 303

"Amorphous hyoscyamine," formerly employed in medicine, appears to have consisted largely of hyoscine. When pure, however, hyoscine may be crystallised as hydrate from water.

Pure hyoscine crystallises in rhombohedral crystals with 1 molecule of water; it is readily soluble in water, alcohol, ether and chloroform, and less readily in light petroleum and benzene. $[\alpha]_D - 28°$ in water. Like hyoscyamine it is an ester of *l*-tropic acid with an alcohol of a basic character. This base, which has been named scopoline or oscine, appears to be optically inactive as in the case of its analogue—tropine. By the action of alkalies or by direct heat hyoscine is racemised to *i*-scopolamine. Hyoscine resembles atropine and hyoscyamine in its physiological action, producing more powerful, but less lasting, paralysing effect upon the peripheral nerve endings and depression of the motor area. It does not, however, stimulate the brain initially and is consequently employed by preference for cerebral affections; it is of great use in acute mania.

Hyoscine hydrobromide, $\overline{B}$,HBr,$3H_2O$, forms colourless rhombic prisms, m. p. of the dried salt 192–194°, $[\alpha]_D$ (in water) $-25.4°$; the commercial salt generally contains 11–12% of water and is variable in its optical rotation owing to the presence of *i*-scopolamine.

Hyoscine picrate forms slender matted needles, m. p. 180–181°.

Hyoscine aurichloride, $\overline{B}$,$HAuCl_4$, crystallises in yellow prisms, m. p. 198–200°.

Hyoscine auribromide, $\overline{B}$,$HAuBr_4$, crystallises from hot water in brown prisms, m. p. 210°.

***i*-Scopolamine, atroscine,** $C_{17}H_{21}NO_4$, is the optically inactive isomer of hyoscine (see footnote to page 299). It forms 2 crystalline hydrates: with $2H_2O$, m. p. 37–38°; with $1H_2O$, m. p. 56–57°. The anhydrous base melts at 82–83°. It forms crystalline salts.

i-Scopolamine hydrobromide, $\overline{B}$,HBr,$3H_2O$, crystallises in rhombic tables, m. p. 181°.

i-Scopolamine hydriodide, $\overline{B}$ HI,$\frac{1}{2}H_2O$, m. p. 192°.

i-Scopolamine picrate forms slender matted needles, m. p. 193°.

i-Scopolamine aurichloride, $\overline{B}$,$HAuCl_4$, m. p. 208°.

i-Scopolamine methobromide forms white needles, m. p. 216–217°.

Mandragorine, $C_{15}H_{19}O_2N$, is a distinctive alkaloid which occurs in *Mandragora vernalis* along with hyoscyamine, pseudo-hyoscyamine

and hyoscine. It has been characterised by Hesse[1] as a brownish oil forming a crystalline *aurichloride*, m. p. 124–126°. On hydrolysis it yields atropic acid and a new base which hitherto has not been characterised.

Meteloidine,[2] $C_{13}H_{21}O_4N$, is a crystalline alkaloid occurring together with atropine and hyoscine in *Datura meteloides*. It forms tabular needles, m. p. 141–142°, readily soluble in alcohol, acetone or chloroform and somewhat sparingly so in water or ether. It forms a crystalline hydrobromide, $B,HBr,2H_2O$, m. p. 250°, and an *aurichloride*, $B,HAuCl_4,\frac{1}{2}H.O$, m. p. 149–150°. On hydrolysis it yields tiglic acid and teloidine.

ARTIFICIAL TROPEINES.

It has already been stated that atropine and atropamine may be synthesised by the interaction of tropine and the respective acid—tropic or atropic. Ladenburg[3] and later Petit and Polonovsky,[4] Merck,[5] Jowett[6] and many others have prepared a large number of esters of tropine with various organic acids, giving rise to an extensive series of tropeines. Many of these tropeines possess mydriatic properties, and one of them—homatropine—is widely employed in ophthalmic surgery; others are quite devoid of mydriatic action.

It was at one time held that for a tropeine to have mydriatic properties, it must contain a benzene residue and an aliphatic *hydroxyl* in the side chain containing the *carboxyl* group. This generalisation, the origin of which has been incorrectly ascribed to Ladenburg, has been shown to be inaccurate.[7]

A few of the more interesting artificial tropeines are enumerated below:

Benzoyl tropeine, $C_6H_5COO.C_8H_{14}N$, m. p. 42°,

Salicyl tropeine, $C_6H_4(OH).COO.C_8H_{14}N$, m p. 58–60°, and

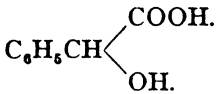
Cinnamyl tropeine, $C_6H_5CH=CH.COO.C_8H_{14}N$, m. p. 36–37°, are all crystalline bases having poisonous properties but possessing little or no mydriatic action.

[1] *J. Pr. Chem.*, 1901 [ii], 64, 274.
[2] Pyman and Reynolds, *Trans.*, 1908, 93, 2077.
Ber., 1880, 13, 106, 1080, 1882, 15, 1025.
[4] *J. Pharm.*, 1894 [v], 28, 529
[5] *Ber. u. d Jahr.*, 1894, 7
[6] *Trans.*, 1906, 89, 357; 1907, 91, 92
[7] Jowett and Pyman, *Trans.*, 1909, 95, 1020.

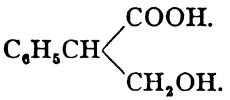
Atrolactyl tropeine, $C_6H_5-C(CH_3)(OH)-COO-C_8H_{14}N$, m. p. 74–75°, and

Atroglyceryl tropeine, $C_6H_5-C(CH_2OH)(OH).COOC_8H_{14}N$, m. p. 124–125°, have well-marked mydriatic properties, but are inferior in this respect to homatropine.

Homatropine, mandelyl tropeine, $C_6H_5CH(OH).COO-C_8H_{14}N$, is a lower homologue of atropine in which the $-CH_2OH$ group is replaced by $-OH$. It is prepared by passing hydrogen chloride gas for 3–4 hours through a mixture of 7 parts of tropine, 10 parts of mandelic acid, and 2 parts of water. Mandelic acid itself is produced by the action of hydrochloric acid on amygdalin, the glucoside of almonds. It is the lower homologue of tropic acid and has the constitution of a phenyl-glycollic acid:



Mandelic acid.



Tropic acid.

Homatropine crystallises from dry ether in prisms, m. p. 99–100°. It is sparingly soluble in water but freely in ether and chloroform. It is also soluble in castor and olive oils, and such solutions are employed in ophthalmic surgery for instilling into the eye.

Homatropine behaves like atropine with Gerrard's test; but with Vitali's test (page 306) it yields a yellow instead of a violet colouration. With picric acid it yields a yellow precipitate which soon becomes crystalline.

Homatropine resembles atropine and hyoscyamine in its general physiological effects, but is much less toxic and in small doses is a true hypnotic. It has a powerful mydriatic effect which is produced and passes off more rapidly than is the case with the natural tropeines.

Homatropine hydrobromide, $\overline{B}$,HBr, crystallises in non-deliquescent flat rhombic prisms or plates which form wart-like aggregations, m. p. 212–214°.

Homatropine hydrochloride, B,HCl, forms small prismatic crystals, m. p. 219–227°.

Homatropine sulphate, $\overline{B}_2,H_2SO_4$, crystallises in silky needles, m. p. 222–226°.

Homatropine aurichloride, $\overline{B}$,$HAuCl_4$, forms yellow prisms, slightly soluble in water; it is precipitated first as an oil, subsequently be-

coming crystalline, when gold chloride is added to a solution of the hydrochloride.

Homatropine methobromide, $\overline{B}$,MeBr, forms glistening crystals, m. p. 192–196°.

DETECTION AND ESTIMATION OF TROPEINES.

Atropine and the allied bases present a close general resemblance alike in their physical, physiological and chemical characters. The following table shows the principal distinctions between the 3 most important alkaloids of this group.

	Atropine	Hyoscyamine	Hyoscine
Appearance	Lustrous needles or acicular prisms	Slender radiating needles or crystalline powder	Oily, or rhombohedral hydrated crystals.
M p	117–118°	107°	56–57°
Specific rotatory power	Inactive	$[\alpha]_D$ −22 in 50% ethyl alcohol	$[\alpha]_D$ −28° in water
Characters of aurichloride	Precipitates as an oil which crystallises M p 137–139° Melts in boiling water	Precipitates in crystals M p 165° Does not melt in boiling water	Yellow prisms, m p 198–200° Does not melt in boiling water
Auribromides	Chocolate coloured prisms, m p of anhydrous salt 120°	Deep red coloured needles M p of anhydrous salt 160°	Chocolate coloured prisms, m p 210°.
Platinichlorides	M p 197–200°	M p 206°	Amorphous
Picrates	Rectangular plates, m p 175–176°	Crusts of needles, m p 165° or quadrangular plates	Slender matted needles, m p 180–181°
Basic product of hydrolysis	Tropine, m p, 63°, b p 233°	Tropine, m p 63°, b p 233°	Scopoline, m p, 109–110°, b p, 241–243°

The reactions of these alkaloids with gold salts and with picric acid form the best distinctions between them. Atropine aurichloride is thrown down from dilute solutions as an amorphous or oily precipitate which gradually becomes crystalline. Under the microscope it appears in rosettes and other characteristic forms. It melts under hot water, and is deposited from its solution in boiling water acidified with hydrochloric acid, as minute crystals, which are lustreless after drying and melt at 137–139°. Hyoscyamine aurichloride is precipitated in brilliant, irregular, golden-yellow scales, appearing under the microscope in quadratic forms. It retains its lustre when dry,

and melts at 165°. Hyoscine aurichloride crystallises in yellow prisms which melt at 198–200°, and are less soluble and less lustrous than the hyoscyamine salt. Homatropine aurichloride is at first oily, but soon crystallises in prismatic forms.

Ladenburg employs the aurichlorides to separate the tropeines from each other. The atropine salt is the least soluble, and in fractional precipitation is thrown down first, while the hyoscyamine salt is the most readily soluble. The alkaloids may be recovered by decomposing the aurichlorides with hydrogen sulphide, adding ammonia to the filtrate, and agitating with chloroform or ether.

The foregoing properties and reactions are almost the only ones affording fairly sharp distinctions between atropine and its allies. The following reactions are (when not otherwise stated) common to the 3 bases, and distinguish them from other alkaloids.

a. By far the most delicate test for the tropeines and scopoleines is their power of producing mydriasis or dilation of the pupil of the eye. Dilation from the application of a solution weaker than 1 in 500 causes little inconvenience to the human eye, but solutions far weaker produce the effect quite distinctly, and even powerfully. The eye of a young cat, dog, or rabbit may be employed for this purpose. In making such an experiment, an aqueous solution must be prepared either of the free alkaloid or of its sulphate or acetate. The solutions should be neutral or only feebly alkaline, not strongly contaminated even with neutral salts, and not alcoholic. A drop or two of such a solution is placed by means of a pipette or glass rod on one of the eyes, and the size of the pupil compared with that of the other pupil from time to time. A solution 1:40,000 produces an effect within 1 hour. E. R. Squibb (*Ephemeris*, 2, 855) states that distinct mydriasis is produced by a solution of 0.000000427 grm. of atropine sulphate in less than an hour. Dogs and cats are more sensitive to mydriasis than man and may serve well for the test. Such an intense effect is quite peculiar to the tropeines and scopoleines (hyoscine is even more powerfully mydriatic), but more or less dilation of the pupil is also produced by cocaine and preparations of hemlock (coniine) and digitalis. Aconitine has a variable effect and nicotine is said first to dilate and then to contract the pupil. Certain ptomaines exert a mydriatic effect.

b. Free atropine, as obtained by evaporating its chloroform or ethereal solution (after liberation of the alkaloid from one of its salts by ammonia), gives a red colour with phenolphthaleïn. This reaction

is common to hyoscyamine and hyoscine, and is also produced by the artificial base homatropine, but is not given by any other alkaloid in common use (except, according to Plugge, the volatile bases coniine and nicotine). Flückiger,[1] who first observed the peculiar behaviour of the tropeines with phenolphthalein, recommends that a minute quantity of the alkaloid to be tested should be placed on phenolphthalein paper, which is then wetted with strong alcohol. No colouration will be produced at first, but on allowing the alcohol to evaporate, and touching the alkaloid with a drop of water, a brilliant red colouration will appear. On adding alcohol the colour is destroyed, but appears again as the alcohol evaporates.[2]

c. When a solution of mercuric chloride in 50% alcohol is cautiously added to free atropine (as obtained by evaporation of a chloroform solution after liberation of the alkaloid by ammonia, avoiding excess), a red precipitate is produced. A. W. Gerrard,[3] who first described this reaction, states that the precipitate consists of mercuric oxide (with a trace of mercurous oxide), and expresses the action by the following equation: $2C_{17}H_{23}O_3N + HgCl_2 + H_2O = HgO + 2C_{17}H_{23}O_3N,HCl$. The atropine hydrochloride reacts with an additional quantity of mercuric chloride to form the double chloride $\overline{B},HCl,2HgCl_2$, which separates in crystalline tufts when the liquid is allowed to stand for a few hours. In a later paper[4] Gerrard modified and more precisely defined the method of making the test as follows: 0.1 grain of the free alkaloid (extracted from a salt by ammonia and chloroform) is placed on a watch-glass or in a test-tube, and 20 minims of a 2% solution of mercuric chloride in proof-spirit gradually added. A red colouration is yielded at once by atropine. Hyoscyamine at first becomes yellow, then darkens a little, and finally, on heating, a well-marked red precipitate is formed. If a large excess of hyoscyamine be used, merely a yellow precipitate is formed, while with a large excess of the reagent no precipitation occurs. Homatropine also yields a red precipitate under the conditions of the test; but hyoscine gives neither a red nor a yellow colouration or precipitate, and hence is sharply distinguished from the others. Gerrard found no red or yellow precipitate to be produced by strychnine, brucine, morphine, codeine, veratrine, aconitine, coniine, gelsemine, caffeine, cinchonine,

[1] *Arch. Pharm*, 1884, 222, 827.
[2] This behaviour is peculiar Alkali hydroxides react perfectly with phenolphthalein in alcoholic solution
[3] *Pharm. J.*, 1884 [iii], 14, 718.
[4] *Pharm J.*, 1891 [iii], 21, 898.

cinchonidine, quinine or quinidine; though most of these substances gave white precipitates which, in the cases of codeine and morphine, became pale yellow on heating. This behaviour has been confirmed by Schweissinger,[1] who also states that with mercuric chloride cocaine gives a white precipitate (only appearing in strong solutions and soluble on warming), while strychnine, caffeine, and sparteine are stated to yield no reaction. Schweissinger suggests that the test might be made quantitative for atropine by determining the mercuric oxide precipitated; but this would only be possible in the absence of alkaloids, glucosides, or other substances giving precipitates of any kind with mercuric chloride. The value of Gerrard's test has also been confirmed by Flückiger,[2] who found cocaine to give a pure white precipitate which very soon turned red.

d. Gerrard[3] has also observed the liberation of mercurous oxide from calomel and other mercurous salts by the action of atropine. If atropine be dissolved in alcohol and 4 volumes of water added, the solution will immediately precipitate black mercurous oxide from a solution of mercuous nitrate free from excess of acid. This is best prepared by adding sodium hydroxide, drop by drop, to a solution of mercurous nitrate until a slight permanent precipitate is produced, and then filtering.

e. D. Vitali[4] has observed that if a minute quantity of solid atropine be treated with a drop of fuming nitric acid, the liquid evaporated at 100° and the residue when cool touched with a drop of freshly prepared solution of potassium hydroxide in absolute alcohol, a magnificent violet colouration is produced, which slowly changes to dark red and ultimately disappears, but can be reproduced by adding more alcoholic potassium hydroxide. The violet colouration is almost peculiar to atropine, hyoscyamine and hyoscine, and is said to be produced by 0.0001 mgrm. of the alkaloid. Out of 60 alkaloids examined, no other was found to give a violet colouration. The colouration is not produced if aqueous potassium hydroxide be substituted for the alcoholic solution. Strychnine gives a yellow passing into a reddish-violet colour, brucine a greenish, and homatropine a yellow colour when similarly treated. Arnold[5] modifies the test by moistening the alkaloid with strong, cold sulphuric acid, and then adding a fragment of sodium

[1] *Arch. Pharm.*, 1884 [*iii*], 22, 827.
[2] *Pharm. J.*, 1886 [*iii*], 16, 601.
[3] *Pharm. J.*, 1886 [*iii*], 16, 762.
[4] *Pharm. J.*, 1881 [*iii*], 12, 459.
[5] *Arch. Pharm.*, 1882, 20, 564.

nitrite. With atropine a yellow colour is produced which, on applying alcoholic potassium hydroxide, changes to a reddish-violet and then to pale rose. Strychnine, veratrine and pseudaconitine give somewhat similar colours, and homatropine behaves like atropine. Alkaloids which yield strong colourations before the application of the alcoholic potassium hydroxide (*e. g.*, morphine, narcotine, narceine) render the test inapplicable. Fluckiger[1] recommends that 0 001 grm. of atropine and about the same quantity of sodium nitrate should be rubbed together with a glass rod, the end of which has been moistened with a very little concentrated sulphuric acid. A saturated solution of sodium hydroxide in absolute alcohol is then added drop by drop; when in presence of atropine a red or violet colour will be produced. When sodium nitrite is substituted for the nitrate in the above test, an orange mixture is obtained, which, on dilution with a strong aqueous solution of sodium hydroxide, turns in succession to red, violet and lilac.

E. Beckmann[2] has pointed out that veratrine behaves somewhat similarly to atropine with Vitali's test, but states that with nitrous acid or a nitrite instead of a nitric acid, and aqueous instead of alcoholic potassium hydroxide, atropine gives a reddish-violet colouration and veratrine a yellow one.

f. When atropine is heated to the b. p. with a mixture of equal volumes of glacial acetic and strong sulphuric acids, no colouration is at first produced; but after a time the liquid exhibits a well-marked yellowish or brownish-green fluorescence. After cooling, the liquid has a pleasant aromatic odour in addition to that of acetic acid. The behaviour of other tropeines with this test, which is due to E. Beckmann, does not appear to have been recorded. Veratrine gives a similar brownish fluorescent liquid, but during the previous heating the solution acquires an intense cherry-red colour.

g. If a small quantity of atropine be evaporated with sulphuric acid and gently heated it gives a pleasant odour resembling orange-flower, which changes to that of hawthorn or bitter almonds when a crystal of potassium dichromate or permanganate is added.

h. A saturated solution of bromine in hydrobromic acid gives with atropine, hyoscyamine and their salts, even in very dilute solutions (1:10,000), a yellow amorphous precipitate which in a short time becomes crystalline. The precipitate from somewhat strong solutions of the alkaloid disappears after a time, but is immediately reproduced

[1] *Pharm. J.*, 1886 [iii], 16, 601.
[2] *Arch. Pharm.*, 1886 [iii], 24, 481.

on adding more of the reagent. The precipitate is insoluble in acetic acid, and only very sparingly soluble in a large excess of the mineral acids or fixed alkali hydroxides. It is even produced from a solution of atropine in concentrated sulphuric acid. The microscopic appearance of the precipitate exhibits under a magnifying power of 75 to 125 diameters bunches of needles or lanceolate, leaf-like crystals, grouped together like the petals of a flower. These forms may be obtained by the spontaneous evaporation of a drop of liquid containing only 1/25,000 grain of atropine. If not produced, a drop of water should be added and evaporation repeated. T. G. Wormley, who is the observer of the reaction, finds that most alkaloids give amorphous yellow precipitates with the reagent, but he considers the formation of crystals quite characteristic of atropine or hyoscyamine. He points out, however, that when the reagent is added to a syrupy solution of hyoscine, reddish-yellow globules result, which crystallise later, but that no crystals are formed in solutions of 1:100 or of greater dilution. (*Amer. J. Pharm.*, 1894, **66**, 513).

These statements require modification. Hyoscine and *i*-scopolamine give with Wormley's reagent, tabular crystals often arranged in leaf-like rosettes, even in dilutions of 1:2,000; and crystals are also given by homatropine and caffeine. Excepting at great dilutions the freshly formed precipitate obtained with atropine hyoscyamine, hyoscine or *i*-scopolamine is amorphous but rapidly crystallises, especially if stirred with a glass rod, that obtained with hyoscine or *i*-scopolamine, however, usually takes a few seconds longer to crystallise, but the difference in the time taken cannot be utilised as a means of identification.

This test cannot, therefore, as has been stated, be relied upon to distinguish hyoscine from other vegetable mydriatic alkaloids.

The most characteristic differences between the precipitates obtained with hyoscine, atropine and hyoscyamine lie in their solubilities, that of hyoscine being the greatest—only a slight cloudiness is produced in solutions of 1:5,000, whereas atropine and hyoscyamine give a distinct precipitate in solutions of 1:10,000.

In the case of H. H. Crippen, who was charged with the murder of his wife in London, and convicted in October, 1910, evidence as to the finding 2/5-grain of hyoscine hydrobromide in the body was submitted. The identification of the alkaloid depended upon the following facts: 1. The alkaloid produced mydriasis. 2. It gave the Vitali test and

was hence a vegetable mydriatic alkaloid. 3. The alkaloid was gummy and did not crystallise. 4. With the Wormley test gummy spheres and no crystals were obtained.

The fact that gummy spheres were obtained with the Wormley test was mainly depended upon to distinguish hyoscine from other vegetable mydriatic alkaloids.

i. Marmé's reagent, made by dissolving 10 grm. of potassium iodide and 5 grm. cadmium sulphide in 100 c.c. of water, gives characteristic crystalline compounds with hyoscyamine and atropine when added drop by drop to solutions of the alkaloids in dilute sulphuric acid. The crystalline forms of the hyoscyamine and atropine compounds are quite distinctive.

j. Mayer's reagent, potassio-iodide of bismuth, phosphomolybdic and phosphotungstic acid precipitate atropine hyoscyamine and the allied alkaloids from somewhat dilute solutions, and may be of service for separating traces of them from other organic matter.

The reactions of atropine and its isomers with other reagents are not characteristic. Potassium thiocyanate, ferrocyanide, ferricyanide and chromate fail to precipitate even concentrated solutions of these alkaloids.

In testing substances suspected of containing these alkaloids it should be borne in mind that atropine and its allies are not removed from acidified solutions by agitation with immiscible solvents. From solutions rendered alkaline by ammonia or an excess of alkali-metal carbonate, they are readily and completely extracted by chloroform, and with less facility by ether. The separated solution may be evaporated, and the residue dried without loss at 100°. The bases thus isolated are distinguished from all other well-known alkaloids by their power of producing mydriasis, reddening phenolphthaleïn (test b), and (with the exception of hyoscine) giving a red precipitate when warmed with an alcoholic solution of mercuric chloride (test c). Nevertheless, where sufficient material is available the m. p. and crystalline form of the alkaloids and the aurichlorides, auribromides and picrates afford the most satisfactory and conclusive evidence as to their identity.

TOXICOLOGICAL DETECTION OF ATROPINE AND ITS ALLIES.

Atropine, hyoscyamine and hyoscine are all highly poisonous. Cases of poisoning by the pure alkaloids are rare, but both criminal

and accidental poisoning by the plants of which they are the active principles have been frequent; and, in India, poisoning by various species of datura is very common and has achieved the position of a profession.

The symptoms of poisoning by atropine and its allies are thus described by A. Swaine Taylor: Heat and dryness of the mouth and throat, nausea, vomiting, giddiness, indistinct or double vision, delirium, great excitement and restlessness, convulsions followed by drowsiness, stupor, and lethargy.[1] The pupils are much dilated and the eyes insensible to light. Occasionally the pupils are contracted during sleep, although dilated in the waking state. The symptoms often come on very soon after taking the poison, while recovery may be delayed for several days or even a week. The symptoms of poisoning by datura are very similar to those produced by belladonna and hyoscyamus, but more severe. Ringing in the ears, dryness of the throat, and flushed face are early symptoms. Delirium of a violent kind, with spectral illusions, come on rapidly and the pupils are widely dilated. There is often paralysis of the lower extremities.

The postmortem indications of poisoning by atropine and its isomers are not characteristic, except that the pupils are dilated. The brain and its membranes are found congested. Where solid parts of a solanaceous plant have been eaten the fragments may often be found in the stomach, and identified by their botanical and microscopic characters.

In the case of a child who died of atropine poisoning Solstein[2] was unable to detect the alkaloid in the liver, kidneys or viscera, but obtained a trace from the urine which gave a good mydriatic effect but no reaction with Vitali's test.

The detection of atropine and its isomers in cases of poisoning may be effected by the Stas-Otto process. Heating with alkalies or mineral acids must be avoided, or the alkaloid may undergo hydrolysis (page 291). Hence tartaric or acetic acid should be used to acidify the matters to be examined. Ammonia or a carbonate of an alkali-metal should be used to liberate the alkaloid, and ether or (preferably) chloroform employed for its extraction.

In extracting animal matter suspected of containing these alkaloids,

[1] The symptoms of atropine poisoning, especially in children, are not unlike those of scarlet fever. Some cases resemble rabies, and the garrulous delirium and hallucinations of an adult are very similar to those of delirium tremens.

[2] *Zeit. öffent. l. Chem.*, 1897, 3, 115.

it has been recommended that they should be acidified with tartaric acid, dried and extracted with chloroform. The most suitable tests for the recognition of atropine and its allies are detailed above (page 304).

Atropine does not appear to suffer rapid change in the body after death. It has been detected after a considerable interval of time. Ptomaines have been found which exert mydriatic action.

PLANTS YIELDING MYDRIATIC ALKALOIDS.

Atropa belladonna, or deadly nightshade,[1] *Scopolia carniolica*, *Hyoscyamus niger*, or henbane,[2] *Hyoscyamus muticus* and *Datura Stramonium* or thorn-apple,[3] are the chief sources of the tropeines; but these or similar alkaloids are found in a number of allied species. A minute proportion of hyoscyamine has been found in lettuce by T. S. Dymond,[4] and Wright[5] states that *Lactuca muralis* contains a minute proportion of a mydriatic alkaloid.

In addition to the alkaloids, which are probably in combination with malic acid, **belladonna root** contains cellulose, starch, sugar, inulin, asparagin, fatty matter, a fluorescent substance[6] and a red colouring matter called atrosin, which is also found in considerable quantity in the berries. The proportion of starch in young belladonna roots is considerable, but it is present only to a limited extent in older and more woody roots, and, according to W. Merz, is almost entirely absent during summer. The following analyses of air-dry belladonna roots are due to E. M. Holmes:

[1] *French*, la Belladone, la Morelle furieuse, *German*, Tollkirsche, Wolfskirsche, Tollkraut.
[2] *French*, la Jusquiame, *German*, Bilsenkraut.
[3] *French*, Stramoine, *German*, Stechapfel.
[4] *Proc*., 1891, 6, 165
[5] *Pharm. J.*, 1905, 74, 549
[6] The fluorescent substance contained in belladonna root, and present also in the leaves and stalk, is called by H. Kunz (*Arch. Pharm*., 1885 [iii], 23, 722) *chrysatropic acid*, and is said to have the formula $C_{11}H_{10}O_5$. H. Paschkis (*Arch. Pharm*., 1885 [iii], 23, 541; 1886 [iii], 24, 155) has isolated what is apparently the same substance from the berries of ripe belladonna, and ascribes to it the formula $C_{10}H_8O_4$. He considers it identical with the scopoletin, obtained by Eykman from Scopolia japonica. It forms pale yellow rhombic prisms or needles, m. p. 198–201°, and subliming without decomposition when carefully heated. It dissolves in about 80 parts of hot water, more sparingly in cold water and ether, but readily in acetic acid, alcohol, chloroform, amyl alcohol and benzene. It is extracted by the last 3 solvents from its aqueous solution. The aqueous, alcoholic and ammoniacal solutions exhibit a splendid fluorescence blue when dilute, and emerald-green when concentrated. The fluorescence is destroyed by acids Ferric chloride gives an emerald-green colouration changing to cobalt-blue. Fehling's solution and ammonio-nitrate of silver are reduced on warming. In moderately concentrated nitric acid the substance dissolves with yellow colour, changed to blood-red by ammonia. (This reaction resembles that of aesculin, observed by Sonnenschein)

Kunz isolated chrysatropic acid by treating the extract of belladonna with acid and agitating with ether. On evaporating the ether, and washing the crystalline residue with cold ether, chrysatropic acid remained, while leuco-tropic acid, $C_{17}H_{21}O_5$, dissolved. The latter is a bitter substance, crystallising in microscopic prisms, m. p. 74°.

	Woody roots	Soft roots
	%	%
Moisture	7.94	10.28
Soluble ash	3 43	2.20
Insoluble ash	4 60	3 68
Alcoholic extract	22.53	29.87
Aqueous extract	15 96	10.50

Belladonna leaves contain cellulose, chlorophyll, alkaloidal salts, fatty and resinous matters, etc. Choline is present, and, according to Biltz, asparagin sometimes crystallises from the extract after long keeping, but the crystals observed by Attfield consisted of potassium nitrate and chloride. By dialysis, Attfield isolated potassium nitrate and square prisms of an organic salt of magnesium. Kunz found 0.6% of succinic acid in an extract prepared from the herbaceous parts of belladonna. Flückiger found the ash of dry belladonna leaves to amount to 14.5% and to consist chiefly of the carbonates of calcium and the alkali-metals.

With regard to the *alkaloids* of belladonna; they consist principally of hyoscyamine and atropine, though a small proportion of hyoscine is also present. Since hyoscyamine is readily converted to atropine in the process of extraction, no great reliance can be placed on the statements made by earlier workers as to the proportions of these two alkaloids present in different parts of the plant and at different seasons; but a large proportion of hyoscyamine occurs in all cases. Schutte[1] has shown that young roots contain only hyoscyamine and older ones a small proportion of atropine.

As the result of published investigations it may be concluded that belladonna roots and leaves of average quality contain 0.4% total alkaloid.

The following figures give the percentages observed in the dried roots and leaves by various workers:

	Percentage of total alkaloid
Dried Roots.	
Lyons	From 0.42 to 0.86
Kordes	From 0.64 ..
Gerrard	From 0.21 0.45
Bryant	From 0.53 ....
Keller	From 0.66 0.67
Gadd	From 0.28 0.62
Caesar and Loretz	From 0.51 0.86
Barclay	From 0.45 0.46
Farr and Wright	From 0.31 0.64
Carr and Reynolds	From 0.29 0.55

[1] *Arch. Pharm.*, 1892, 229, 492.

	Percentage of total alkaloid
Dried Leaves.	
Lefort	From 0.436
Dragendorf	From 0.66
Schmidt	From 0.26 Cultivated. / From 0.40 Wild.
Lyons	From 0.23 to 0.87
Gerrard	From 0 22 0.58
Kordes	From 0 64
Gunther	From 0.84
Farr and Wright	From 0.55
Beckurts	From 0 2 0.6
Carr and Reynolds	From 0.23 1.08

According to the results of certain investigators the plant produces more alkaloid under cultivation than when wild, but just the reverse has been observed by others. These discrepant results are no doubt explained by the observations of recent workers that different seasonal conditions, differences of soil and the different ages of the plant considerably modify the amount of alkaloid contained in it. J. Rippetoe[1] has shown that the dried leaves of first and second year's plants contained respectively 0.32% and 0.68%. Carr and Reynolds[2] have shown that dried leaves and stems grown on the same plot in 3 successive years contained 0.38%, 0.66% and 0.33%. Chevalier[3] has shown that nitrogenous manures have the effect of increasing the percentage of total alkaloids while other manures have very little effect.

J. Henderson[4] has observed that imported roots often contain very small percentages of alkaloid and that handsome starchy roots are frequently deficient in alkaloid.

Gunther found that the dried fruit contained 0.82% of alkaloid, while the unripe fruit contained rather more, viz. 0.95%; and this observation has been confirmed by Williams.[5]

Both the leaves and roots of *Phytolacca decandra* have been employed in adulterating belladonna, which they superficially resemble. According to Holmes the leaves are to be recognised by their upper surface being without hairs, the epidermal cells being polygonal and the stomata elliptical; whereas belladonna leaves have hairs on the upper surface, irregular epidermal cells and round stomata. The roots resemble belladonna in colour and in appearance but are readily distinguished from the latter by their concentric rings of woody tissue and by

[1] *Amer. J. Pharm.*, 1907, 79, 523.
[2] *Pharm. J.*, 1908, 80, 542.
[3] *Compt. Rend.*, 1910, 150, 344.
[4] *Pharm J.*, 1905, 75, 191.
[5] *Pharm. J.*, 1909, 83, 493.

separation of these rings into fibrous laminæ when the root is broken. No standard of alkaloidal content for belladonna leaves or root is imposed by the *British Pharmacopœia*, but that of the United States imposes one for both the dried leaves and roots. The *German* and *Swiss Pharmacopœias* require wild plants only to be employed. In both the *British* and *United States Pharmacopœias* galenical preparations are standardised.

The following table summarises the standards adopted by these two pharmacopœias.

Galenical preparation	Total alkaloid	
	B P, 1898	U. S P, 8th rev.
Belladonna leaves	No standard	0.3%.
Belladonna leaves, extract of		1 4%
Belladonna leaves, tincture of		0 035% w/v
Belladonna leaves, green ext	No standard	
Belladonna root	No standard	0.45%
Belladonna root, liq extract of	0 75% w/v	0 4% w/v
Belladonna root, tincture	0 05% w/v	
Belladonna root, alcoholic ext	1 0%	
Belladonna plaster	0 5%	0 4%
Belladonna liniment	0 375% w/v	0 38% (about)

The green extract of the *British Pharmacopœia* contains on an average 1% of total alkaloid, but has been shown to vary very considerably. Naylor and Bryant found as little as 0.55% in one sample and as much as 1.80% in another.

For the assay of belladonna root, Dunstan and Ransom[1] recommend extraction in the following manner: 20 grm. of the dry and finely-powdered root are extracted by hot percolation with a mixture of equal volumes of chloroform and absolute alcohol. If an extraction apparatus be used about 60 c.c. of the mixture will be required. The solution is agitated with 2 successive quantities of 25 c.c. of distilled water, adding a small quantity of dilute sulphuric acid. The separation of the aqueous liquid from the chloroform occurs promptly and completely on warming the liquid slightly. The chloroform retains nearly the whole of the colouring-matter, while the alcohol and alkaloids (as salts) pass into the water. The aqueous layer is separated, and agitated once with chloroform to remove the last traces of colouring matter; after which it is rendered alkaline with ammonia, and agitated twice with chloroform, using 25 c.c. each time, to extract the alkaloid. The separated chloroform is agitated once with water rendered faintly

[1] *Pharm. J.*, 1884 [iii], 14, 623.

alkaline with ammonia, and then evaporated, the residue being dried at 100° till constant in weight. The alkaloid thus isolated is obtained as a perfectly transparent fused mass.

Alcohol alone may be used for the extraction, but dissolves more extractive which impedes the subsequent extraction with chloroform.

A modification of this method may also be used for the assay of galenical preparations of belladonna. The method of procedure should be as follows: a quantity of the preparation equivalent to about 20 grm. of the root or leaves is employed (*i. e.*, 10 grm. of soft extract, 15 c.c. of liquid extract and 200 c.c. of tincture). To the soft extract 10 c.c. of water are added, the tincture is gently concentrated to about 20 c.c. and the liquid extract may be taken as it is. After transferring to a separating funnel, a small, though a decided, excess of dilute ammonia solution is added, then 30 c.c. of chloroform, and after shaking and separating it is again extracted with 2 successive quantities of 20 c.c. of chloroform. If much trouble with emulsification is encountered it may be necessary to add a little alcohol and to give it further shakings with chloroform. The alkaloid contained in the mixed chloroform solution is extracted by shaking with 20 c.c. of 2% sulphuric acid and separating and again rinsing twice with water. The acid solution is now rendered alkaline with ammonia and completely extracted with chloroform, and the chloroformic extract, after rinsing, is evaporated, dried and weighed; it should be crystalline. It is then dissolved in $N/10$ sulphuric acid and titrated back with $N/100$ sodium carbonate, using hæmatoxylin or cochineal as indicator.

Belladonna plaster, *British Pharmacopœia*, is prepared from the "liquid extract" of the root and contains lead oleate, soap, resin and other ingredients, which render the assay somewhat troublesome to carry out. The following process, based on that recommended by Bird,[1] obviates these difficulties: 15 grm. of the plaster are dissolved with gentle heat in a mixture of 35 c.c. of chloroform and 5 c.c. of glacial acetic acid; 70 c.c. of 4% sulphuric acid are added and the mixture warmed and stirred. It is filtered under pressure and the cake of lead sulphate disintegrated and warmed with a mixture of chloroform 10 c.c. and 4% sulphuric acid 10 c.c. and again filtered; the chloroform layer is separated from the mixed filtrates and washed twice with 5 c.c. of 4% sulphuric acid. The mixed aqueous portions are then rendered alkaline with ammonia and extracted with chloro-

[1] *Analyst*, 1899, 24, 175.

form and again shaken into acid and back into chloroform, proceeding as in the assay of other belladonna preparations.

Belladonna plaster, *United States Pharmacopœia,* is prepared from extract of belladonna leaves and contains rubber, hard paraffin and lead oleate. The method of assay laid down by the *United States Pharmacopœia* is shortly as follows:

10 grm. of the plaster, 50 c.c. of chloroform and 3 c.c. ammonia water are macerated together, the liquid is poured off and the cloth washed with a mixture of 25 c.c. of chloroform and 1 c.c. of ammonia solution, this operation being repeated if necessary. The cloth is dried and weighed and this weight deducted from that originally taken. To the chloroform solution four-fifths of its volume of 95% alcohol is added, and the rubber allowed to separate. The supernatent liquid is extracted with 20 c.c. of a mixture of 40 c.c. sulphuric acid and 60 c.c. of water; and this is repeated, using 10 c.c. of the mixture, until all the alkaloid is extracted. The combined acid extract is rendered alkaline with ammonia and completely extracted with chloroform, the chloroform evaporated and the residue titrated.

Many small modifications of the method of assaying belladonna and its preparations (designed principally to obviate emulsification in the first stages of extraction) have been proposed, see:

H. W. and S. C. Gadd (*Pharm. Jour.*, 1905, **75**, 438).

Rupp (*Pharm. Zeit.*, 1908, **53**, 737).

Naylor (*Pharm. Jour.*, 1907, **78**, 393).

Lyons (*Pharm. Rev.*, 1908, 22).

HYOSCYAMUS.

The flowering tops and leaves of *Hyoscyamus niger* or henbane contain principally hyoscyamine, probably some atropine, and certainly some hyoscine.

The amount of alkaloid present in different samples has been shown to vary considerably, the following results having been recorded for the analyses of dried leaves and tops:

Hyoscyamus Leaves and Tops.

Schmidt	0.286%
Kordes	0.15%
Dohme	0.173%
Beckurt	0.089%

Gerrard	0 065%
Parke, Davis & Co. .	0.073% to 0.13%
Barclay. . . .	0.09%
Umney	0.07% to 0.1%
Carr & Reynolds . .	0 06% to 0 21%
Farr & Wright	0 064% to 0.12%

The *British* and *United States Pharmacopœias* require that second year's leaves and tops alone should be employed, but it has been shown[1] that first and second year's leaves grown in the same locality contain the same amount of alkaloid.

A. W. Gerrard[2] has employed substantially the same process as the above for the assay of belladonna for the root and leaves of henbane. The substance is dried at 100°, powdered and exhausted with proof-spirit. The spirit is distilled off, and the semi-fluid extract treated with water containing 1 per 1,000 of hydrochloric acid, filtered, and the filtrate further diluted to 100 c.c. The alkaloids are extracted by ammonia and chloroform in the usual way, purified by solution in ether, and agitated with hydrochloric acid; again liberated by ammonia, extracted by ether, and determined in the alkaloidal residue by titration with $N/10$ hydrochloric acid.

The following results are recorded:

Variety of henbane	Part used	Where grown	Yield of alkaloids per 1,000
Biennial . .	Roots	Middlesex	1 602
Biennial	Roots	Sussex .	1 550
Biennial	Roots	Lincolnshire .	1 729
Biennial	First year's leaf	Lincolnshire	0 690
Biennial	First year's leaf	Sussex	0 667
Biennial	First year's leaf	Middlesex	0 592
Biennial	Second year's leaf	Middlesex	0 672
Biennial	Second year's leaf	Sussex	0 680
Biennial .	Second year's leaf	Lincolnshire	0 656
Annual	Leaves and tops	Leicestershire	0 641
Annual	Leaves and tops	Surrey	0 689
Annual .	Leaves and tops	Middlesex	0 701
Annual	Entire herb	Germany	0 295
Biennial .	First year's leaves	France	0 398
Biennial	First year's leaver (old)	England	0.390
Biennial	Second year's tops (old)	England	0 451

Gerrard considers that bright-coloured, well-preserved henbane, whether annual or biennial, can be relied on to yield good preparations, while old and dark-coloured leaves, containing stalks and fruit, should

[1] Carr and Reynolds, *Pharm. J*, 1908, 80, 542.
[2] *Pharm J.*, 1890 [iii], 21, 212, 1891 [iii], 22, 213.

be avoided. He regards the first year's root of biennial *Hyoscyamus niger* as much richer in alkaloids than the herbaceous portions of the plant, but both as much poorer than the respective parts of belladonna. These conclusions are in opposition to the experience of E. Thorey (Dragendorff's *Quelques Drogues Actives*), who found henbane to contain alkaloid in greatest quantity in the leaves, next in the fruit, then in the roots, and lastly in the stalk.

Ransom found 0.058% of alkaloid in the seeds of biennial henbane grown at Hitchin; but a sample of seeds examined by Farr and Wright contained 0.12% of alkaloid.

No standard of alkaloidal content is required by the *British Pharmacopœia* in *hyoscyamus* or in any of its preparations. A good *British Pharmacopœia* extract should contain 0.2% and a good tincture 0 01% w/v of alkaloid. The *United States Pharmacopœia* (8th Rev.) adopts the following standards:

Leaves and flowering tops not less than. .	0.08%
Liquid extract	0.075% w/v.
Tincture	0 007% w/v.
Extract	0 3%.

The *Swiss Pharmacopœia* requires that the leaves should contain 0.1% of alkaloid.

The method of assaying hyoscyamus and its preparations is substantially the same as that described above for belladonna; about 5 times as much of the leaves or preparation should, however, be employed on account of the smaller proportion of alkaloid present.

Hyoscyamus albus, a closely allied plant, contains about the same percentage of alkaloid as *Hyoscyamus niger*. Though it is not officially recognised by the pharmacopœias of Great Britain or the United States, the French Codex allows *H. niger* or *H. albus* to be employed indiscriminately, and the latter variety is actually much used in the south of Europe.

Hyoscyamus muticus is a species of henbane growing in certain districts from Egypt to India, where it is employed medicinally. Dunstan and Brown[1] found the dried stem and leaf of the plant grown in India to contain 0.1% of mydriatic alkaloid, while that grown in Egypt was shown to contain 0.59%. Gadamer found even more in the Egyptian plant, namely, 1.34% in the seeds and capsules, 1.39%

[1] *Trans.*, 1899, 75, 72; 1901, 79, 71.

in the leaves and 0.57% in the stem. The alkaloid consists almost entirely of hyoscyamine.

DATURA.

The alkaloids of *Datura Stramonium* consist of hyoscyamine, atropine and hyoscine. The amount found in the dried leaves varies from 0.12% to 0.35% and that in the seeds from 0.16% to 0.37%, while an analysis of the dried roots gave 0.15%. The dried leaves from India were found to contain 0.26%.

From stramonium seeds Hartz[1] obtained 0.167% of alkaloid by extracting the fat from the dried substance by petroleum spirit, then removing the alkaloid with proof-spirit, and proceeding in the usual way. Farr and Wright found from 0.16 to 0.24% of alkaloid in stramonium seeds. E. Schmidt found, in 4 samples of stramonium *seed* from different sources, 0.25, 0.37, 0.05, and 0.20% of total alkaloids. From 50 to 70% of these consisted of pure atropine melting at 115°. The remainder, which were more difficult to crystallise, consisted of hyoscyamine, and probably other bases and their decomposition-products. But the relative proportions of the alkaloids are probably very variable, as Ladenburg found hyoscyamine to preponderate and Schutte found that both fresh and old stramonium seeds yielded chiefly hyoscyamine, with small quantities of ready-formed atropine and scopolamine. A. B. Lyons (*Manual of Pharmaceutical Assaying*) found in 5 specimens of *stramonium seeds* proportions of alkaloid (titrated by Mayer's solution) ranging from 0.45 to 0.55%, the extractive matter yielded to strong alcohol by the same samples varying from 3.3 to 7.5%. In eight samples of *stramonium leaves*, Lyons found from 0.40 to 0.52% of alkaloid (titrated), and from 19.5 to 25.3% of extractive matter yielded to spirit of 66%. Farr and Wright extracted from 0.12 to 0.22% of alkaloid from stramonium leaves.

The dried leaves and seeds of D. stramonium are employed for making tinctures and extracts, and are officially recognised in most pharmacopœias. The *British Pharmacopœia* requires no standard in either the drug or its preparations, but the *United States Pharmacopœia* requires the following:

D. stramonium leaves	0.25%
D. stramonium extract	1.0%

[1] *Pharm. J.*, 1884 [iii], 15, 203.

D. stramonium liquid extract ..... 0.25 % w/v.
D. stramonium tincture ..... 0.025% w/v.

Stramonium leaves form an important ingredient of smoking mixtures which are employed as a sedative in asthma.

D. stramonium[1] and its preparations may be assayed by the same method as that employed for belladonna.

D. metel[2] contains about 0.5% of alkaloids, consisting mainly of hyoscine with a small amount of atropine and hyoscyamine. *D. meteloides* contains about 0.4% of alkaloids from which 0.13% of hyoscine, 0.03% of atropine and 0.07% of meteloidine have been obtained. *D. arborea* contains both hyoscine and hyoscyamine. *D. quercifolia* contains 0.4% of total alkaloid consisting of hyoscine and hyoscyamine.

The seeds of *D. alba* have been mistaken for those of capsicum, which they resemble in general appearance. They may be readily distinguished by their faint, bitter taste and by the absence of the intense burning effect upon the tongue and lips which is such a characteristic property of capsicum.

SCOPOLIA.

The dry rhizome of Scopolina atropoides or Scopolia carniolica contains about 0.5% of mydriatic alkaloid consisting principally of hyoscyamine. Although it is not recognised by most pharmacopœias it is official in those of the United States and Japan. The former of these requires that the rhizome should contain not less than 0.5% of total alkaloid. The same pharmacopœia requires that the soft extract of scopolia should contain 2% of total alkaloid and the liquid extract 0.5%.

The method of assay of Belladonna and its preparations, as described above, may be employed in the analysis of scópolia and its preparations.

Andrews, *Trans*, 1911, 99, 1871
Schmidt, *Arch Pharm*, 1905, 243, 309, 1910, 248, 641.

COCAINE.

By SAMUEL P. SADTLER.

Cocaine. Benzoyl methyl-ecgonine. Methyl benzoyl-ecgonine.

$$C_{17}H_{21}O_4N; \text{ or } C_8H_{13}N \begin{cases} O.C_7H_5O \\ CO.OCH_3 \end{cases}$$

Cocaine is the characteristic alkaloid of coca leaves, and of great medicinal value as a local anesthetic. It may be extracted from the plant by the usual processes with associated alkaloids, avoiding as much as possible treatment with acids and alkalies, as it undergoes hydrolysis with great facility with formation of objectionable decomposition-products. Instead of endeavoring to separate cocaine from accompanying ecgonine and other ecgonine derivatives, the crude extract is converted into ecgonine by hydrolysis, and then treated with benzoic anhydride and methyl iodide as referred to below or a similar method.

The synthesis of cocaine was effected by Merck by heating together ecgonine, benzoic anhydride and methyl iodide to 100° for 10 hours in a sealed tube. The industrial production of cocaine from ecgonine has been effected and patented by Liebermann (page 337).

Cocaine crystallises from a strong alcoholic solution in colourless monoclinic prisms, melting at 98°, and subliming with partial decomposition at a higher temperature.

Cocaine is very slightly soluble in water,[1] but dissolves readily in alcohol, ether, chloroform,[2] benzene, benzin, carbon disulphide and volatile and fixed oils. 100 parts of carbon tetrachloride dissolve 18,503 parts of cocaine at 17°. It is readily removed from its solutions by adding ammonia and agitating with ether or other immiscible solvent.

[1] The solubility of cocaine in cold water is probably near to 1 in 1300 (B H Paul), but is commonly greatly over-estimated, owing to the ease with which cocaine is decomposed by hot water with formation of soluble products.

[2] The solubility of cocaine in chloroform enabled B H Paul to separate it from morphine, and prove a product introduced under the name of hopeine, and said to be a natural narcotic alkaloid from American hops, to be, in fact, an artificial mixture of cocaine and morphine (*Pharm Jour*, 1886 [*iii*], 16, 877).

An aqueous solution of cocaine has a strong alkaline reaction to litmus, cochineal, iodeosin and methyl-orange, but does not affect phenolphthaleïn. The free base may be titrated with the aid of either of the former indicators. An aqueous solution of cocaine, if not very carefully prepared and secluded from air, or preserved by an antiseptic, rapidly decomposes with formation of vegetable growths.

Cocaine produces on the tongue a sudden and characteristic cessation of feeling, which lasts only a few minutes. One drop of a 4% solution (of the hydrochloride), if placed on the tongue, soon produces a decided numbness, the effect being evanescent unless the application be repeated. Cocaine also produces an intense local anæsthetic and blanching effect on the mucous membrane. A single drop of a 4% solution suffices to blanch the conjunctiva of the eye. Anæsthesia of the eye, of much value in ophthalmic operations, can be produced by a somewhat larger dose. Dilation of the pupil is generally produced by cocaine, whether applied locally to the eye or otherwise introduced into the system; but the mydriasis produced by cocaine is not so invariable and is far less intense than that characteristic of atropine and its isomers.

In large doses, cocaine has marked poisonous properties. The fatal dose for dogs is from 2 to 5 grains. The hypodermic injection of 1/20 grain has caused dangerous symptoms in a girl 12 years of age (see *Pharm. Jour.*, 1886 [*iii*], **16**, 721).[1]

Cocaine is levorotatory, the specific rotation in chloroform solution being about $-15.8°$ for the sodium ray; while the rotation of the hydrochloride at 20° in dilute alcohol is $[\alpha]_D = -(52.8+0.1588+q)$ where q = the percentage of alcohol in the solution.

Qualitative Tests.

Cocaine is precipitated from its solutions by alkali hydroxides, alkaline carbonates and ammonia. It is almost insoluble in excess of ammonia, which is to be preferred as a precipitant.[2] Precipitated cocaine is amorphous when thrown down from strong solutions, but rapidly becomes crystalline.

[1] For various alarming symptoms produced by cocaine in dental practice, see remarks by Stockman (*Pharm Jour.* [*iii*], **18**, 791). A resumé of the pharmacology of cocaine and its allies appeared in the *Pharmaceutical Journal* [*iii*], **21**, 161.

[2] If a solution of cocaine salt be precipitated with sodium hydroxide or sodium carbonate, the filtrate will be found to contain a distinct trace of benzoic acid resulting from decomposition of the alkaloid, but this is not the case if ammonia be substituted (B. H. Paul).

Mayer's solution precipitates cocaine from extremely dilute solutions, and A. B. Lyons has attempted to employ the reaction for the estimation of cocaine, but with results which are wanting in exactness.

Iodised potassium iodide gives a rose-coloured precipitate with a solution of 1 part of cocaine hydrochloride in 7,500 of water; in stronger solutions the precipitate appears brown, and under the microscope assumes the form of black globules.

Tannin produces a distinct cloud in neutral solutions of cocaine containing 1:25,000, and a distinct precipitate with twice that proportion. Picric acid produces in strong solutions a yellow precipitate, rapidly becoming crystalline, and appearing under the microscope in sheaf-like forms. Phosphomolybdic acid produces a faint turbidity in solutions of 1:50,000, and a distinct precipitate with 1:12,500. Phosphotungstic acid gives a gelatinous white precipitate, soluble in ammonia.

Platinic chloride produces at once, in solutions of cocaine hydrochloride containing 1:400, a yellow precipitate consisting of plumose needles, mostly of stellate pattern. In solutions of 1·600 most of the crystals resemble carpet-tacks, consisting of short, well-formed prisms, with a single branch from the centre, joined at an oblique angle and tapering to a point. The characters of the platinichloride distinguish cocaine from the amorphous base associated with it in coca-leaves, the platinum salt of which is far less soluble in water, and crystallises in rosette-like forms, contrasting strongly with the feathery appearance of the cocaine salt.

Cocaine aurichloride is precipitated on adding auric chloride to a solution of cocaine hydrochloride. In solutions containing 1:3,000 an immediate precipitate is produced, which appears under the microscope in forms resembling fern-fronds, generally with a stellate arrangement. In solutions of 1:12,000 similar crystals form after a short time. "Cocaïdine" aurichloride forms minute prismatic crystals, having a microscopic appearance quite different from that of the cocaine salt (A. B. Lyons, *Amer. Jour. Pharm.*, 1885, **57**, 10).

According to Lerch and Scharges, if a drop of ferric chloride be added to a solution of cocaine and the liquid boiled, an intense red colour will be developed "owing to the formation of benzoic acid." Benzoyl-ecgonine also gives the reaction.

H. Siemssen (*Pharm. Ztg.*, 1903, **48**, 53, 534) has observed that when an aqueous solution of a cocaine salt is treated with a con-

centrated hot solution of sodium molybdate, a white precipitate is produced which, when examined under a lens of 50 to 60 diameters, appears of a light green colour. This apparent change in colour is characteristic of cocaine and is not exhibited by other alkaloids.

Potassium dichromate does not precipitate cocaine except from neutral solutions, unless they are very concentrated (1:25); but Metzer states that from a solution containing hydrochloric acid, chromic acid precipitates the chromate, $C_{17}H_{21},O_4NH_2CrO_4$, in silky, lustrous plates (compare page 331). If 0.05 grm. of cocaine hydrochloride be dissolved in 5 c.c. of water, and 5 drops of a 5% aqueous solution of chromic acid added, each drop produces a distinct precipitate, which immediately redissolves; but if 1 c.c. of strong hydrochloric acid be now added, a heavy yellow precipitate of cocaine chromate is produced. If cocaine be present, reduction of the chromic acid will ensue. Ecgonine, sparteine, atropine, caffeine, pilocarpine, codeine and morphine do not form yellow precipitates with chromic acid or potassium chromate. Quinine, quinidine, cinchonine cinchonidine, hydroquinine, apomorphine, brucine, strychnine and veratrine form precipitates with 5% chromic acid if the solutions are neutral; but, according to K. Metzer (*Pharm. Zeit.*, 1889, **34**, 697), cocaine is singular in being precipitated only after addition of hydrochloric acid.

F. Giesel (*Pharm. Zeit.*, 1886, **31**, 132) has observed that cocaine permanganate is very stable compared with the corresponding salts of the majority of alkaloids. Hence, if 0.01 grm. of cocaine hydrochloride be dissolved in 1 or 2 drops of water, and about 1 c.c. of a 3% solution of potassium permanganate be added, a purple-violet crystalline precipitate of cocaine permanganate is produced, the supernatant liquid acquiring a purple-violet tint. A. B. Lyons recommends that a strong solution of the cocaine salt should be used, and the permanganate employed in $N/10$ solution (3.162 grm. per litre). The precipitate is unstable, and decomposes in a few hours even at the ordinary temperature, leaving a brown hydrated manganese dioxide. If the liquid containing the precipitate be heated to boiling, decomposition occurs at once but without the production of any peculiar odour. But if examined under the microscope when first thrown down, the precipitate is found to consist, wholly or in part, according to the strength of the cocaine solution, of translucent, violet-red, rhombic (nearly rectangular) plates of great beauty, often grouped together to form rosettes. A 5% solution of cocaine gives a copious precipitate at once,

and a 2% solution after a short time; but with a 1% solution the crystals only form as evaporation takes place.

The behaviour with potassium permanganate serves to detect an admixture of methyl cinnamyl-ecgonine and certain other impurities in cocaine hydrochloride. The presence of these causes an immediate reduction of the permanganate in the cold. The first drop or two of the reagent produces a brown discolouration, while the precipitate thrown down by a further addition is more or less brown, instead of a distinct violet-purple or red. If a limited quantity of the reagent be employed, and the liquid heated to boiling, in presence of impurities a distinct odour will be developed in some cases resembling that of bitter-almond oil, and in others like that of crude cocaine (A. B. Lyons *Amer. Jour. Pharm.*, 1886, **58**, 240). The behaviour of other alkaloids with potassium permanganate is described on page 107.

According to F. da Silva (*Compt. Rend.*, **111**, 348; *Pharm. Jour* [*iii*], **21**, 162), when treated by Vitali's test for atropine (page 306) even a minute quantity of cocaine (0.0005 grm.) develops a distinct and peculiar odour, recalling that of peppermint or citronella. No other alkaloid extracted by benzene from an ammoniacal solution behaves at all similarly, though atropine, hyoscyamine, strychnine codeine and eserine give colour-reactions, and the last-named alkaloid develops a disagreeable smell resembling phenyl-carbamine Delphinine, brucine, and veratrine develop slight odours which cannot be mistaken for that produced by cocaine. A. C. Stark (*Pharm Jour.*, 1891 [*iii*], **21**, 848) has confirmed Da Silva's statements, but considers the odour scarcely distinctive enough to render the test completely reliable.

According to Henriques (*Germ. Pat.*, 77,437) cocaine may be precipitated from dilute solutions by means of zinc tungstate.

C. Reichardt (*Chem. Ztg.*, 1904, **28**, 299) describes a number of new and characteristic reactions for cocaine. On the addition of concentrated solution of sodium nitroprusside, drop by drop, to moderately-concentrated cold solution of cocaine, an immediate turbidity is produced which, when examined under a lens of moderate power, is seen to be due to the formation of well-formed reddish crystals. These consist of cocaine nitroprusside and may be produced in moderately dilute solutions of cocaine salts. If, in the same manner a cold saturated solution of uranium nitrate is added to a fairly strong cold solution of cocaine hydrochloride, an immediate yellow crystallin

precipitate is produced, the composition of which has not been determined, but which is possibly a double salt. It is also obtainable from fairly dilute solutions of the alkaloid. If a little perfectly pure titanic acid is dissolved in a few drops of concentrated sulphuric acid by the aid of heat and allowed to cool, the addition of a trace of cocaine hydrochloride to the cold solution is without effect upon it; but if the mixture is heated while stirring, so that oily drops are separated and are non-adherent to the sides of the porcelain container, the liquid gradually assumes a handsome violet to blue colour. This reaction, which is quite characteristic, is due to a reaction of the titanic acid. If a little potassium ethyl-sulphate is rubbed with a trace of cocaine hydrochloride and a few drops of concentrated sulphuric acid are added to the mixture, no reactions of any kind are manifested until, on applying heat, a distinct odour of peppermint is developed. This manifests itself even in the presence of the smallest trace of cocaine. Finally, if a trace of cocaine hydrochloride is rubbed with urea and concentrated sulphuric acid is added, the mixture remains unchanged in the cold; but on heating it, a blue colour, gradually increasing in intensity, is developed.

Toxicological Identification of Cocaine.—Siemssen (*Pharm. Ztg.*, 1903, 941), referring to the reaction of cocaine with sodium molybdate (page 324), states that he has found it unsuitable for forensic examinations. He finds bromine water, however, to be admirably suited for its identification in toxicological cases, as shown by the following experiment: A segment of intestine was extracted with ether in a Soxhlet apparatus and then impregnated with 1 c.c. of a 0.01% solution of cocaine by setting it aside during 2 days. The segment was then repeatedly shaken with ether-alcohol and then transferred to a glass cylinder containing 2 c.c. of bromine water which had been saturated at 20°. A light yellow voluminous precipitate resulted immediately, which proved to be insoluble in the precipitant. Atropine, brucine, morphine, strychnine and several other alkaloids, treated in the same way, afforded reactions which were in each case characteristic and could be distinguished from each other and from cocaine. The individual observations made will be the subject of a future paper.

Salts of Cocaine.

Cocaine Hydrochloride. Hydrochlorate of Cocaine. $C_{17}H_{21}O_4N,HCl$. This salt, which is readily prepared by neutralising

cocaine by hydrochloric acid, crystallises from alcohol in short prisms melting at 189.9°. The crystals from the aqueous solution contain, according to A. B. Lyons, 9.6% of water, while those from the alcoholic solution are anhydrous. The salt is not hygroscopic, but is soluble in less than its own weight of water, forming a thick syrupy liquid. It is readily soluble in spirit, but with less facility in absolute alcohol, chloroform, and amyl alcohol; and is practically insoluble in ether, petroleum spirit, and fixed and volatile oils. Ether precipitates cocaine hydrochloride from its solutions in absolute alcohol[1] and chloroform.

Cocaine hydrobromide, $BHBr,2H_2O$, crystallises readily from its aqueous solution in transparent prisms, stable in the air.

Cocaine acetate is readily soluble in water. It is difficult to obtain it in a crystalline condition, as acetic acid is given off during the evaporation of its solution.

Cocaine oleate readily crystallises, and is soluble in oleic acid and fixed oils.

Cocaine gives crystalline salts with sulphuric, boric and oxalic acids. The *citrate* is hygroscopic, and crystallises with difficulty.

Cocaine benzoate, $C_{17}H_{21}O_4N,C_7H_6O_2$, may be prepared by mixing molecular proportions of cocaine and benzoic acid. It is a very soluble salt, obtainable with difficulty in acicular crystals, the solution usually drying up to a gummy mass, which gradually acquires a crystalline structure. A sample of commercial cocaine benzoate of French origin was found by B. H. Paul to give no precipitate of cocaine with ammonia, and no benzoic acid with hydrochloric acid. It consisted of benzoyl-ecgonine (*Pharm. Jour.* [*iii*], **16**, 817). According to A. Bignon (*Pharm. Jour.*, 1886 [*iii*], **16**, 721), the anesthesia produced by a 5% solution of cocaine benzoate lasts during 4 consecutive hours and is not preceded by the sensation of pain produced by the hydrochloride.

Cocainemethobromide, $C_{17}H_{21}O_4N.CH_3Br$, is prepared by heating cocaine and methyl bromide together for 2 hours at 100°. Crystallises out of alcohol.

Cocainemethiodide is prepared similarly to the brommethylate.

Cocaineethylester, $C_9H_{13}(C_2H_5O)O_2N.C_7H_5O$, is prepared by heating benzoylecgonine and ethyl alcohol at 100° (Merck, *Ber.*, 1885, **18**, 2954), m. p. 108–109°.

[1] Stockman (*Pharm. Jour*, 1887 [*iii*], 17, 862) gives the solubility of pure cocaine hydrochloride in chloroform, absolute alcohol, and amyl alcohol as 1 in 48, 1 in 34, and 1 in 70 respectively; but B. H. Paul does not find such large proportions of solvent necessary.

The propyl and isobutyl esters (m. p. 78–79.5° and 61–62°) respectively have been described by Novy (*J. Am. Chem. Soc.*, 1887, **10**, 147), as well as the bromethyl-ester, made by heating benzoyl-recgonine, ethylene bromide and alcohol to 95°.

Derivatives of dextrorotatory cocaine are similar to the laevo derivatives.

Examination of Commercial Cocaine and its Salts.

The absolute purity of cocaine and cocaine salts intended for medicinal use is essential, as various undesirable and even dangerous symptoms are produced by certain impurities liable to be present. Fortunately the tests now given in the pharmacopœias (especially the *United States Pharmacopœia*) are sufficient to prove the purity of cocaine.

Crude cocaine has for some time been manufactured in South America for export to European markets in place of coca leaves, which have been found to be liable to deterioration in transit. B. H. Paul (*Pharm. Jour.*, 1888 [*iii*], **18**, 782) describes it as a white or yellowish pulverulent substance compressed into thin cakes. It contains not only earthy substances, sodium carbonate and lime salts, but also a waxy substance and traces of petroleum. Its manufacture has probably been effected by extracting the coca leaves with petroleum spirit, washing out the alkaloid with an acid, and then precipitating it with lime or sodium carbonate. It is represented as containing from 80 to upwards of 90% of alkaloid, but the proportion of crystallisable cocaine present varies considerably, in one instance not exceeding one-half of the total alkaloid present (85%). The remaining portion was precipitated on adding ammonia to its solution in hydrochloric acid in oily globules, which after a time collected at the bottom of the liquid as a viscid semi-transparent layer, which ultimately became more or less crystalline. In all cases the liquid remained quite milky for a considerable time, in this respect presenting a marked contrast to the rapid clearing of the liquid, which takes place when pure cocaine is precipitated from the solution of its hydrochloride.

The analysis of a sample of crude cocaine by E. R. Squibb showed: Moisture, 3.25%; residue insoluble in ether, 5.25; impurity soluble in ether, 0.50; pure alkaloid, 89.94; and loss, 1.06% (*Jour. Soc. Chem. Ind.*, 1889, **8**, 724, 1013).

A convenient method of purifying cocaine is to recrystallise it several time from strong alcohol, and, when a certain degree of purity has been attained, precipitate the base from its solution in 10 parts of strong alcohol by addition of 5 volumes of water.

Paul and Cownley have pointed out that the solubility of a sample of cocaine in petroleum spirit cannot be relied on as a proof of its purity, since cinnamyl-cocaine behaves similarly.

John Williams (*Year-book Pharm.*, 1887, 502) proposed to purify and assay commercial cocaine hydrochloride by dissolving it in the smallest possible quantity of absolute alcohol (sp. gr. 0.795), and adding to this solution 6 times its volume of dry ether, when the cocaine hydrochloride is precipitated in a finely-divided but perfectly crystalline condition. Unfortunately, as pointed out by B. H. Paul, the hydrochlorides of the amorphous bases and of benzoyl-ecgonine are precipitated under the same conditions; and hence the method is useless for the assay of crude cocaine hydrochloride or for the elimination of impurities, though serviceable for improving the appearance of a pure salt and converting it into a convenient form for use.[1]

Cocaine hydrochloride should be perfectly colourless, and soluble in water to a perfectly colourless solution, which ought to be absolutely neutral to litmus-paper. The solution of the pure salt keeps fairly well, but in presence of common impurities is decomposed with great facility. In the dry solid state, cocaine hydrochloride undergoes no change by keeping. It ought to be perfectly free from odour; but as sold it not infrequently retains the odour of a solvent used in its preparation, or has a peculiar butyric or mousy smell, or even a distinct benzoic odour. In any case, a sample having a distinct odour must be regarded with suspicion.

Pure cocaine hydrochloride is always distinctly crystalline, though much of the commercial article presents an amorphous or granular appearance. The tendency to crystallise is so marked that B. H. Paul (*Pharm. Jour.*, 1888 [III], **18**, 781) regards an amorphous condition, or even difficult crystallisability, as an indication of the presence of impurity. Paul states that on dissolving 5 to 10 grains of a pure sample in 1 dram of water and rapidly evaporating the solution (in

[1] Paul adds that it is a mistake to attempt the purification of cocaine hydrochloride at all. The free alkaloid is much more susceptible of purification, and may be obtained in very fine crystals either from ether or alcohol. From pure cocaine the hydrochloride can be readily prepared, as the neutral solution may be evaporated to dryness without decomposition, and the resultant dry salt can be readily vertecond into a good-looking crystalline condition by Williams' method.

a glass basin) on a water-bath, the dry residue obtained will be white and opaque, presenting a radiating crystalline structure, while in the case of an impure mixed salt the residue will be more or less yellow, translucent, and of a gummy or resinoid character.

The most definite test for the purity of cocaine hydrochloride is said by Antrich (*Ber.*, **20**, 310) to be the optical activity. In dilute alcoholic solution, at 20°, the specific rotatory power is $[\alpha]_D = -(52.18° + 0.1588q)$, and $[\alpha]_D = -(67.982 - 0\ 15827c)$; where q is the weight of dilute alcohol of .9353 sp. gr. at 20°/4° (which corresponds to a mixture of 6 parts by weight of absolute alcohol with 9 parts of water) in 100 parts by weight of the solution, and c is the weight of hydrochloride in 100 volumes of the solution. When $q = 0$, or, in other words, the solution is aqueous, $[\alpha]_D = -52.2°$; when q is 100, $[\alpha]_D = -68.06°$.

The characteristics of cocaine hydrochloride should be, according to Beckurts, that it should give a clear and colourless solution in water; leave no residue on ignition; give a colourless solution in concentrated sulphuric acid, when dissolved in the proportion of 0.020 grm. to 1 c.c.; that a concentrated aqueous solution should be absolutely neutral (to litmus); not immediately reduce potassium permanganate; and when heated with the latter reagent give off no odour of bitter-almond oil.

The *British Pharmacopœia* (1905) prescribes the following tests for cocaine hydrochloride: It occurs in colourless crystals or as a white crystalline powder and has a bitter taste, which is succeeded by numbness. Very soluble in water (2 in 1), alcohol (1 in 3), and glycerin (1 in 3); soluble in chloroform (1 in 20), insoluble in oils and almost insoluble in ether. It should contain no more than traces of sulphates or amorphous alkaloid, nor lose more than 1% of water when dried at 100°. It melts at 180° to 186, and should leave no residue on complete ignition. A solution containing 0.1 grm. of the salt dissolved in 5 c.c. of distilled water, 0.15 c.c. of dilute sulphuric acid, and 0.1 c.c. of solution of potassium permanganate should not fade in half an hour (absence of cinnamyl-cocaine, cocamine, or other products derived from cocaine). 100 c.c. of a 0.1% solution of the salt affords with 0.25 c.c. of ammonia a clear solution, from which a crystalline deposist, free from amorphous flocks, should separate gradually on continued and vigorous stirring (limit of amorphous alkaloid).

The *German Pharmacopœia* (1900) prescribed the following tests

for cocaine hydrochloride: A mixture of equal parts of cocaine hydrochloride and mercurous chloride is blackened when moistened with dilute alcohol.

If 5 drops of chromic acid solution are added to a solution of 0.05 grm. of cocaine hydrochloride in 5 c.c. of water, each drop produces on precipitation which, however, on rotating the mixture redissolves, but on the further addition of 1 c.c. of hydrochloric acid it separates out again.

The *United States Pharmacopœia* (8th Rev., 1900) is here quoted at length with reference to tests for cocaine hydrochloride because of its complete treatment of the subject:

Colourless, transparent, monoclinic prisms, flaky, lustrous leaflets or a white crystalline powder; permanent in the air, containing no water of crystallisation; odourless; of a saline, slightly bitter taste, and producing on the tongue a tingling sensation followed by numbness of several minutes' duration.

Soluble in 0.4 parts of water, 2.6 parts of alcohol, and in 18.5 parts of chloroform at 25°; soluble on 0.1 part of water at 80°, and in 1.4 parts of alcohol at 60°; insoluble in benzene, petroleum benzin, and ether.

It melts at about 189.9°. Minute quantities of impurities may reduce the m. p. to 180° or less. It leaves no residue on incineration.

Its aqueous solution is neutral to litmus paper and is lævogyrate.

If silver nitrate solution be added to the aqueous solution of the salt (1 in 100), a white precipitate is produced, which is insoluble in nitric acid.

On adding 5 drops of a solution of chromium trioxide (1 in 20) to 5 c.c. of a solution of cocaine hydrochloride (1 in 50), a yellow precipitate will be produced, which redissolves on shaking, on now adding 1 c.c. of hydrochloric acid, a permanent, orange-coloured crystalline precipitate will be formed.

On adding a solution of potassium chromate (1 in 20) to a hydrochloric acid solution of the salt, orange-yellow leaflets of cocaine chromate are precipitated.

If mercuric chloride solution be added to an aqueous solution of the salt, a white flocculent precipitate is produced.

Cocaine hydrochloride is not coloured by cold sulphuric acid, but if a crystal be heated with sulphuric acid, in a test-tube, vapours are produced from which benzoic acid is deposited on cooling.

If 3 drops of palladous chloride, with 3 c.c. of chlorine water, be added to 3 drops of an aqueous solution of the salt (1 in 20), a red precipitate is produced.

When 0.01 grm. of the salt is dissolved in 2 drops of water, the addition of 1 c.c. of a solution of potassium permanganate (1 in 30) produces a violet precipitate, which appears brownish-violet when collected on a filter.

A crystal of the salt dissolved in alcohol yields when stirred with a piece of potassium hydroxide an odour of ethyl benzoate.

If 0.1 grm. of the salt be dissolved in 5 c.c. of distilled water containing 3 drops of diluted sulphuric acid, the addition to this solution of 3 drops of $N/10$ potassium permanganate solution will produce a violet colour, which should not fade in half an hour (limit of cinnamyl-cocaine).

If 0.1 grm. of the salt be dissolved in 85 c c. of cold distilled water in a beaker and 4 drops of ammonia water added and the solution stirred vigorously for 15 minutes, with occasional rubbing of the sides of the beaker with a stirring rod, a crystalline precipitate of cocaine should be formed, and the supernatant liquid should be perfectly clear (limit of isatropyl-cocaine). The presence of 0.5% of isatropyl-cocaine will prevent the formation of nearly all of the precipitate, and will cause the supernatant liquid to be opalescent.

The following test is due to H. Maclagan (*Amer. Drug.*, 1887, 22; *Pharm. Jour.*, 1887 [*iii*], **17**, 686): 1 grain of cocaine hydrochloride is dissolved in 2 oz. of water, 2 drops of strong ammonia are added, and the walls of the containing vessel rubbed from time to time with a glass rod; in a quarter of an hour a good crop of glistening crystals separate. When the cocaine is not very pure the solution remains clear, or else deposits only a small crop. With a good sample a dense precipitate is produced either at once or on stirring, and soon acquires a crystalline condition, the liquid rapidly clearing. When the cocaine contains more than 4% of amorphous alkaloid the solution becomes milky.

B. H. Paul (*Pharm. Jour.*, 1888 [*iii*], **18**, 783) has pointed out that the precipitate of cocaine produced in Maclagan's test redissolves if left for a long time in the ammoniacal solution, owing to its conversion into the soluble base benzoyl-ecgonine. He describes a quantitative application of the ammonia test (using a 2% solution of the salt) which, in the case of good samples free from odour and colour, will fairly

indicate the purity and value; but, in the case of bad samples, regard must also be paid to the character of the precipitated alkaloid. This is done by adding the ammonia gradually, with constant stirring, as long as a crystalline precipitate forms and the liquid clears promptly. When the precipitate begins to form clots which adhere to the sides of the beaker, and the liquid remains milky, the precipitate already formed is separated, and the amorphous precipitate produced on further addition of ammonia collected separately.[1] The following results were obtained by B. H. Paul by the examination of commercial cocaine hydrochloride by the above process:

Sample number	Water, %	Ammonia precipitate, %	
		On sample	= On dry salt
1	.90	85 6	86 3
2	.50	84 3	84 7
3	. .	84.0	84
4	1 00	83 6	84 00
5	43	82 6	82 95
6	1.19	81 35	82 33
7	.43	81 04	81.40
8	9 47	74 9	82 75
9	2 00	Cryst 66 4 Amorph 12 2	80 2
10	0 57	Cryst 43 28 Amorph 34 93	78 66
11	2 93	Cryst 41 7 Amorph 31 7	75 5
12		65 3	...

The ammonia precipitates from the first 8 of these samples were perfectly crystalline, without any trace of stickiness; they deposited rapidly, and left the supernatant liquid quite clear and bright. In the case of samples 9, 10 and 11, a considerable proportion of the alkaloid was of an amorphous sticky nature, quite different from that obtained from a pure salt. No. 12 was so impure that it was impossible to effect a fractional precipitation quantitatively.

Paul states that the principal impurity in the last 4 samples was undoubtedly the hydrochloride of the amorphous alkaloid associated with cocaine in coca leaves (see page 341), the salts having been probably produced by evaporating the solution of the mixed bases in hydrochloric acid; and it is questionable whether the presence of this amorphous base should be tolerated in a product which purports to be "*cocaine* hydrochloride."

[1] The amorphous alkaloid when freed from colouring matter is a clear yellowish transparent substance, resembling thick canada balsam, and the hydrochloride forms a varnish-like mass that cannot be reduced to powder

Tests for Cocaine in the Presence of the Eucaines.—In the course of an examination of a 1% β-eucaine solution for the possible presence of α-eucaine or cocaine G. Eigel (*Apoth. Ztg.*, 1903, **18**, 603) made the observation that the reactions heretofore employed for distinguishing between the hydrochloride of these 3 bases gave different results if solutions of different strengths (0.1, 1.0 or 5.0%) were under examination. (1) He finds that if 1 drop of solution of ammonia is added to 10 c.c. of 0.1% solution of α-eucaine hydrochloride, a white precipitate is produced, while solutions of β-eucaine and cocaine hydrochloride solutions of the same strength are not so affected. (2) 1 drop of 1.0% α-eucaine solution if mixed with 1 drop of solution of potassium iodide (1:10) produces in a few minutes large crystals of α-eucaine hydriodide, while no crystals are produced under identical conditions with either cocaine or β-eucaine hydrochloride. (3) 1 drop of 1.0% solution of α-eucaine or of cocaine hydrochloride yields a white precipitate with 1 drop of solution of mercuric chloride (1:20); β-eucaine does not. (4) But all 3 salts produce a precipitate with the solution of mercuric chloride if they are in solution of 5% strength.

On these observations Eigel bases the following method of distinction: 1 drop of a 1% solution is mixed with solution of mercuric chloride (1:20). If precipitate results it is α-eucaine or cocaine; if no precipitate results it is β-eucaine. If now equal drops of this solution and of solution of potassium iodide (1·10) are mixed, the formation of crystals indicates α-eucaine while if no crystals are formed, the solution contains cocaine.

G. L. Schaefer (*Pharm. Jour.*, 1899, 336) suggested the following test for ascertaining the purity of salts of cocaine and it has been the subject of several papers, several of which are given in outline herewith. The test is compared as to merit chiefly with Maclagan's test (page 332): 0.05 grm. of cocaine hydrochloride are dissolved in 20 c.c. of distilled water and 5 c.c. of a 3% chromic acid solution added and then to this mixture 5 c.c. of a 10% solution of hydrochloric acid are added. The temperature of the mixture should be kept at about 15°. If the salt be pure, a clear solution will result. If more than traces of salts of other coca bases are present, the solution becomes cloudy at once or in a few minutes according to the amount of impurity present. (See Metzer's test, page 324.)

Schaefer's Chromic Acid Test.—In the course of a reply to

criticisms on his chromic-acid test for determining the purity of cocaine, by A. J. Cownley, P. H. Squire and E. Merck (see proceedings, 1899, 758–740), Schaefer insists on its superiority over Maclagan's ammonia test. He says that in applying the test it is important that the temperature of the cocaine solution be maintained at 15°, the chromates of both pure cocaine and the amorphous alkaloids being influenced by rise and fall of temperature, heat increasing and cold diminishing their solubility. The test produces no turbidity when the acid is added to a solution of a pure specimen of cocaine, the temperature being 15°. If, however, the solution be subjected to a considerably lower temperature, it becomes turbid, and if it be preserved at this reduced temperature for several hours, a crystalline deposit will be found, consisting of long, needle-shaped crystals of cocaine chromate. A solution of impure cocaine rendered turbid by the reagent at 15°, and exposed to a lower temperature remains turbid for several hours, then slowly deposits a yellowish-brown amorphous sediment. These reactions are characteristic and serve to distinguish between cocaine and the amorphous alkaloids, especially isatropylcocaine. If a stronger acid is used the alkaloids will separate out more quickly. In order to show the superiority of the chromate test over Maclagan's test, the author prepared a series of specimens of cocaine of different degrees of purity. These, as well as the various brands of cocaine in the market, he subjected to Maclagan's and the chromate test. As a result, he found specimens which gave negative results with Maclagan's test to be impure by the chromate test, and those specimens which reacted with Maclagan's test yielded a decided turbidity upon the addition of even less than 5 c.c. of the 10% hydrochloric acid. (*Amer. Drugg.*, 1899, page 4).

On the other hand, A. J. Cownley, in a note to the foregoing, maintains that for commercial purposes Maclagan's test is to be preferred to the chromic-acid test for ascertaining the purity of cocaine hydrochloride, and that this opinion is corroborated by Schaefer's own statement that epecimens of cocaine hydrochloride, which were condemned by Maclagan's test, were shown to be impure by the chromate test. As has been previously stated by Cownley, the results mentioned by Schaefer only go to show that probably the only salt that would pass the chromic acid test would be one prepared from synthetic cocaine. (*Pharm. Jour.*, 1899, 66.)

Decomposition-products of Cocaine.

Benzoyl-ecgonine. $C_{16}H_{19}O_4N$; or $C_8H_{13}N(O.C_7H_5O).COOH$. This base may be prepared by the action of benzoic anhydride or benzoic chloride on ecgonine, and is also a product of the action of acids or water on cocaine. Hence it occurs as a by-product of the manufacture of cocaine.[1] On a large scale, benzoyl-ecgonine is prepared by gradually adding a little more than 1 molecule of benzoic anhydride to a hot saturated aqueous solution of 1 molecule of ecgonine, and heating the mixture on the water-bath for about an hour. After cooling, the product is shaken with ether to remove unchanged benzoic anhydride and acid, and the residual benzoyl-ecgonine washed with a little water to dissolve unaltered ecgonine. The yield is about 80% of the ecgonine employed, and an additional quantity can be obtained by concentrating the mother-liquor and again treating it with benzoic anhydride.

Benzoyl-ecgonine crystallises with $4H_2O$ in transparent, flat, trimetric prisms, resembling ammonium oxalate, which melt at a variable temperature ranging from 87–140°. When fusion occurs at the lower temperature (as happens when the heat is rapidly applied), the substance resolidifies on further heating, and melts again at 195°, turning brown at the same time.

Benzoyl-ecgonine is sparingly soluble in cold water, but readily in hot water, alcohol, and dilute alkalies and acids. It is almost insoluble in ether.

The *acetate* and *sulphate* of benzoyl-ecgonine crystallise in prisms. $BHAuCl_4$ forms small, yellow, anhydrous scales, soluble in alcohol but only sparingly so in water.

When heated with alkalies or with hydrochloric acid to 100° in sealed tubes, the base is decomposed into benzoic acid and ecgonine. By treatment with methyl iodide it yields cocaine.

Benzoyl-ecgonine does not appear to have much, if any, anæsthetic effect when applied to the eye, and exerts only a moderate dilating action on the pupil. R. Stockman states that it is very irritating to the mucous membranes, and when injected subcutaneously produces tetanic spasms. In many respects its action resembles that of caffeine,

[1] Benzoyl-ecgonine is easily produced by heating cocaine with about 20 parts of water in a closed tube. The cocaine melts at about 90°, but gradually dissolves on maintaining the temperature at 100°. The change is facilitated by agitation, and in about twelve hours a clear solution is obtained, which is only faintly acid if pure cocaine was employed.

but paralysis of the sensory nerves is quite absent (*Pharm. Jour.*, 1886 [*iii*], **16**, 898).

Ecgonine. $C_9H_{15}O_3N$; or $C_8H_{13}N(OH).COOH$. Ecgonine is obtained, together with benzoic acid and methyl alcohol, by heating cocaine with concentrated hydrochloric acid to 100° in sealed tubes.[1] Also, when cocaine or its hydrochloride is heated with 20 parts of water and 10 of baryta to 120° in sealed tubes, it is decomposed according to the equation:

$$C_{17}H_{21}O_4N + 2H_2O = C_7H_6O_2 + C_9H_{15}NO_3 + CH_4O.$$

The actual products are methyl alcohol, barium benzoate, and a compound of barium benzoate with the barium compound of ecgonine ($2Ba(C_9H_{14}O_3N)_2 + Ba(OBz)_2 + xH_2O$), which forms slender prismatic needles, very soluble in water and alcohol, but only slightly soluble in ether. This compound is a convenient source of ecgonine. On subjecting it to dry distillation it yields an isotropine, the platinichloride of which forms bulky, orange-red, deliquescent crystals containing $(C_8H_{15}ON)_2H_2PtCl_6$.

Ecgonine crystallises from absolute alcohol in monoclinic prisms containing $1H_2O$, m. p. 198°; or, after drying at 140° to expel the water of crystallisation, at 205°. Ecgonine is very soluble in water, sparingly in absolute alcohol, and insoluble in ether. It has a slight bitter-sweet taste.

When ecgonine is heated with moderately strong sulphuric acid, neither carbonic oxide nor formic acid is formed, but a base is produced which bears the same relation to ecgonine that ether bears to alcohol. It unites both with acids and bases.

C. E. Merck (*Ber.*, 1886, **19**, 3002) states that ecgonine, when distilled with nearly dry barium hydroxide, yields methylamine and not ethylamine as one of the products, thus agreeing with the behaviour of tropine when similarly treated.

When ecgonine (or anhydro-ecgonine) is oxidised with potassium permanganate, or nitric acid, succinic acid is formed (Einhorn, *Ber.*, 1888, **21**, 47), a fact which shows that the side-chain in the molecule of ecgonine must be either in the α- or β-position.

Ecgonine contains a carboxyl-group, and hence behaves at once as an acid and a base. It has a neutral reaction, but reacts with

[1] Liebermann and Giesel obtain ecgonine on a large scale by boiling the amorphous base obtained in the manufacture of cocaine for about an hour with hydrochloric acid. The filtered solution is evaporated to dryness, the residue treated with a little alcohol to remove impurities, and the residual ecgonine hydrochloride decomposed by sodium carbonate, the liberated base being recrystallised from alcohol.

alkalies to form gummy compounds of faint alkaline reaction, which crystallise with difficulty and are very soluble in water and alcohol. *Ecgonine hydrochloride*, $C_9H_{15}O_3N.HCl$, forms triclinic tables, difficultly soluble in alcohol and melting at 246°. $B_2H_2PtCl_6$, after drying at 140°, melts at 226°. It is extremely soluble in water, and is deposited in orange-red prisms on adding excess of alcohol to its aqueous solution. $BHAuCl_4$ is a greenish-yellow, gummy substance, very soluble in water and alcohol.

With iodised potassium iodide, ecgonine yields a reddish-brown precipitate, rapidly changing to reddish-yellow, microscopic tables or prisms. In dilute solutions the precipitate is formed only after concentration. In the animal system, cocaine is converted into ecgonine, which may be detected in the urine by this test.

Anhydro-ecgonine. $C_9H_{13}O_2N$; or $C_5NH_7Me.CH:CH\,COOH$. This base is formed by the action of phosphorus oxychloride or pentachloride on ecgonine, or by heating cocaine for 8 hours to 140° with glacial acetic acid which has been saturated with hydrogen chloride acid gas. It forms colourless crystals, m. p. 235°, soluble in water and alcohol, but insoluble in ether, chloroform, benzene and petroleum spirit.[1] When anhydro-ecgonine is heated with water to 150°, methylamine is liberated. It combines directly with bromine to form a base containing $C_9H_{13}Br_2O_2N$, the hydrochloride of which melts at 184°. The salts of anhydro-ecgonine are crystallisable. BHCl crystallises from absolute alcohol in white needles, m. p. 240–241°.

Bases Allied to Cocaine.

Dextro-cocaine. $C_{17}H_{21}O_4N$. Einhorn and Marquardt (*Ber.*, 1890, **23**, 469, 979) have found that by warming with aqueous potassium hydroxide for 24 hours, ecgonine is converted into a base which differs from ordinary ecgonine in being much less soluble in absolute alcohol, and having a much higher m. p. (254°); but especially in being dextrorotatory.

From this dextro-ecgonine a synthetic dextro-cocaine may be prepared as a colourless oil, which solidifies on standing, and is readily soluble in ether, alcohol, benzene, and petroleum spirit.

Dextro-cocaine may be obtained in crystals, m. p. at 43–45°,

[1] Hence it is best isolated by treating the solution of its hydrochloride with argentic oxide (compare page 22). It may be purified by precipitation from its alcoholic solution by ether.

by treating its solution with a crystal of benzoyl-dextro-ecgonine ethyl-ester.

The salts of dextro-cocaine crystallise well. BHCl is much more difficultly soluble than the hydrochloride of ordinary cocaine, and melts at 205° instead of 189.9°. BHO_3N is especially characteristic. 100 parts of water at 20° dissolve 1.55 parts of the nitrate, which is precipitated in crystals on adding nitric acid to solutions of other salts of the base. This behaviour distinguishes dextro-cocaine from ordinary cocaine. $B_2H_2PtCl_6$ crystallises from hot water in yellowish needles. $BHAuCl_4$ crystallises from dilute alcohol in needles, m. p. 148°.

Dextro-cocaine was found to resemble ordinary cocaine in its physiological effects, except that local anæsthetic action commenced more rapidly, and disappeared in a shorter time.

With chromic acid, potassium permanganate, and auric chloride, dextro-cocaine behaves like cocaine.

Cocethyline, Homococaine, or benzoyl-ecgonine ethyl-ester, $C_{18}H_{23}O_4N$, is the higher homologue of cocaine, which base it closely resembles. It is prepared by heating benzoyl-ecgonine with ethyl iodide and alcohol for 8 hours at 100°. It crystallises from alcohol in vitreous prisms melting at 108–109°, and is also soluble in ether but nearly insoluble in water. The *platinichloride* forms bright yellow, rhombic plates, resembling the cocaine salt but more crystalline. Physiologically, homococaine is similar in its effects to cocaine, but is weaker and less toxic, and does not appear to be mydriatic.

The higher homologues of cocethyline, containing propyl and isobutyl groups, have been prepared by similar means; and also by passing hydrochloric acid gas into a solution of benzoyl-ecgonine in the corresponding alcohol.

Cinnamyl-cocaine. $C_{19}H_{23}O_4N$; or $C_8H_{13}(CH_3)(C_9H_7O)O_3N$. This base has been obtained synthetically by passing dry hydrochloric gas into a solution of cinnamyl-ecgonine (prepared by heating ecgonine with cinnamic anhydride and water). It forms large colourless crystals melting at 121°, and is almost insoluble in water, but readily soluble in alcohol, ether, etc. When boiled with hydrochloric acid it is decomposed readily and quantitatively into cinnamic acid, ecgonine, and methyl alcohol. BHCl is precipitated as an oil which solidifies after a time on adding a large volume of ether to a strong acidified solution of the salt in alcohol. $B_2H_2PtCl_6$ crystallises in microscopic

needles, m. p. 217°. When treated with a cold solution of potassium permanganate cinnamyl-cocaine and its salts immediately evolve a strong odour of benzaldehyde (bitter-almond oil).

Cinnamyl-cocaine has been proved to occur naturally in coca leaves from various sources. Paul and Cownley (*Pharm. Jour.*, 1890 [*iii*], **20**, 165) examined a sample of leaves containing 1.75% of total alkaloid, nearly 0.5% being crystallisable from petroleum spirit, but which, nevertheless, contained very little real cocaine. On oxidation by permanganate the crystallisable alkaloid yielded abundance of benzaldehyde, and in other respects corresponded with cinnamyl-cocaine (methyl cinnamyl-ecgonine).

Cocamine. α-Truxilline. $C_{38}H_{46}O_8N_2+H_2O$. This base is contained in notable quantity in Truxillo coca leaves. Hesse found 0.6% in leaves of a different kind, and states that East Indian coca leaves, and especially those from Java, contain cocamine in considerable amount. Liebermann regards cocamine as identical with the base originally described by him as γ-isotropyl cocaine, and afterward as α-truxilline; but Hesse contends that Liebermann's product was a mixture, of which cocamine was a leading constituent.[1]

Cocamine has a bitter taste. Hesse and Stockman found its physiological effect to be similar to that of cocaine, but somewhat weaker, and its anæsthetic action especially weak. On the other hand, G. Falkson alludes to γ-isatropylcocaine (cocamine) as a "deadly alkaloid," and Liebermann describes it as a heart-poison which does not produce anæsthesia. To its presence as an impurity, the occasionally highly toxic effects of commercial cocaine are not improbably due.

Cocamine is precipitated by alkali hydroxides and ammonia from solutions of its salts, and after exposure at the ordinary temperature in a desiccator retains 1 molecule of water. It is readily soluble in alcohol, ether, benzene and chloroform, but differs from cocaine in being very sparingly soluble in petroleum spirit. Neither the free base nor its salts have been obtained crystallised. Repeated solution in hydrochloric acid and reprecipitation by soda was the process employed by Liebermann to purify the cocamine from the co-occurring isococamine (β-truxilline), which is also bitter, and produces numbness of the tongue very slowly by reason of its sparing solubility.

Both cocamine and its isomeride have been obtained synthetically.

[1] The composition of cocamine and its allies has formed the subject of an embittered controversy between Liebermann and Hesse (*Pharm. Jour.*, 1891 [*iii*], **21**, 1109, 1129, **22**, 61, 101).

When hydrolysed by mineral acids they yield ecgonine, methyl alcohol, and cocaic and isococaic acids respectively.

Cocaic acid, $C_9H_8O_2$, or $C_{18}H_{16}O_4$, called by Liebermann γ-isatropic acid or α-truxillic acid, is produced by boiling cocamine with hydrochloric acid. The isomeric *isococaic acid* (δ-isatropic or β-truxillic acid) is the similar product from isococamine. Cocaic acid melts at 274°, is tasteless and odourless, insoluble in water, and nearly insoluble in ether, from which, however, it crystallises in forms resembling benzoic acid. Isococaic (β-truxillic) acid melts at 206°. Both cocaic and isococaic acids yield cinnamic acid and other products on distillation.

Benzoyl-pseudotropine, $C_8H_{14}ON.C_7H_5O$, is a base isolated by Giesel from a narrow-leaved coca plant cultivated in Java (*Ber.*, **24**, 2336). It somewhat resembles dextrococaine, but is optically inactive, and differs from other coca-bases in not yielding methyl alcohol on hydrolysis; for, when heated with hydrochloric acid under a reflux condenser for some hours, it is completely decomposed into benzoic acid and pseudotropine, $C_8H_{15}ON$. In this respect the base resembles atropine and the other tropeïnes.[1] Benzoyl-pseudotropine is obtained as a milky precipitate which does not become crystalline on adding sodium carbonate to the solution of one of its salts. The base may be extracted by ether, and on evaporating the solution is obtained as an oil which, when quite dry, solidifies in radiating crystals melting at 49°. It has a strong alkaline reaction, and is easily soluble in alcohol, ether, chloroform, benzene and petroleum spirit. BHCl, obtained by passing hydrogen chloride gas into an ethereal solution of the base, crystallises in white needles, m. p. 271°. The solution gives a bulky crystalline precipitate with mercuric chloride. $B_2H_2PtCl_6$ is a flesh-coloured precipitate, insoluble in hot water, alcohol and ether. $BHAuCl_4$ crystallises from water in sparingly soluble yellow needles, melting at 208°. The *picrate* forms fine yellow needles, difficultly soluble in water. With potassium dichromate, benzoyl-pseudotropine yields a crystalline precipitate, instead of an oily one like cocaine and dextrococaine.

Amorphous Bases of Coca.

In isolating cocaine there is found in the mother-liquors a variable quantity of a basic substance commonly known as "amorphous

[1] Liebreich finds that benzoyl-pseudotropine introduced into the eyes of rabbits occasions strong local anæsthesia and a slight enlargement of the pupil, in this respect acting more like cocaine than atropine.

cocaine," while the names cocaicine and cocainoïdine have also been applied to it. Amorphous cocaine is described by R. Stockman (*Pharm. Jour.*, 1887 [*iii*], **17**, 861) at ranging in colour from dark yellow to dark brown, and consistence from that of treacle to a sticky tenacious solid, having a peculiar smell resembling that of nicotine, and a bitter and aromatic taste. Stockman concludes that "amorphous cocaine" is in reality a solution of ordinary crystalline cocaine in hygrine, the liquid alkaloid said to have been found in coca leaves by Lassen. The amorphous alkaloid is extracted from the coca in greater or less amount by the process now employed by manufacturers, and its presence is considered by Stockman to account for certain disagreeable effects resulting from the employment of cocaine containing the impurity. Thus if the hydrochloride of the impure alkaloid be used to produce anæsthesia of the conjunctiva considerable irritation ensues.

W. C. Howard (*Pharm. Jour.*, 1888 [*iii*], **18**, 71) to a certain extent agrees with Stockman's view as to the nature of amorphous cocaine. He found that when the solution of the bases of coca in hydrochloric acid was completely precipitated with platinic chloride, and the liquid filtered after standing over-night, the mixed platinum salts obtained were amorphous or semi-crystalline, and somewhat light in colour. When the precipitate was washed with a large quantity of water at a temperature not exceeding 80°, the cocaine platinichloride dissolved, and the alkaloid could be obtained therefrom in a crystalline state. The fraction of the platinum salt insoluble in water when decomposed by hydrogen sulphide, and extracted with ammonia and ether, left on evaporating the ether a liquid base which thickened considerably on keeping, but in which no crystals appeared even after a week. It had an intensely bitter taste, formed an uncrystallisable hydrochloride, and a platinichloride containing 18.5% of platinum (against 19.3% in the cocaine salt)[1] and not affected by hot water, all of which characters distinguish the base from the description of hygrine given by Lossen (*Annal. der Pharm.*, **71**, 374).

O. Hesse states that when working on the bases from the broad-leaved coca, separating the cocaine as hydrochloride "by a special process," and ascertaining the absence of cocamine, the residual mixture was dissolved in dilute hydrochloric acid and the solution treated with ammonia in excess. This process of solution and repre-

[1] Hesse (*Pharm Jour.*, 1888 [*iii*], **18**, 71, 437) considers that Howard's platinum salt was hydrated, being in reality the chloroplatinate of an amorphous base isomeric with cocaine.

cipitation being repeated until the precipitate dissolved in hydrochloric acid gave a solution which showed no fluorescence on dilution with water, thus proving its freedom from hygrine. The precipitate, after being further washed with water at 80°, gave a melted mass which was spread on glass plates and dried at 60°, by which means it was obtained in transparent, brittle, hygroscopic laminæ which were nearly insoluble in water and alkaline liquids, but dissolved readily in alcohol, ether, chloroform, benzene and petroleum spirit. The solution was alkaline to litmus, but without effect on phenolphthaleïn (*Pharm. Jour.*, 1888 [*iii*], **18**, 71, 437). When boiled with alcoholic barium hydroxide, or heated in a sealed tube with hydrochloric acid, the amorphous base yields benzoic acid and another product not yet identified.

From a later investigation (*ibid.*, **19**, 867), Hesse concludes that the amorphous bases from true coca consist chiefly of benzoyl compounds of an oily non-volatile base, together with some cocamine; while, on the contrary, those obtained from Truxillo leaves consist essentially of cocamine, and the cinnamyl compounds of the beforementioned oily base; and the cocamine is in each case accompanied by a base containing H_2 less than cocamine.

A specimen of the amorphous base from coca examined by B. H. Paul (*Pharm. Jour.*, 1888 [*iii*], **18**, 784) is described by him as being pale yellow, and of the consistence of thick Canada balsam. It had a faint odour at once suggestive of benzoïn and butyric acid, and a distinctly bitter taste, but produced no anæsthetic effect on the tongue until after the lapse of some minutes, and then very slight compared with that produced by cocaine.

A residue from the preparation of "cocaidine" from amorphous cocaine has been loosely given the name of "hygrine."

Hesse points out that hygrine probably does not pre-exist in coca leaves, but is a product of decomposition. He states that when sound coca leaves are moistened with ammonia, shaken with ether, and the ether treated with dilute hydrochloric acid, the acid liquid on dilution at first shows no fluorescence, but after a time exhibits this character distinctly.

R. Stockman (*Pharm. Jour.*, 1888 [*iii*], **18**, 701) states that "hygrine" exists in coca leaves in very minute quantity only, and some manufacturers never meet with it. He found it in cocaine mother-liquors given him by Messrs. Howard & Sons, and notably in the alcoholic tincture of *fresh* coca leaves. Stockman finds "hygrine" to distil very imper-

fectly with steam in presence of cocaine. The whole of the statements respecting "hygrine" require confirmation.

Hygrine properly speaking has the formula $C_8H_{15}ON$ and is obtained form the *Cusko bark.*

Assay of Fluidextract of Coca. *Gravimetric Alkaloidal Determination.* Th. Roder (*Pharm. Ztg.*, 1906, 322) recommends the following method for estimating the alkaloid in fluidextract of coca: Place 15 grm. of the fluidextract into a 250 c.c. flask, add 120 grm. of petroleum ether and 10 c.c. of ammonia water, and shake 2 hours. After subsidence, decant 100 c.c. of the petroleum ether solution carefully, shake it out successively with 30, 20, 10 and 10 c.c. of 0.5% hydrochloric acid, supersaturate the united acid liquids with ammonia water, add 100 c.c. of ether, and shake the mixture frequently during one or two hours. Carefully decant 80 grm. of the ether solution, filtering if necessary through a dry folded filter, into a dry weighing flask, carefully evaporate the ether, dry for 3 hours at 100°, and weigh. The weight so ascertained, multiplied by 10, gives the alkaloid content in 100 grm. of the fluidextract.

Coca Leaves.

The coca leaves occurring in commerce are chiefly of two kinds, the one being obtained from *Erythroxylon coca,*[1] which was the original trade-product, and the other, which is of more recent importation, derived from Jamaica and St. Lucia. Coca leaves contain, in addition to the ordinary plant-constituents and the characteristic alkaloids, cocatannic acid.

Cocatannic acid (C. J. H. Warden, *Pharm. Jour.*, 1888 [*iii*], **18**, 985) has the probable composition $C_{14}H_{18}O_8$. It forms a sulphur-yellow powder, which appears under the microscope in filiform crystals interlaced in masses. It melts at 189–191° to a deep red liquid, and is only slightly soluble in cold water, cold absolute alcohol, ether and chloroform. In hot water it dissolves more readily, and rather freely in boiling absolute alcohol. A hot aqueous solution of cocatannic acid has an acid reaction. It yields no reaction with ferrous salts (according to some observers, green), but with ferric gives a dark green colouration, and reduces silver nitrate slowly in the cold and immediately on heating, but not Fehling's solution. It does not precipitate gelatin. The

[1] The coca plant is a small shrub from 4 to 6 feet in height, growing and largely cultivated in Peru and Bolivia, and, to some extent, in Bazirl and the Argentine Republic.

alcoholic solution gives, with alcoholic lead acetate, a precipitate varying from yellow to orange-red. When heated with hydrochloric acid to 100°, cocatannic acid yields a glucose and a phlobaphene. The products of potassium hydroxide fusion do not appear to be characteristic. They are said to include butyric and traces of benzoic acid.

C. J. H. Warden (*Pharm. Jour.*, 1888 [*iii*], **18**, 1010, 1027) has observed that coca leaves which are rich in cocatannic acid also contain much alkaloid, and suggests, with much probability, that the cocaine and allied alkaloids of coca leaves exist in combination with cocatannic acid. Warden, in 9 specimens of the dry leaves from plants grown in different parts of India, found from 6.36 to 12.64% of *ash* (average 8.85%), and from 0.358 to 1.671% of "crude alkaloid" (average 0.982%). Warden did not succeed in obtaining a crystalline alkaloid from Indian coca, but does not consider the non-crystalline character detracts from its physiological activity (?).

A. G. Howard (*Pharm. Jour.*, 1889 [*iii*], **19**, 569) has published analyses of a large number of coca leaves from different sources. His results show that while *Erythroxylon coca* yields about 0.75% of alkaloid, the proportion obtainable from most other species of *Erythroxylon* is extremely insignificant, and in some cases the alkaloid is wholly absent. In Brazil alone there are upward of 80 species of *Erythroxylon*.

The following is the method of assay of coca leaves of the *United States Pharmacopœia*, Eighth Revision (1900). This method should be used in all work that in any way comes under the U. S. Food and Drugs Act.

Coca, in No. 60 powder, 10 grm.,
Chloroform,
Ether,
Normal sulphuric acid V. S.,
Ammonia water,
Distilled water,
N/10 sulphuric acid,
N/50 potassium hydroxide,
Cochineal or iodeosin solution, each, a sufficient quantity.

Place the coca in an Erlenmeyer flask, add 50 c.c. of a mixture of chloroform 1 volume and ether 4 volumes and insert the stopper

securely. Allow the flask to stand 10 minutes, then add 2 c.c. of ammonia water mixed with 3 c.c. of distilled water, and shake the flask well, at frequent intervals, during 1 hour. Then transfer as much as possible of the contents of the flask to a small percolator which has been provided with a pledget of cotton packed firmly in the neck, and insert in a separator containing 6 c.c. of $N/1$ sulphuric acid, diluted with 20 c.c. of distilled water. When the liquid has passed through the cotton, pack the coca firmly in the percolator with the aid of a glass rod, and, having rinsed the flask with 10 c.c. of chloroform-ether mixture, transfer the remaining contents of the flask to the percolator by the aid of several small portions (5 c.c.) of a chloroform-ether mixture, using the same proportions as before, and continue the percolation with successive small portions of the same liquid (in all 50 c.c.). Next, shake the separator well for 1 minute, after securely inserting the stopper, and when the liquid has completely separated, draw off the acid liquid into another separator. Add to the chloroform-ether mixture 10 c.c. of a sulphuric acid mixture, using the same proportions as before, agitate well and again draw off the acid liquid. Repeat this operation once more, drawing off the acid solution as before into the second separator, introduce a small piece of red litmus paper, add ammonia water until the liquid is distinctly alkaline, and shake out with 3 successive portions of ether (25, 20 and 15 c.c.). Collect the ether solutions in a beaker, place it on a water-bath filled with warm water, and allow the ether to evaporate entirely. Dissolve the residue in 3 c.c. of ether, and let this also evaporate completely. To the alkaloidal residue add 4 c.c. of $N/10$ sulphuric acid, and five drops of cochineal or iodeosin solution, then titrate the excess of acid with $N/50$ potassium hydroxide. Divide the number of cubic centimetres of $N/50$ potassium hydroxide used by 5, subtracting this number from 4 (the 4 c.c. of $N/10$ sulphuric acid taken), and multiply the remainder by 0.03 and this product by 10, to obtain the percentage of ether-soluble alkaloids contained in the coca.

The following method is given as it is a gravimetric method, based upon the well known and much used Keller method of alkaloidal assay:

K. de Jogg (*Chem. C. Bl.*, 1905, **2**, 16) recommends the following modification of Keller's method for the assay of coca leaves: 25 grm. of the dried and powdered leaves are moistened with 10 c.c. of ammonia and shaken during half an hour with 20 c.c. of ice-cold ether in a well-

closed flask. The mixture is then shaken with 60 c.c. of ice water, and filtered through cotton, and 100 c.c. of the filtrate are shaken out in a separatory funnel successively with 50 and .25 c.c. of 0.5% hydrochloric acid, filtering the acid solutions through a well wetted filter. After shaking the filtrate once with ether, it is neutralised with ammonia, and shaken out successively with 50 and 25 c.c., and a third and fourth portion of a few c.c. of ether is distilled off from the united solutions, and the last traces of adhering water are removed from the residue by alternately heating and passing air through the weighing flask. The residual alkaloid represents all the bases contained in the coca leaves except the benzoyl-ecgonine (*Pharm. Ztg*, 1, 87.)

Although not presented in the form of a regular assay method of coca alkaloids the following experimental work of William Garsed (*Pharm. Jour.*, 1903, 784–791) is worthy of consideration. Several alternative methods are discussed and the author's conclusions are given.

Process No. 1. The crude alkaloid is weighed, dissolved in dilute sulphuric acid and subjected to the action of potassium permanganate. The unoxidised alkaloid is re-extracted and weighed; the loss in weight represents the amount of cinnamyl-cocaine present. The re-extracted alkaloid is then subjected to alkaline hydrolysis, and the truxillic and benzoic acids separated by taking advantage of the insolubility of the former in water. From the quantity found of each, the respective amounts of truxilline and cocaine originally present can be calculated. This process admits of the direct determination of the benzoic acid.

Process No. 2. The crude alkaloid is at once subjected to alkaline hydrolysis, the cinnamic acid determined by the bromination method, and the truxillic acid by taking advantage of its insolubility in water. The amount of truxilline and cinnamyl-cocaine present is then calculated, and the difference between the combined weight and the weight of crude alkaloid originally taken represents the amount of cocaine present.

Each process was tried on 2 samples of crude alkaloid, extracted respectively from Truxillo and Java leaves. The results are given in the following table:

ALKALOID FROM TRUXILLO COCA.

Crude alkaloid taken	Process No. 1	Process No. 2
	0.1540 grm.	0.1232 grm.
	Grm. %	Grm %
Truxilline found	0.0280 = 18.2	0.0220 = 17.8
Cinnamyl-cocaine found	0 0356 = 23 1	0.0165 = 13.4
Cocaine found	0.0800 = 52 0	0 0847 = 68.8 (by difference)
Total found	0 1436 = 93.3	0 1232 = 100.0

ALKALOID FROM JAVA COCA.

Crude alkaloid taken	Process No 1	Process No. 2
	0 2010 grm	0 2108 grm.
	Grm %	Grm %
Truxilline found	0 0164 = 8 1	0 0197 = 9.3
Cinnamyl-cocaine found	0 1024 = 51 0	0 0801 = 38.0
Cocaine found	0 0740 = 37 0	0 1110 = 52 7 (by difference)
Total found	0 1928 = 96 1	0 2108 = 100 0

The process appeared equally good as far as the determination of truxilline is concerned; process No. 2 has the advantage that the cocaine is estimated by difference; in process No. 1 the cinnamyl-cocaine is estimated by difference, and comes out considerably higher than in process No. 2. This is what may be expected, as any impurities oxidisable by permanganate would be calculated as cinnamyl-cocaine. The sum of the presentage results in process No. 1 is over 90, and as it is certain that cocaine and truxilline are practically unaffected during the oxidation of the cinnamyl-cocaine, preference must be given to the permanganate process. In process No. 2, the fact that cinnamic acid readily absorbs bromine, while benzoic and truxillic acids, being saturated substances, do not absorb any bromine, is utilized for the direct estimation of the cinnamyl-cocaine.

H. T. Pfeiffer (*Chem. Zeit.*, 1887, 11, 783, 818) has described the following process of manufacturing crude cocaine hydrochloride direct from coca leaves: The disintegrated leaves are digested in closed vessels at 70° for 2 hours, with a very weak solution of sodium hydroxide and petroleum boiling between 200–250°. The mass is filtered, pressed while still tepid, and the filtrate allowed to stand until the

petroleum has completely separated from the aqueous liquid. The former is then drawn off and carefully neutralised with very weak hydrochloric acid, when a bulky, white precipitate of cocaine hydrochloride is obtained, together with an aqueous liquid from which a further quantity of the salt can be recovered by evaporation.

The dried product contains about 75% of real alkaloid, besides traces of "hygrine," gum, and other matters. A repetition of the process proved that the whole of the alkaloid was removed by a single treatment. The sodium hydroxide cannot be substituted by lime, nor the hydrochloric acid by other acid.

For the assay of coca,[1] v. d. Marck (*Jour. Pharm.* [*v*], **20**, 500; *Analyst*, 1889, **14**, 115), after a trial of various processes, recommends that 50 grm. of the leaves should be mixed with 20 grm. of calcined magnesia and moistened with a little water, dried at 60°, and the mixture exhausted with ether. The ether is distilled off, and the residue treated with 30 c.c. of 2% hydrochloric acid. The solution is filtered, and repeatedly shaken with ether to remove colouring-matters. Ammonia is then added, and the cocaine extracted by shaking 3 times with 25 c.c. of ether. After standing for a short time over some fragments of calcium chloride, the ether is evaporated, and the residual alkaloid weighed.

A more detailed method based upon the decomposition of the cocaine-containing substance (glucoside) with magnesia and estimation of total alkaloids has been proposed by E. Leger (*J. Pharm. Chim.*, 1904, **19**, 334). Having determined the amount of moisture in a small amount of the powdered leaves, a quantity of the same powder equivalent to 25 grm. of the dried leaves is intimately mixed into a mortar with 5 grm. of magnesia and 15 c.c. of distilled water. The mixture

[1] Cocaine Estimation as Di-iodo-cocaine hydriodide. W. Garsel and J N. Collie (*Proc Chem. Soc*, 1907, 17, 89) have communicated the result of their researches undertaken with the object of finding a method for the fairly accurate estimation of cocaine in small quantities, either when free or mixed with benzoyl ecgonine and ecgonine, the products of hydrolysis of pure cocaine. The estimation of cocaine in presence of cinnamyl cocaine and isatropyl cocaine, and other substances with which it is associated in coca leaves has, however, not been attempted. When a solution of cocaine in the form of a salt containing about 1% of cocaine base is titrated by adding excess of N/10 iodine solution till the supernatant liquid contains excess of iodine, a precipitate of Di-iodo-cocaine hydriodide, $C_{17}H_{21}O_4N, HI_3$, is formed. The excess of iodine in solution can then be estimated by a N/10 sodium-thiosulphate solution The precipitate di-iodo-compound can be collected and weighed, or the cocaine estimated by the amount of iodine used. Any cocaine salt can be used, since the potassium iodide in the solution reacts with the salt producing the iodide. Di-iodo-cocaine hydriodide is a remarkably stable and crystalline compound, crystallising in large glistening crystals of constant composition. Cocaine can be estimated in presence of ecgonine, as ecgonine forms a soluble iodo-compound Benzoyl ecgonine, however, interferes to a considerable extent with the estimation of cocaine. Making use of the fact that both benzoyl ecgonine and ecgonine are insoluble in ether or light petroleum, a separation can be effected, as cocaine is soluble in both these solvents. The extracted cocaine can then be weighed directly or titrated with iodine.

is introduced into a 1-litre wide-mouthed, glass-stoppered flask and treated with 625 c.c. of ether, sp. gr. 0.721, saturated with water. The flask is then stoppered, tied down with a piece of cloth, well shaken up, and set aside for 12 hours, with frequent agitation. The whole is then shaken up, transferred to a filter, the filtrate collected in a 500 c.c. graduated flask, the funnel being covered with a glass plate during filtration. The 500 c.c. of filtrate thus collected, equivalent to 20 grm. of dry powder, is distilled in several portions from a dry 250 c.c. flask by plunging the latter in warm water. The green residue is dissolved in 20 c.c. of neutral ether, 10 c.c. of $N/10$ hydrochloric acid, and 20 c.c. of water are added, the flask covered with a rubber stopper and agitated. The whole contents are then transferred to a separator, and the acid liquid, after separation, withdrawn into a conical flask. The ether layer is then twice shaken out with 25 c.c. of distilled water, these washings being added to the acid liquid in the flask. This acid solution is filtered through a moistened double filter into a wide-mouthed glass-stoppered 500 c c. flask, and the filter thoroughly washed through into the same. Sufficient distilled water is added to make up the volume to 150 c.c., when sufficient neutral ether to give a layer 1 cm. deep is added. 5 or 6 drops of 0.2% iodeosin solution are then added, and the amount of free acid titrated back in the usual manner with $N/10$ potassium hydroxide solution. The number of c.c. of acid thus found to be combined with the coca alkaloids, multiplied by 0.1535, gives the percentage of these in the powder.

For the estimation of the *cocaine* in coca leaves, A. B. Lyons (*Jour. Pharm.* [v], **13**, 197) recommends that the finely-powdered leaves should be macerated for 24 hours with 8 times their weight of a mixture of 95 volumes of ether with 5 of ammonia. From an aliquot part of this liquid the alkaloid is extracted by agitation with acidified water, the ether separated, and the alkaloid liberated from the aqueous liquid by means of ammonia and again extracted with ether, which is then evaporated to dryness and the cocaine weighed. The associated bases, being soluble in water and insoluble in ether, remain in the ammoniacal liquid. Lyons states that coca leaves do not contain more than 0.8% of cocaine, and sometimes the proportion is as low as 0.15%. The leaves rapidly deteriorate in value, so that in 6 months they are practically worthless. The product from deteriorated leaves is always more or less coloured, and very little of it is crystallisable; while that from good leaves is almost colourless, and easily crystallises.

M. Bignon (Lima) states that coca leaves dried in dry weather, with frequent turning, and sheltered from dew and moisture, yield easily 0.8% of alkaloid, and the finer sorts can give 1.0% and upward under exceptional circumstances. Coca leaves dried in damp weather, or pressed into sacks before being completely dried, undergo a gradual fermentation which ends in the complete destruction of the cocaine.

OPIUM ALKALOIDS.

By FRANK O. TAYLOR.

Opium, the nature and character of which are described at length on page 354, is remarkable for the large number of nitrogenised organic principles contained in it. Over 20 alkaloids have been isolated from opium and the list is probably still incomplete. Most of these substances have well defined basic properties, and the majority are poisonous. Some of them, as morphine and narcotine, occur in opium in considerable quantity, but the greater number are present in small proportions, and are entirely absent from some samples.

The following table exhibits the leading character of the alkaloids which have been recognised in opium. In some cases the basic character is slight, while certain of the alkaloids (*e. g.*, pseudo-morphine, oxynarcotine) are probably decomposition products. The quantities given as present are only approximate and may vary widely, particularly for morphine and narcotine.

In addition to the alkaloids in the above list, deuteropine, opionine, papaverosine, and porphyroxine have been described, but their existence as individuals is very doubtful.

With one or two exceptions, the alkaloids of opium are strictly peculiar to *Papaver somniferum;* while, on the other hand, the poisonous alkaloid sanguinarine, which is present in all other papaveraceous plants, does not appear to exist in *Papaver*. Indeed, with the exception of protopine, which is probably identical with the interesting alkaloid macleyine, $C_{20}H_{19}O_5N$, obtained by Eykman (*Year-book Pharm.*, 1882, p. 33) from *Macleya cordata* (a poisonous Japanese plant), none of the nitrogenised substances found in opium appear to be identical with any of those extracted from other plants of the family.[1]

[1] A base identical with, or similar to, narcotine was isolated by T. and H. Smith from the fresh juice of the roots of *Aconitum Napellus*, but other observers have not confirmed this result.

LIST OF OPIUM BASES.

Alkaloid	Average percentage in opium	Formula	Discoverer	Date	M p.	Optical activity	Basic character	Physiological action
Morphine	9 0%	$C_{17}H_{19}O_3N$	Serturner	1816	254° (about)	L	Strong	Powerful narcotic poison
Codeine .	0 3	$C_{18}H_{21}O_3N$	Robiquet	1832	155°	L	Strong .	Narcotic poison
Thebaine	0 4	$C_{19}H_{21}O_3N$	Thiboumèry	1835	193°	L	Strong .	Violent tetanic poison
Papaverine	0 8	$C_{20}H_{21}O_4N$	Merck	1848	147°	I	Very feeble	Tetanising, feebly narcotic.
Mecomidine	.	$C_{21}H_{23}O_4N$	Hesse	1870	58°		Strong	
Codamine	0 002	$C_{20}H_{25}O_4N$	Hesse	1872	121–126°		Strong	
Laudanine	0 01	$C_{20}H_{25}O_4N$	Hesse	1870	166°	L	Strong	Active tetanic poison
Laudanidine		$C_{20}H_{25}O_4N$	Hesse	1894	177°	L	Strong	Active tetanic poison
Laudanosine	0 0008	$C_{21}H_{27}O_4N$	Hesse	1871	89°	R	Strong	Tetanic poison
Lanthopine	0 006	$C_{23}H_{25}O_4N$	Hesse	1870	200°		Very feeble	
Protopine	0 003	$C_{20}H_{19}O_5N$	Hesse	1871	202°		Strong	Narcotic
Cryptopine	0 08	$C_{21}H_{23}O_5N$	T and H Smith	1864	313°	I	Strong	Hypnotic and mydriatic
Papaveramine		$C_{21}H_{21}O_5N$	Hesse	1886	142°			
Rhœadine	. ..	$C_{21}H_{21}O_6N$	Hesse	1865	232°		Well-marked	Not poisonous
Narcotine	5 0	$C_{22}H_{23}O_7N$	Derosne	1803	176°	L	Very feeble	Feebly poisonous.
Gnoscopine	. .	$C_{22}H_{23}O_7N$	T and H Smith	1878	233°		Weak	
Oxynarcotine		$C_{22}H_{23}O_8N$	Brown	1875			Feeble	
Narceine .	0 2	$C_{23}H_{27}O_8N$	Pelletier	1832	170°	I	Feeble	Purely hypnotic
Pseudomorphine	0 02	$C_{34}H_{362}O_6N$	Pelletier and Thiboumèry	1835	Not fusible	L	Very feeble	Not poisonous
Tritopine	0 0015	$(C_{21}H_{27}O_3N)_2O$	Kauder	1890	182°		Diacid base	
Hydrocotarnine	.	$C_{12}H_{15}O_2N$	Hesse	1871	50°		Well-marked	Similar to narcotine
Xanthaline.....	.....	$C_{47}H_{36}O_9N_2$	T and H Smith	1893	206°			

Constitution of Opium Bases.

Some of the opium bases are isomeric, while others are homologous, or else differ from each other by the increments C_2H_2, CO, H_2, HO, or multiples of these.

The tendency to combine with each other to form stable crystalline compounds, which renders the isolation and study of the cinchona bases so difficult (see Homoquinine), does not seem to exist in the case of the opium alkaloids.

The various opium alkaloids may be classified broadly in 2 groups, the morphine group, comprising morphine, codeine, pseudomorphine and thebaine; the second, the papaverine group, consisting of most of the remaining alkaloids. The first group are strong bases and very poisonous while the second group alkaloids possess as a whole little physiological action.

Since the last previous edition of this volume an immense amount of work has been done on the constitution of the opium alkaloids and the most important ones are fairly well understood, though even yet there remain questions such as the exact manner of attachment of the nitrogen-containing chain in the morphine molecule on which there is not complete agreement by the most prominent investigators. Some alkaloids of opium, as for example papaverine and landanosine, may be considered as having their constitution established, and while morphine, codeine and thebaine still remain of slightly uncertain character, yet their relation is well understood and large numbers of derivatives of known constitution have been obtained.

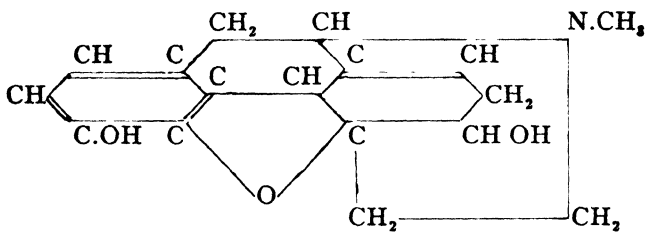
A theoretical discussion of the constitution of these alkaloids is out of place in a work of this kind except as it may have direct bearing on analytical work and hence only very brief statements will be included regarding their structure.

Morphine, codeine and *thebaine* are so closely related that they may be considered together. The early researches of Wright, Grimaux, Hesse, Skraup and Knorr began the attack on this problem and the later work of Knorr, Pschorr, Vongerichten and others has brought it to almost complete solution. It was early shown that morphine contained 2 hydroxyls, 1 phenolic and the other groups alcoholic in character. By methylation of the phenolic hydroxyl, codeine is formed and in a similar manner related derivatives may be produced. Like-

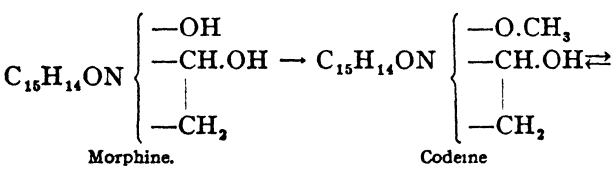
wise acid radicals may be introduced at this point giving substances such as acetyl morphine.

Codeine by oxidation with potassium permanganate in acetone solution or by chromic acid in sulphuric acid is converted from a secondary alcohol into the ketone *Codeinone*, as shown by Ach and Knorr (*Ber.*, **36**, 3067), and this body may be reconverted into codeine. Freund (*Ber.*, **39**, 844) has shown that *thebaine* may be converted into codeinone by first converting into bromcodeinone by treatment with bromine in chloroform solution and subsequent reduction of this substance, while Knorr and Horlein (*Ber.*, **39**, 1409) accomplish the same result by boiling with $N/1$ sulphuric acid for 6 or 7 minutes and shaking out this solution with ether after making alkaline.

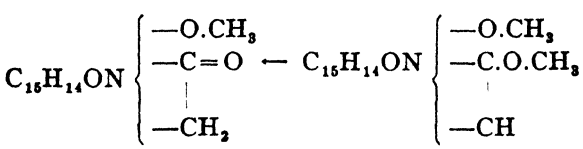
The most probable formula for morphine may be considered, as given by Knorr and Horlein (*Ber.*, **40**, 3341),



and the relationship of these three important alkaloids may be indicated as follows:



Morphine. Codeine



Codeinone. Thebaine.

The poisonous characters of morphine, which are both narcotic and tetanic, are shared qualitatively by its derivatives, such as codeine,

ethyl-morphine, amyl-morphine, mono- and diacetyl-morphine, and benzoyl-morphine.[1]

When further substitution takes place, as in chlorocodeine and methocodeine, the product is not merely a nerve-poison, but a muscle-poison. By dehydration with hydrochloric acid or zinc chloride, *apomorphine* is produced which has a physiological action much different from morphine, being strongly emetic instead of narcotic.

Pseudomorphine was formerly represented by the formula $C_{17}H_{19}O_4N$ as given by Hesse, but more recently Polstorff (*Ber.*, **19**, 1760) has shown that it has the formula $C_{34}H_{36}O_6N_2$ or $(C_{17}H_{18}O_3N)_2$, with which Hesse agreed. Its formation by oxidation of morphine may be indicated as follows:

$$\underset{\text{Morphine.}}{2C_{17}H_{19}O_3N} + O = \underset{\text{Pseudomorphine}}{(C_{17}H_{18}O_3N)_2} + H_2O.$$

It probably has therefore the constitution of an oxydimorphine and as a tetracetyl derivative is produced by heating pseudomorphine with acetyl chloride. The four hydroxyl groups evidently remain intact and the hydrogen atoms lost in the formation from morphine must have been united with carbon. On the other hand, M. P. Cazeneuve (*Compt. Rend*, 1891, **112**, 805) has obtained a violet colouring matter of definite composition by acting on morphine with *p*-nitroso-dimethylaniline. This dye appears to be an indamine, analogous in constitution to Bindschedler's Green; whereas, if pseudomorphine were derived from two molecules of morphine, the colouring matter would have contained two morphine residues, and have had the constitution of a safranine.

Narcotine, $C_{22}H_{23}O_7N$, contains three methoxyl groups, the first two methyl groups of which may be successively removed by heating the alkaloid with strong hydrochloric acid, forming, first, dimethylnornarcotine and then methylnornarcotine. By further heating with fuming hydriodic acid the third group is removed, forming nornarcotine and methyl iodide. It is a tertiary base and contains neither carboxyl nor aldehyde group.

By heating with water under pressure at 150°, or with sulphuric acid or baryta-water, it is decomposed into the base *hydrocotarnine* and the non-nitrogenous *opianic acid*,

[1] By heating anhydrous morphine with excess of benzoyl chloride to 100–110° a dibenzoy derivative may be obtained and the diacetyl derivative may be produced in like manner with acetyl chloride.

$$\underset{\text{Narcotine}}{C_{22}H_{23}O_7N} + H_2O = \underset{\text{Opianic acid.}}{C_{10}H_{10}O_5} + \underset{\text{Hydrocotarnine.}}{C_{12}N_{15}O_3N}.$$

By reduction with zinc and hydrochloric acid, a similar change is produced giving, however, in place of the opianic acid another product, *meconin*,

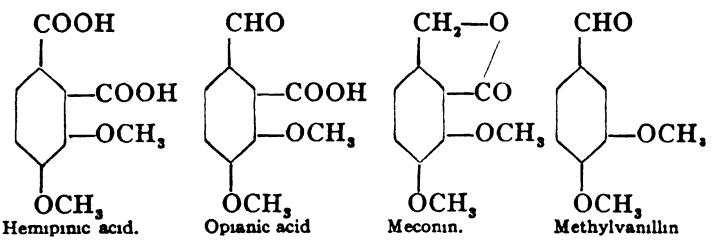
$$\underset{\text{Narcotine.}}{C_{22}H_{23}O_7N} + 2H = \underset{\text{Meconin}}{C_{10}H_{10}O_4} + \underset{\text{Hydrocotarnine}}{C_{12}H_{15}O_3N}.$$

By strong oxidation, as with nitric acid, there is formed opianic acid and *cotarnine*,

$$\underset{\text{Narcotine}}{C_{22}H_{23}O_7N} + H_2O + O = \underset{\text{Opianic acid}}{C_{10}H_{10}O_5} + \underset{\text{Cotarnine}}{C_{12}H_{15}O_4N},$$

and by the further oxidation of opianic acid the related *hemipinic acid*, $C_{10}H_{10}O_6$, results. By nascent hydrogen opianic acid is reduced to meconine and by the action of soda-lime gives methyl-vanillin, $C_9H_{10}O_3$, which by boiling with hydrochloric acid yields vanillin, $C_8H_8O_3$ (see Dott, *Pharm. Jour.* [*iii*], **14**, 641).

The relation of these products may be shown as follows:



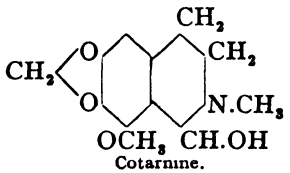
Hemipinic acid. Opianic acid Meconin. Methylvanillin

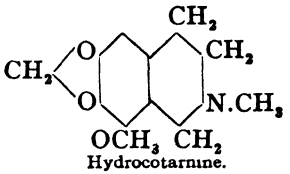
The first three bear the relation of acid, aldehyde and lactone having the phthalide form.

Cotarnine was considered by Roser to have an aldehyde form, which was also favored by Freund and Becker, but Dobbie, Lauder and Tinkler (*Proc. Chem. Soc.*, **19**, 75) from an investigation of its spectrum consider it to have a carbinol form in the solid state or in solution in ether or chloroform, which changes to an ammonium hydroxide form in water or alcohol.

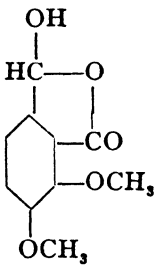
Salway (*J. Chem. Soc.*, 1910, **97**, 1208) has synthesised cotarnine so that now the synthesis of narcotine is complete.

The formula of cotarnine of the carbinol form and of *hydrocotarnine* may then be given as follows:

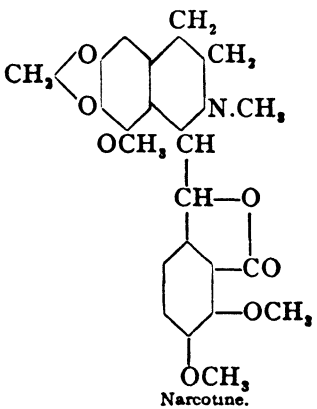

Cotarnine.


Hydrocotarnine.

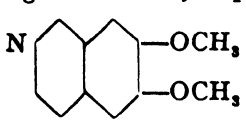
Taking the oxyphthalide form for the formula of opianic acid



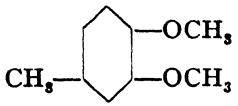
we may then consider the formula of narcotine to be


Narcotine.

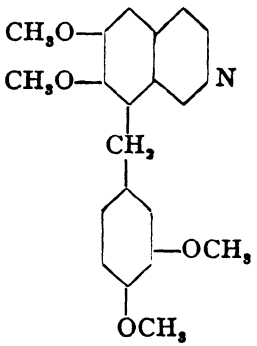
Papaverine is a weak tertiary base which was the first natural alkaloid shown to be an isoquinoline derivative. It contains 4 methoxyl groups and on heating with concentrated hydrochloric acid at 130° is decomposed into methyl chloride and homopyrocatechin. Fusion with alkali gives *dimethoxylisoquinoline.*



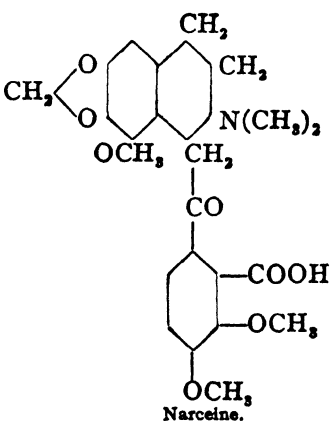
and *dimethylhomopyrocatechin*



From this and other considerations, the formula of papaverine may be given as below, which indicates its relationship to narcotine,



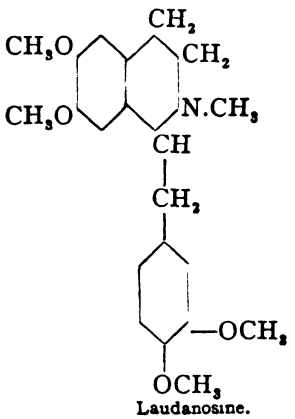
Narceine is closely related to narcotine and was originally assigned the formula $C_{23}H_{29}O_9N+2H_2O$, but the more recent researches of Freund and Frankforter (*Ann.*, **277**, 20) indicate that it should be $C_{23}H_{27}O_8N.3H_2O$. Roser (*Ann.*, **247**, 167) succeeded in converting narcotine into narceine by treating narcotine methylchloride with aqueous solution of sodium hydroxide when narcotine methylhydroxide is precipitated. On heating with steam this changes into the base $C_{23}H_{27}O_8N.3H_2O$. The constitution of narceine may be expressed by the following formula:



Narceine.

Two other alkaloids related to narcotine are *Oxynarcotine* and *Gnoscopine*. The former has the formula $C_{22}H_{23}O_8N$ and differs from narcotine in that by treatment with ferric chloride it decomposes into cotarnine and hemipinic acid, whereas opianic acid results from narcotine. The latter is probably a stereoisomer of narcotine. Dobbie and Lauder (*Jour. Chem. Soc.*, **83**, 605) have shown that the absorption spectra of the 2 alkaloids is the same.

Laudanosine may be produced by reduction of papaverine methylchloride with tin and hydrochloric acid and the separation of the racemic mixture thus formed by use of the quinic acid salts. It has been shown by Pictet and Athanasescu (*Ber.*, **33**, 2346) to be *dextro-n-methyl-tetrahydropapaverine*,



Laudanosine.

Related to laudanosine are *Laudanine* and *Laudanidine*, the former being *n-methyl-trimethylpapaveroline*, according to Hesse's work on laudanosine and production of the monomethyl ester of laudanine, and the latter is apparently its levo-modification.

General Characters of Opium Bases.

Morphine, codeine, thebaine, papaverine, narcotine and narceine are the most important of the alkaloids of opium. The opium alkaloids form a group of which all the members exert a more or less narcotic and tetanising action, but in very varying degree. Thus morphine is almost purely narcotic and thebaine almost purely tetanising in its action.[1] Morphine, codeine and thebaine have strongly

[1] Thebaine appears to be the most poisonous of the leading alkaloids of opium. Papaverine appears to possess only very slight poisonous properties, if any.

marked basic characters. They are strongly alkaline to litmus, and afford stable salts.[1] Papaverine, narcotine and narceine, on the contrary, are very weak bases (compare page 354).

The free alkaloids of opium are generally but slightly soluble in water, but dissolve more readily in alcohol. In many instances the solutions of the free alkaloids are strongly alkaline to litmus. On the other hand, certain of them (*e. g.*, morphine, narceine, laudanine) exhibit a distinct phenoloid character, and form definite compounds with the alkalies. The different behaviour of the opium bases to slovents affords a valuable means of distinguishing and separating them. They are precipitated from concentrated solutions of their salts by alkali hydroxides and alkali carbonates, some of the precipitates dissolving in excess of the reagent. Most of the opium alkaloids (except papaverine and laudanosine) have a lævorotatory action on polarised light, but the specific rotatory power varies so greatly with the solvent and the concentration of the solution that the fact has a very limited practical value. Many of the opium alkaloids furnish characteristic colour-reactions when treated with strong acids and oxidising agents, which, with observations of their m p , crystalline form, and behaviour with solvents, will suffice for the recognition of most of them when in an unmixed state. Their separation is described on page 370, *et seq.*

Behaviour of Opium Bases with Solvents.

The following table shows the recorded behaviour of the opium bases with solvents though in some cases, notably morphine, widely discrepant statements exist so that the figures are in some cases only approximate. Several of the more important derivatives of morphine and codeine are included in a second table. The figures are the number of parts of the solvent required for the solution of 1 part of the alkaloid.

The solubility of opium bases, as of other substances, is much affected by the physical condition of the alkaloids, and to some extent by the manner of making the experiment.

[1] Codeine is distinctly more strongly basic than morphine, and a method of determining the former alkaloid has been based on the fact.

Alkaloids	Water		Water with		Alcohol	Amyl alcohol	Ether	Chloroform	Benzol	Petroleum ether
	Cold	Hot	Sodium hydroxide	Ammonia						
Morphine. .	3330	1040 at 80°	Very soluble	Very slightly soluble	168 76 at 60°	125 50 hot	4464	1780	Insoluble	1176
Pseudomorphine	Insoluble		Soluble	Slightly soluble	Insoluble		Insoluble	Insoluble	Soluble	
Codeine	120	59 at 80°	Soluble	Same as with water	1 6 0 92 at 60°	7	12 5	Very soluble	12	
Thebaine	Very slightly soluble		Very slightly soluble	Very slightly soluble	10	60	140	18	19	Insoluble
Papaverine	Insoluble	Very slightly soluble	Insoluble	Insoluble	45 cold 4 hot	70	250	Soluble	36	Slight soluble cold, very soluble hot
Meconidine ,	Insoluble		Soluble	Soluble in excess	Very soluble		Very soluble	Very soluble	Very soluble	
Codamine	Slightly soluble	.	Soluble	Soluble	Very soluble		Soluble .	Soluble .	Soluble	
Laudanine			Very soluble	Soluble	540 cold		650	Very soluble	Very soluble	
Laudanidine			Very soluble	Soluble	Slightly soluble	. .....	Slightly soluble	Very soluble	Very soluble	
Laudanosine	Insoluble		Insoluble	Insoluble	Very soluble		19	Very soluble	Sol cold, Very sol hot	
Lanthopine			Soluble	Insoluble	Slightly soluble		Slightly soluble	Soluble	Slightly soluble	

Alkaloids	Water		Water with		Alcohol	Amyl alcohol	Ether	Chloroform	Benzol	Petroleum ether
	Cold	Hot	Sodium hydroxide	Ammonia						
Protopine	Insoluble		Insoluble	Soluble	Very slightly soluble		Slightly soluble	Soluble	Very slightly soluble	
Cryptopine	Insoluble		Insoluble	Insoluble	1265		Insoluble	Very soluble	Very slightly soluble	Insoluble.
Papaveramine	Insoluble		Insoluble	Insoluble	Very soluble		Slightly soluble	Very soluble	Slightly soluble	
Rhoeadine	Very slightly soluble		Insoluble	Very slightly soluble	Very slightly soluble		1280	Very slightly soluble	Very slightly soluble	
Narcotine	25,000	7000	Insoluble cold Soluble hot	Insoluble	189 cold 13 hot	310	165	3	25	Very slightly soluble
Gnoscopine	Insoluble	Insoluble	Insoluble	Insoluble	1500	Insoluble	Very slightly soluble	Very soluble	Slightly soluble	Insoluble
Oxynarcotine	Insoluble	Slightly soluble	Soluble	Soluble	Slightly soluble		Very slightly soluble	Very slightly soluble	Very slightly soluble	
Narceine	1284 (13°)	60	Soluble, insoluble in strong NaOH	Soluble	300 cold Very soluble hot	Very slightly soluble	Insoluble	Very slightly soluble	Insoluble	Insoluble
Tritopine			Soluble, pptd by large excess		Soluble		Slightly soluble	Very soluble		
Hydrocotamine	Insoluble		Insoluble	Insoluble	Very soluble		Very soluble	Very soluble	Soluble	
Xanthaline	Insoluble		Insoluble	Insoluble	Slight solution boiling			Soluble	Soluble	

COLOUR REACTIONS OF OPIUM BASES.

Alkaloid	Nitric acid (sp gr. 1 42)	Concentrated sulphuric acid		Formaldehyde and sulphuric acid	Erdmann's reagent	Fröhde's reagent	Ferric chloride
		Alone	On adding $KClO_3$ or HNO_3				
Morphine	Orange-red turning yellow on heating	Cold, no colour or faint pink, on heating, variable	See page 379	Purple		Fine violet, turning blue or dirty green	Greenish-blue.
Apomorphine	Blood-red, or reddish-violet	No colour (or violet to brown)		Violet		Deep green, turning violet	Rose pink, changing to violet and black
Heroine	Yellow, turning blue with heat			Reddish-violet becoming bluish Red with heat		Purple	
Pseudomorphine	Orange-red, changing to yellow	Cold, no colour, or olive green; on heating, dingy green (or purple), and finally red				Violet, changing to blue and green.	Blue
Codeine	Yellow, not changing to red	No colour, dirty brownish-green on heating	Blue on warming	Violet	Blue, on warming	Dirty green, changing to blue and pale yellow	No colour
Thebaine	Yellow	Blood-red, turning orange-yellow, olive-green on heating	Same as with sulphuric acid alone	Red	Orange-red	Blood-red, turning orange-yellow and colourless	No colour
Papaverine	Yellow	No colour if pure		Yellowish-green to rose and then brown	No colour if pure	No colour if pure	No colour
Narcotine	Red	Darkens, changing to orange and brick-red on gently heating	Carmine-red		On warming, pink, changing to orange-red and violet	Pink, changing to green, yellow, and orange.	No colour
Narceine	Yellow rapidly fading	Brown, dissolving to yellow solution (changing to dark red) [1] If impure, red or blue colour	No change	Yellow	Brown-yellow, becoming mahogany brown on heating [1]	Brownish-green, changing to yellow and reddish[1] (yellow-brown to blue)	

[1] Great discrepancies occur in the description of this reaction

Colour Reactions of Opium Bases.

Many of the opium alkaloids give brilliant, and in some cases characteristic, colour reactions with mineral acids, with or without the aid of heat and the addition of oxidising agents. The colours obtained vary somewhat with the mode of applying the test and with the oxidiser employed. The colours obtained are modified in a marked manner by very slight traces of oxidising agents in the sulphuric acid used, and hence this reagent should be scrupulously free from iron and oxides of nitrogen. E. Kauder recommends that the purity of the sulphuric acid should be tested by codeine, which should give no colour even on heating, while in presence of the faintest trace of iron, such as may be taken up from long keeping in a bottle of common glass, a violet colouration is produced.

The colour reactions of the opium alkaloids are best observed in the manner described in detail on page 367 *et seq.*

Many of the colour reactions of the opium bases defy classification, and such of these as appear of value are described under the alkaloids to which they refer; but the accompanying tables show many of the better-known reactions of the more important opium bases, according to the most reliable observers.

If a trace of narceine be evaporated with dilute sulphuric acid at 100° a beautiful violet-red colouration appears as soon as the liquid is sufficiently concentrated, changing to cherry-red by continued heating. After cooling, the addition of a trace of nitric acid or a nitrite produces bluish-violet streaks in the red liquid. The test, which is due to Plugge (*J. Chem. Soc.*, 1888, **52**, 870), is said to be very delicate and characteristic. With traces of morphine, codeine, or papaverine the liquid remains quite colourless; with larger quantities of either of the two former bases a faint rose-red tint is obtained, with thebaine a greenish-yellow to brown colour, and with narcotine a red to reddish-brown.

According to Serena (*Analyst*, 1885, **10**, 149), the following colour reactions are produced on treating certain of the opium alkaloids *successively* with a few drops of concentrated sulphuric acid and a very small quantity of a dilute solution of ferric chloride, with the aid of slight heat

Alkaloid	With sulphuric acid	On adding ferric chloride
Apomorphine	Not changed	Violet streaks at point of contact, the bluish-green mass becoming light violet on heating
Codeine	Light violet-red, deepened by heat	Sky-blue
Papaverine[1]	Purplish-red	Colourless, on heating, violet
Opionine	No colouration	Green, rapidly becoming deep blue
Narceine	Coffee-brown	Bluish-green
Codamine		Green-blue, at 100°, violet

The following table shows the colour reactions observed by Hesse (*J. Chem. Soc.*, **24**, 1905, 1064) when certain of the opium bases are treated with pure concentrated sulphuric acid, and with acid containing traces of oxide of iron or oxides of nitrogen. The reactions with ferric chloride are also shown

Alkaloid	With pure sulphuric acid		With acid containing oxide of iron		With ferric chloride
	At 20°	At 150°	At 20°	At 150°	
Codeine	Colourless	Dirty green[2]	Blue	Dirty green	No reaction
Codamine	Colourless	Dirty red-violet	Intense green-blue	Deep violet	Dark green
Lanthopine	Colourless	Brownish-yellow			No reaction
Laudanine	Very faint rose-red	Deep red-violet	Intense rose colour	Green, changing to deep violet	Emerald-green[3]
Laudanosine	Faint rose-red	Deep red-violet	Brownish-red (resembling cobalt nitrate solution)	Green, changing to deep violet	No reaction
Protopine	Yellow, changing to red and bluish-red	Dirty greenish-brown	Deep violet	Dirty greenish-brown	No reaction.
Cryptopine ..	Violet, changing to green and yellow	Dirty green	Deep violet	Dirty green .	No reaction.
Hydrocotarnine	Yellow .	Crimson-red changing to dirty red-violet		Dirty red-violet	

[1] According to Pictet and Kramers (*Ber.*, 1910, 43, 1329) no colour with sulphuric acid is pure If containing some cryptopine, bluish-violet changing to green and yellow

[2] According to E Kauder (*Pharm Journ* [III], **18**, 250), if the sulphuric acid be quite pure no colouration is yielded with codeine even on heating, but a blue colour is produced if traces of iron be present

[3] According to Merck, laudanine gives a violet colour with ferric chloride.

Cryptopine dissolves with violet colour, changing to deep blue, and fading to greenish on standing or heating to 150°. In presence of oxide of iron, cryptopine is said to dissolve in sulphuric acid with deep violet-rose colour, changing to violet and deep blue, and becoming greenish on heating to 150°. The hydrochloride gives a yellow coloration when first treated with acid.

A. Pfister (*Chem. Ztg. Rep.*, 1908, **32**, 494) gives the following colour reactions for several opium alkaloids and derivatives.

Alkaloid	Reagents and reactions					
	Frohde's	Mandelin's	Marquis'	Lafon's	2% aqueous furfurol	Ferric chloride
Apomorphine....	Green changing to blue.	Green changing to blue	Violet ..	Blue to greenish-brown	Evanescent red, violet with heat	Red coffee color
Codeine.....	Green to blue	Green, with heat blue	Violet .	Green.		
Dionine	Green; with heat blue.	Greenish-yellow, with heat green	Blue	Green	Purple	
Heroin.....	Purple...	Light green	Mulberry colour, with heat red	Green		
Morphine.... ..	Purple	.	Purple	Green	Purple	Green
Narceine	Yellowish-brown; with heat brick red	Orange, with heat red	Yellow .	Bluish-brown		
Narcotine.....	Intense green; with heat greenish-brown.	Red .	.	Greenish-brown; with heat blood-red		
Thebaine.....	Red	Red . .	Red .	. . . .	Evanescent red	

Hesse employs the colour reactions of the opium bases with pure sulphuric acid as a means of grouping them, thus:

Colouration at 150°.	Alkaloids
Dirty dark green...	Codeine, morphine, pseudomorphine
Dirty red-violet	Codamine, laudanine, laudanosine, narcotine, hydrocotarnine.
Dirty green to green-brown	Thebaine, cryptopine, protopine
Dark violet or blue.	Papaverine [1]
Black-brown to dark brown . .	Narceine, lanthopine

With acid containing iron, codamine, laudanine and laudanosine are stated to give a dark violet colour, while narcotine and hydrocotarnine react in the same way as with pure acid.

It will be seen that several of the reactions described by Hesse differ in a marked manner from those recorded by other observers. As in the case of other colour observations, the only safe way is to compare the substance under examination side by side with products of known purity.

Lafon's reagent, prepared by dissolving 1 grm. of ammonium selenite in 20 c.c. of conc. sulphuric acid, is stated by da Silva (*Compt. Rend.*, 1891, **112**, 1266) to give the following colour reactions with the opium bases: *Codeine*, magnificent green colouration; *morphine*, greenish-blue, changing to chestnut-brown; *narcotine*, blue, turning violet and then reddish, with slight reddish precipitate after long standing; *narceine*, yellowish-green, changing to brown and red, with red precipitate on standing; *papaverine*, blue, passing to dull green, violet and red, with a slight bluish precipitate on standing.

By heating *morphine* with sulphuric acid and then adding a little chloral, on stirring a violet colour appears. With *codeine* a bluish-green colour is produced which gradually changes to red and the change may be made more rapid by adding water or solution of sodium hydroxide. By the same treatment *dionin* reacts as does codeine, and *heroin* gives a brownish-red colour. The other papaveraceæ alkaloids are stated not to give colour tests in this manner.

With a reagent consisting of a 10% solution of hexamethylenetetramine in sulphuric acid colour tests are given as follows: *Morphine*, purple; *Codeine*, bluish, turning green; *Apomorphine*, bluish-violet;

[1] Hesse states that, when absolutely pure, papaverine dissolves in small quantities of sulphuric acid without colouration, but, generally, on warming a crystal of papaverine with concentrated sulphuric acid, a dark blue colour is produced Dott also obtains no colouration in the cold, and the blue colour on strongly heating only A red colouration before heating is generally due to thebaine See also note 1, on page 367.

Narceine, saffron yellow; *Papaverine*, lilac, turning violet; *Heroin*, light yellow, becoming deep yellow and finally dark blue.

For a description of several colour tests for narceine, narcotine and papaverine, some of which are similar to those already described, see Reichard (*Pharm. Centr.-h.*, 1906, **47**, 1028; 1907, **48**, 44, 288, 313, and 334 or *J. Chem. Soc.*, 1907, **92**, 319, 414 and 592.)

Estimation and Separation of Opium Bases.—Morphine, codeine, and thebaine may be titrated with ease and accuracy by a standard mineral acid, using litmus or methyl-orange as an indicator. On the contrary, they have little or no action on phenolphthaleïn, the reaction with which, however, is not sharp in the case of morphine.

Papaverine, narcotine and narceine, on the contrary, do not affect litmus, and their salts may be titrated with litmus and standard alkali, just as if the acid were uncombined (Plugge, *Pharm. Jour.*, 1890 [*iii*], **20**, 401); and the first two of them evince their feeble basic characters by the fact that they are extracted by chloroform from acid solutions. Their salts, especially with certain organic acids (*e. g.*, acetic, benzoic), are very unstable, many of them being decomposed slowly by cold and rapidly by hot water. Hence, when a compound of the alkaloid with a mineral acid is treated with a neutral solution of acetate of sodium, or even with a slightly acid solution, the free alkaloid is precipitated.[1] A faintly acid solution of sodium acetate will indicate 1 part in 40,000 of narcotine, 1 in 30,000 of papaverine, and 1 in 600 of narceine, none of the other opium bases being precipitated.

On the foregoing and similar facts, P. C. Plugge (*Analyst*, 1887, **12**, 197) has based the following process of separating the leading alkaloids of opium. The aqueous solution of the hydrochlorides is mixed with a concentrated solution of sodium acetate, and filtered after 24 hours. The precipitate, consisting of pure narcotine and papaverine, is washed with a little water, and dissolved in a minimum of dilute hydrochloric acid. The liquid is diluted till it contains not more than 1/400 of narcotine, when potassium ferricyanide is added. This precipitates papaverine very perfectly. After standing 24 hours the liquid is filtered, and the precipitate of *papaverine* hydroferricyanide either weighed as such, or washed with a little water, decomposed by dilute sodium hydroxide, and the liberated alkaloid dissolved in dilute

[1] This observation is due to P. C. Plugge (*Arch Pharm.* [*iii*], 24, 994; *Analyst*, 1887, 12, 197). The reaction not only distinguishes papaverine, narcotine and narceine from morphine, codeine, and thebaine, but also from caffeine, cocaine, conine, atropine, pilocarpine, strychnine, brucine, quinine, cinchonine and cinchonidine. The cinchona bases are precipitated if the sodium acetate is at all alkaline.

acid and reprecipitated with ammonia. In the filtrate from the precipitate produced by the ferricyanide the *narcotine* is precipitated by ammonia. The filtrate from the precipitate produced by sodium acetate is concentrated to a small volume at 100°, cooled thoroughly, and filtered after 24 hours. The deposited *narceine* is filtered off, and washed with a little water. The filtrate is mixed with a strong solution of sodium salicylate, and the crystalline precipitate of *thebaine* salicylate separated after 24 hours, and washed with a little water, dried at 100°, and weighed. On subsequent treatment on the filter with dilute sodium hydroxide or ammonia, till the washings are free from salicylic acid (as indicated by evaporating to dryness, and the non-production of a violet colouration on moistening the residue with ferric chloride), pure *thebaine* is left. The filtrate from the thebaine salicylate is acidified with hydrochloric acid, the precipitated salicylic acid filtered off, and the filtrate repeatedly shaken with chloroform. This dissolves the remaining salicylic acid, and traces of narceine and thebaine, which may be recovered by evaporating the chloroform. The acid liquid separated therefrom is concentrated somewhat, made exactly neutral to litmus, and mixed with potassium thiocyanate (sulphocyanide), which throws down the *codeine* as an acid thiocyanate. 24 hours should be allowed for its complete separation [1] The filtrate should be treated with a slight excess of ammonia, and time allowed for the separated morphine to become crystalline. The liquid is then shaken with chloroform or ether to remove the remainder of the codeine and traces of other bases. After separation it is acidified to dissolve the morphine, heated to 60°, and the morphine shaken out with hot amylic alcohol, after addition of a slight excess of ammonia or carbonate of sodium. Plugge's results, obtained in test experiments, except in the separation of codeine and morphine, were very satisfactory, considering the difficult nature of the problem to be solved.[1] But the methods are not to be regarded as having the same quantitative accuracy as those for the separation of the metals.

Another method of separating the principal alkaloids of opium consists in treating the solution with an alkaline carbonate or ammonia and agitating with benzene, when morphine and narceine are lef

[1] The separation of codeine and morphine by this process is very imperfect If the solution be too strong, morphine is precipitated with the codeine, and if this condition be avoided the precipitation of the codeine is incomplete In test-experiments Plugge only recovered 70% of the codeine used Hence it is better to omit the precipitation with thiocyanat altogether, precipitate the morphine with ammonia, and extract the codeine from the filtrat by ether or chloroform, after adding sodium hydroxide (compare page 393)

insoluble, the remainder passing into the benzene. Much the same separation occurs with chloroform, except that pseudomorphine is left with the insoluble alkaloids.

D. B. Dott communicated to A. H. Allen the following method of separating the chief bases of opium: Treat the solution of their mixed hydrochlorides with a 10% solution of sodium hydroxide, and wash the precipitate, which will consist of narcotine, papaverine and thebaine, the alkaline solution containing morphine, codeine and narceine. On agitating the filtrate with chloroform, the *codeine* will be extracted; and on separating the alkaline liquid, acidifying it, and rendering it faintly alkaline with ammonia, the *morphine* will be precipitated, the *narceine*, from its greater solubility, remaining dissolved It can be recovered by evaporating the liquid to dryness and treating the residue with strong alcohol. From the bases precipitated by sodium hydroxide, the *thebaine* can be separated fairly well by crystallisation as acid tartrate.

Narcotine and papaverine may also be separated from thebaine (and codeine) by dissolving the free bases in dilute alcohol, rendering the liquid faintly acid with acetic acid, and adding 3 volumes of boiling water, when the narcotine and papaverine are precipitated; or sodium acetate may be used as already described. Narcotine and papaverine may likewise be separated by solution in boiling water containing 1/3% of oxalic acid, when an acid papaverine oxalate crystallises out on cooling. The process should be repeated several times, and the narcotine finally precipitated by ammonia and crystallised from boiling alcohol.

The following is an epitome of Hesse's method of separating the rarer opium bases from the mother-liquors left from the preparation of morphine by the Robertson-Gregory process.[1] The aqueous extract of opium is first precipitated by calcium chloride, the filtrate from the calcium meconate concentrated, and the hydrochlorides of morphine, pseudomorphine and codeine separated by crystallisation. The mother-liquor is diluted with an equal bulk of boiling water, excess of ammonia added, the precipitate removed by filtration and dissolved in acetic acid. The filtrate is agitated with ether, the ethereal layer shaken with excess of acetic acid, and the acetic solution mixed with that of the ammonia precipitate. The acetic acid solution is then

[1] For E. Kauder's modification of Hesse's method, see *Arch. Pharm.*, 1890, **228**, 419, and *J. Chem. Soc.*, 1891, 60, 227.

treated with excess of sodium hydroxide, which precipitates papaverine, narcotine, thebaine, some cryptopine, protopine, laudanosine and hydrocotarnine; while lanthopine, laudanine, codamine, meconidine, and a portion of the cryptopine remain in solution. The alkaline liquid is neutralised, ammonia added, the bases again extracted by ether, and shaken out with acetic acid. The acetic acid is neutralised with ammonia, when a little lanthopine separates out in 24 hours, and the filtrate is treated with more ammonia. The precipitate formed is dissolved in a very small quantity of boiling dilute alcohol, which on cooling deposits white crystals of mixed *laudanine* and *cryptopine*. On evaporating the alcoholic solution,[1] and treatment of the residue with ether, a solution is obtained from which *codamine* may be isolated, either by addition of fused calcium chloride (which causes water, colouring-matter, and crystals of codamine to separate), or by conversion into the acetate, and this into the hydriodide.

The mixture of bases insoluble in sodium hydroxide is digested with dilute alcohol, and acetic acid added till the liquid is faintly acid to litmus. On adding 3 measures of boiling water, a crystalline precipitate of *papaverine* and *narcotine* is thrown down. The filtrate, freed from alcohol by evaporation, on adding strong hydrochloric acid, will give a precipitate of cryptopine hydrochloride; but in order to avoid the conversion of thebaine into its non-crystalline isomer thebaicine, it is preferable to add tartaric acid, which throws down crystalline *thebaine* acid tartrate. The mother-liquor of this is neutralised with ammonia, and mixed with 3% of its weight of sodium bicarbonate made into a paste with water. After standing about a week, a black, pitchy mass separates, the filtrate from which gives with ammonia a precipitate which is treated with boiling benzene, the filtrate being also extracted by agitation with benzene. On shaking the united benzene solution with a saturated aqueous solution of sodium bicarbonate, *laudanosine* crystallises out; and the benzene filtered from this yields *hydrocotarnine* hydrochloride on passing hydrochloric acid gas. The portion of the ammonia precipitates left undissolved by benzene contains cryptopine and protopine. These bases are converted in hydrochlorides, and the solution treated with strong hydrochloric acid, when the *protopine* hydrochloride forms a horny deposit

[1] Hesse could obtain no meconidine from this solution, and hence concludes that it had been decomposed by the preceding operations, as he had previously obtained it from a similar source by another process (*Ann Chem Pharm*, 153, 47).

which adheres to the sides of the glass, and is easily freed from the gelatinous *cryptopine* salt by washing with a little water.

Narceine is mentioned as existing in the liquors, but the stage at which it is separated is not stated.

Morphine.

$C_{17}H_{19}O_3N$ or $C_{17}H_{17}ON(OH)_2$.

Morphine is the most important of the bases contained in *opium*, in which it exists in combination with sulphuric and meconic acids.

The mode of preparing morphine may be gathered from the methods of assaying opium and from the preceding methods for separating the opium alkaloids.

Morphine crystallises in transparent, colourless, trimetric prisms, which are usually very short. They contain are molecule of water, which is given off slowly at a temperature of 90° and more rapidly at 100° (*Pharm. Jour.*, 1888 [*iii*], **18**, 701, 801; **19**, 61, 148, 180). At or above 200° morphine turns brown, melts with partial volatilization at about 254° according to some authorities and 247° according to others, becoming carbonised at a somewhat higher temperature.

Morphine is inodorous, has a persistent bitter taste, and is a powerful narcotic poison.

Morphine is peculiarly difficult of solution in the organic liquids which are in most cases excellent solvents of the alkaloids; only in alcohol is it soluble with some degree of readiness. Remarkable variations are found in the recorded solubilities of morphine as observed by different investigators.

The solubility of morphine in water is given by A. Seidell as 1:3,330 at 25° and 1:1,040 at 80°. W. Muller gives its solubility as 1:3,533; and in water saturated with ether 1:2,239 The old figures of Chastaing of 1:33,333 at 3° and 1:4,545 at 22° are most probably too high. At 42° the solubility is stated to be (*Year-book of Pharm.*, 1882, 30) 1:2,380 and in boiling water 1:460. The solution in water has an alkaline reaction. Morphine dissolves in 30 parts of boiling or 50 parts of cold absolute alcohol and according to Seidell in 168 parts of 95% alcohol at 25° or 76 parts at 60°. In ether and chloroform it is almost insoluble when in a crystallized state, but dissolves sparingly when freshly precipitated and amorphous. Its solubility in ether

is given by Seidell as 1:4,464 at 25°; by Prescott as 1:6,148 in the crystalline form; by Muller as 1:7,632 and by the same authority as 1:10,622 in ether saturated with water. Marchionneschi (*Pharm. Ztg.*, 1907, **52**, 747) gives the solubility of the crystals in ether at 5.5° as 1:20,400; in anhydrous ether, 1:3,800; and of anhydrous morphine in anhydrous ether 1:1,785, chloroform is stated by Prescott to dissolve 1 part of morphine in 4,379 parts; 1,525 by Muller, and is also given as 1:1,800 and 1:2,500.

Muller (*Apoth. Ztg.*, 1903, **18**, 257) gives the following solubilities for crystallised morphine: 1 part of morphine dissolves in 3,533 parts of water, 7,632 parts ether, 10,622 parts ether saturated with water, 2,239 parts water saturated with ether, 1,599 parts benzol, 1,525 parts chloroform, 537 parts ethyl acetate, 1,170 parts petroleum ether, and 6,396 parts carbon tetrachloride. A useful solvent for morphine is a mixture of equal volumes of ether and acetic ether (ethyl acetate); but even in this its solubility is limited, especially in the crystalline state. Amylic alcohol dissolves morphine sparingly (1:150) in the cold, but when heated is a fairly good solvent for it (1:50). The alkaloid dissolves best when liberated from one of its salts in presence of amylic alcohol.

Florio (*Gaz. Chim. Italiano*, **13**, 496) gives the following solubilities of morphine:

Solvent	Morphine dissolved by 100 of solvent		
	At 10–11°	At 56°	At 78°
Alcohol, absolute	1 132	. . .	8 623
Alcohol, 90%	0 377	. . . .	2.991
Alcohol, 75%	0 223	. . . . .	1 985
Wood-spirit	1 675	8.466	. . . . .
Fusel-oil	0 268	. .	2.247
Benzene. . . .	0 020	1 235	. . . . .
Chloroform	0 040		
Ether, absolute	0 023		

A. B. Prescott (*J. Amer. Chem. Soc.*, 1907, **29**, 405) has pointed out the great influence the physical condition of morphine has upon its relation to solvents, and has determined the proportion of different solvents requisite for the solution of morphine in the crystalline, amorphous, and "nascent" conditions; by the last term meaning that in which the alkaloid exists when liberated by ammonia or an alkaline carbonate from the aqueous solution of one of its salts. The following are Prescott's figures:

Condition of the morphine	Parts of solvent required			
	Ether	Chloroform	Amylic alcohol	Benzene
Crystallised....	6148	4379	91	8930
Amorphous powder	2112	1977		
"Nascent" state....	1062	861	91	1997

Solutions of sodium and potassium hydroxides dissolve morphine readily, as also do barium hydroxide and lime water, and, to a limited extent, ammonia also. Solutions of alkali hydroxides dissolve quantities of morphine equivalent to the bases contained in them, with the formation of unstable morphinates which are decomposed by carbonic acid and assume a dark brown colour on exposure to air. Crystalline morphinates of potassium, barium, and calcium have been obtained. From these facts, and the blue reaction with ferric chloride, Chastaing (*Jour Pharm.* [V], **4**, 19) inferred that morphine possessed a phenoloid character, and this view has been fully borne out by later researches.

Solutions of morphine are strongly levorotatory. Hesse gives the following results in alkaline solutions:

1 mol. morphine + 1 mol. Na_2O $C=2$, $[\alpha]^{22.5}_D = -67.5°$.
1 mol. morphine + 5 mol. Na_2O $C=2$, $[\alpha]^{22.5}_D = -70\ 2°$.
1 mol. morphine + 2 mol. Na_2O $C=5$, $[\alpha]^{22.5}_D = -71.0°$.

Tykociner gives its rotation in absolute alcohol where $C=1.0$ to 1.8 as $[\alpha]^{20}_D = -140.5°$. The rotation of the salts of morphine is referred to under their respective headings.

Morphine is very sensitive to the action of oxidising agents, a fact which is often used for its detection (page 382 *et seq.*). It reduces salts of gold and silver, permanganates, ferricyanides, iodic and periodic acids, etc. The reactions of morphine with strong sulphuric and nitric acids are described on page 382.

When morphine is heated with strong hydrochloric acid or zinc chloride it loses the elements of water and is converted into apomorphine, $C_{17}H_{17}O_2N$ (page 357).

Salts of Morphine.

Morphine dissolves readily in dilute acids, forming salts which are perfectly neutral in reaction to litmus and methyl-orange, and hence

it may be titrated with accuracy by the aid of standard hydrochloric acid and either of these indicators. With phenolphthaleïn morphine does not give a sharp reaction, but the point of neutrality is approximately the same as if the acid of the morphine salt were in a free state.

The salts of morphine are mostly crystallisable, and are all bitter and very poisonous. They are generally soluble in water and in alcohol, but are insoluble or only slightly soluble in amyl alcohol, ether, chloroform, benzene, or petroleum spirit. Morphine is not removed from its acid or neutral solutions by agitation with any of the above solvents, except imperfectly by amyl alcohol.

The following table shows the formulæ of the more important salts of morphine, the percentage of crystallised morphine in each, the relative dose based on this percentage and their solubility in water.

Morphine salt	Formula	Morphine hydrate, %	Relative dose	Solubility in water	
				at 15 5°[1]	at 25°[2]
Hydrochloride	$BHCl+3H_2O$	80 71	1 00	1 in 24	1 in 16½
Sulphate	$B_2,H_2SO_4+5H_2O$	79 94	1 00	1 in 23	1 in 15⅔[3]
Acetate .	$B,C_2H_4O_2+3H_2O$	75 94	1 04	1 in 2½	1 in 2½
Lactate.	$B,C_3H_6O_3$	80 80	1 00	1 in 8	
Tartrate . .	$B_2,C_4H_6O_6+3H_2O$	78 30	1 02	1 in 9½	
Meconate	$B_2,C_7H_4O_7+5H_2O$	70 45	1 14	1 in 34	1 in 25

Morphine hydrochloride, $BHCl+3H_2O$, crystallises in white silky needles or minute cubical crystals which according to Seidell are soluble at 25° in 17 parts of water or 42 parts of alcohol. It is soluble at 80° in half its weight of water, at 60° in 35.5 parts of alcohol, and is also soluble in about 19 parts of glycerin. At 100° it becomes anhydrous and on heating to 250° it turns brown and with greater heat chars without melting. The blackening in color at a much lower temperature is indicative of traces of impurity.

Hesse gives its optical rotation as follows:

In water $-C=1$ to 4, $[\alpha]_D^{18} = -100.67+1.14\ C.$

With 10 molecules HCl + water $-C=2-[\alpha]_D^{15} = -94.3°.$

Morphine hydriodide, $BHI+3H_2O$, is obtained as a compact mass of hair-like needles on mixing a concentrated alcoholic solution of potassium iodide with a concentrated solution of morphine hydro-

[1] B. D. Dott (*Pharm. Jour.*, 1883 [iii], 13, 404; 1886, 16, 653).
[2] A. Seidell (Solubilities, page 205)
[3] Power gives solubility as 1 in 24 at 15°.

chloride. The product only slowly redissolves on adding more spirit, and is very sparingly soluble in water, especially in presence of potassium iodide. The *hydrobromide* can be obtained similarly.

Morphine sulphate, $B_2H_2SO_4+5H_2O$, crystallises in bundles of transparent silky needles. It loses 3 molecules of water at 100° and the remaining 2 molecules at 110°. It exists naturally in opium. It is soluble at 15° in 20 parts of water, at 25° in 15 parts of water and at 80° in 0.6 parts of water. At 25° it dissolves in 465 parts of alcohol. On heating it behaves similarly to morphine hydrochloride. Its optical rotation according to Hesse is

In water, C=1 to 4, $[\alpha]_D^{15} = -100.47+0.96$ C.

Morphine acetate, $BHC_2H_3O_2+3H_2O$, is a white or more frequently slightly yellowish, obscurely crystalline powder. It is also very soluble in water, differing, however, from the sulphate or hydrochloride in that it is very nearly as soluble in cold water as in hot. At 15° it dissolves in 2.5 parts of water, at 25° in 2.25, and at 80° in about 2 parts. It is soluble in about 22 parts of alcohol at 25° and 100 parts of 90% alcohol at 15°, solubility being very much greater in hot alcohol. It is also soluble in about 5 parts of glycerin and is slightly soluble in chloroform. On exposure to the air it gradually loses acetic acid and is partially decomposed by boiling or evaporating its aqueous solution, crystals of morphine being deposited.

Its optical rotation is given by Oudemans as follows:

In water, C=2.5 $-[\alpha]_D = -77°$.
In alcohol (sp. gr. −0.865) C=0.97 $-[\alpha]_D = -98.9°$.
In absolute alcohol, C=1.2 $-[\alpha]_D = -100.4°$.

Morphine tartrate, $B_2C_4H_6O_6+3H_2O$, is readily soluble, but the acid tartrate, $BC_4H_6O_6$, only sparingly so. Their solutions are not precipitated by alkali hydroxides, alkaline carbonates, or chloride of calcium. The tartrate is best detected by precipitating the concentrated solution with potassium acetate and acetic acid in presence of alcohol. After boiling off the alcohol, the morphine can be precipitated from the filtrate by an alkaline carbonate or ammonia.

Morphine meconate, $B_2C_7H_4O_7+5H_2O$, is interesting as being the form in which morphine largely exists in opium. When morphine and meconic acid are dissolved in absolute alcohol, and the solution evaporated, an amorphous, hygroscopic, very soluble residue is

obtained, which in concentrated solution deposits crystals of neutral morphine meconate containing 5 molecules of water, even in presence of sufficient meconic acid to form the acid salt. It is soluble in alcohol and also in about 25 parts of water.

Detection and Estimation of Morphine.

Free morphine, when pure or in the form of one of its ordinary salts, is readily detected. Its determination is easy when unmixed with interfering substances, but as it exists in opium is attended with considerable difficulties. Most of the colour reactions of morphine are best observed by operating on the solid substance, but for certain qualitative tests and for all quantitative methods the alkaloid must be in solution.

A. **Reactions of Solid Morphine.**—For observing these reactions a minute fragment or crystal of the solid alkaloid or its salt should be employed, and the experiment should be conducted in a small porcelain basin or crucible. The residue obtained by the evaporation of the solution of morphine in alcohol or amylic alcohol is well-suited for the operation.

1. Solid morphine treated with a drop of a *perfectly neutral* solution of ferric chloride or iron-alum gives a very characteristic deep greenish-blue colour, changed to green by excess of the reagent. The colouring matter is not taken up by chloroform. The colour is destroyed by free acid, by heat, or by contact with alcohol.[1] *Pseudomorphine* also gives a blue colour with ferric chloride, and *codamine* a dark green.

2. Nitric acid (1.42 sp. gr.) added to solid morphine turns it an orange-red colour, which is changed to yellow on heating, and destroyed on adding sodium thiosulphate (hyposulphite). The colouration is said to be due to the formation of a substance of the formula $C_{10}H_9NO_9$, which yields picric acid when heated with water to 100°.

3. Solid morphine, when pure, is commonly said to yield no colouration in the cold on adding pure concentrated sulphuric acid; but according to Dott (*Pharm. Journ.*, 1882 [*iii*], **12**, 615) a distinct, though

[1] The colouration is produced in strong solutions of morphine, but becomes imperceptible with moderate dilution. J. L. Armitage (*Pharm. Journ*, 1888 [*iii*], **18**, 761) has pointed out that even in solutions far too dilute to give the reaction, the morphine may be detected by adding potassium ferricyanide, which produces a blue or green colouration. Armitage attributes this reaction to the reduction of the iron to the ferrous state, and the reaction of this with the ferricyanide to form Turnbull's blue, but it is more probable that the ferricyanide is reduced to ferrocyanide, and then reacts with the ferric salt to form Prussian blue.

faint, pink colour is produced. On heating to 150°, a dirty green (or rose-red) colour is developed, and on raising the temperature still further the solution becomes almost black. On allowing it to cool and diluting with water, a greenish-blue colour is produced, which on addition of ammonia in excess becomes green.

4. On adding *oxidising agents* to the solution of solid morphine in cold concentrated sulphuric acid, the following reactions are produced.[1] *a*. After adding a drop of two of water to heat the mixture, the subsequent addition of nitric acid will produce a rose-red colouration, changing to brown. The reaction is very delicate. *b*. Potassium chlorate gives reactions similar to those with nitric acid. If the alkaloid be first heated with concentrated sulphuric acid to 100° for half an hour, and a crystal of potassium chlorate or nitrate added to the previously cooled violet-red solution, a beautiful violet-blue colour is produced, which passes into a dark blood-red, changing to yellow. *c*. If the sulphuric acid solution be heated on the water-bath to 100°, and a minute fragment of pure potassium perchlorate[2] be added, a deep brown or reddish-brown colouration is produced, which rapidly spreads through the liquid. The colour is destroyed on dilution. L. Siebold, to whom the test is due, did not observe a similar reaction with any other alkaloid. *d*. Potassium dichromate is reduced with production of green colour. (No colour reaction is produced if for the dichromate be substituted the dioxide of lead or manganese. Distinction from strychnine.) *e*. On adding sodium or potassium arsenate, and warming gently, a slate-blue colour is produced, which on raising the temperature passes into green, then into deep blue, and finally, when the acid begins to volatilise, again into dark olive-green. On diluting moderately with water, a reddish-brown colouration is produced, changing to dirty bluish and green on further dilution; and on agitating with chloroform the latter liquid is coloured violet-blue (Donath). If sodium phosphate be substituted for the arsenate[3] and heat applied till acid fumes appear, the mixture becomes violet, changing to brown or olive-green. If, after cooling, water be gradually added, a reddish-brown colouration appears, changing to dirty bluish-green on further dilution. On now shaking with chloroform, the

[1] The reactions in question were verified in the laboratory of A. H. Allen by W. H. Barraclough, and the description given in the text is in accordance with his results.

[2] The perchlorate must be free from chlorate, which is ensured by heating it with hydrochloric acid as long as chlorine is evolved. The salt is then washed with cold water and dried.

[3] For convenience, this test is described here, but it seems improbable that the reaction is due to oxidation.

latter liquid acquires a fine blue colour. *f.* Sodium or ammonium molybdate added to the sulphuric acid solution gives a fine violet colouration, changing to blue and dirty green, and finally almost vanishing. The reaction of morphine with sulphomolybdic acid may be observed with more certainty by adding previously prepared Frohde's reagent to the solid morphine. *Papaverine, heroin, peronin,* and a few glucosides give a similar reaction.

5. If solid morphine be mixed with from 2 to 8 parts of powdered cane-sugar, or solutions of the two substances be mixed and evaporated to dryness, addition of a drop of concentrated sulphuric acid will produce a beautiful purple colour, changing gradually to blood-red and brownish-red, becoming olive-brown on dilution with water. The colouring matter is not soluble in chloroform. The test may be applied to a solution of morphine by saturating the liquid with sugar, and pouring it carefully on to some concentrated sulphuric acid, when a purple or rose-red colouration will be observed at the junction of the two fluids. Codeine gives a very similar reaction (Schneider). According to H. Weppen the delicacy of this test is much increased by adding a drop of bromine-water after the sulphuric acid, this modification rendering the reaction equal if not superior to reactions 3 and 4 *c*, and less dependent on the purity of the morphine.

M. Robin mixes the alkaloid with twice its weight of powdered sugar, and adds 1 or 2 drops of pure sulphuric acid, and states that morphine hydrochloride gives a beautiful rose colour, changing first to the tint of a solution of potassium permanganate, and then to violet and dark green, while codeine gives a cherry-red colour changing to violet, and narcotine a beautiful and very persistent mahogany-brown colour.[1]

Reichard states that if morphine is warmed gently with concentrated sulphuric acid containing arsenous or arsenic acid an intense and permanent purple colour is produced. The reaction is best obtained by dissolving the arsenous acid in a little strong solution of sodium hydroxide, then add the morphine and finally excess of concentrated acid. A red colour is given by adding morphine to sulphuric acid containing antimonous or stannous chloride.

[1] Atropine gives with sugar and sulphuric acid a violet colouration, changing to brown; veratrine, a deep green; santonin, a red colour, changing to coffee-black. Salicin gives a vivid red. Pure aconitine gives no reaction, but mixed aconite alkaloids as extracted from the root give a fine cherry-red colouration, changing to crimson No reaction is given by strychnine, brucine, cocaine, pilocarpine, caffeine, beberine, apomorphine, cupreine, or the cinchona bases (J. F. Burnett).

Morphine is stated by Rosenthaler to give a reddish-violet colour on heating with a 1% solution of vanillin in hydrochloric acid; a similar colour, however, is given by many ketones and phenols.

Reichard also gives the following reactions (*Zeit. Anal. Chem.*, 1903, **42**, 95): To 2 or 3 c.c. of a 0.1% solution of ammonium vanadate add concentrated sulphuric acid drop by drop until the yellow colour disappears. On now adding a soluble morphine salt a stable green colour is produced.

On shaking a 0.1% solution of sodium tungstate with a few drops of concentrated sulphuric acid and morphine a bright blue or violet colour is produced which is less stable than the colour with ammonium vanadate. A stronger solution of sodium tungstate cannot be used because of the separation of tungstic acid.

On adding morphine to a cold solution of titanic acid in concentrated sulphuric acid a blood-red colour appears on shaking but disappears on dilution with water.

According to G. Fleury (*Ann. de Chim. Anal.*, 1901, **6**, 417) a solution of morphine in dilute sulphuric acid causes a faint rose colour when shaken for several minutes with a small quantity of lead dioxide. If the mixture is filtered and an excess of ammonia added to the filtrate a deep brown colour is produced, which is due to the formation of protocatechuic acid.

On warming morphine with strong sulphuric acid until a pink colour develops and adding with stirring either chloral or bromal a violet colour is produced.

B. **Reactions of Morphine in Solution.**—The following reactions are yielded by an aqueous solution of the hydrochloride or acetate of morphine:

1. On adding to a tolerably concentrated solution of a salt of morphine a fixed alkali hydroxide, an alkaline carbonate, ammonia, or lime-water, hydrated morphine, $C_{17}H_{19}O_3N+H_2O$, is thrown down as a white precipitate speedily becoming crystalline. The precipitate is almost insoluble in perfectly cold water, but dissolves in excess of ammonia or lime-water, and very readily in excess of alkali hydroxide. The alkaline carbonates, used in excess, redissolve the precipitate somewhat, but it is insoluble in excess of bicarbonates. Excess of magnesia precipitates the alkaloid completely. The morphine precipitated by the foregoing reagents, and allowed time to become crystalline, presents a characteristic appearance under the microscope.

A fairly accurate determination of morphine may be made in the absence of interfering substances, by precipitating the tolerably concentrated, cold, aqueous solution with sodium acid carbonate, allowing time for the precipitate to become crystalline, filtering, washing moderately with very cold water (preferably saturated with morphine), drying at 100 or 120°, and weighing the anhydrous morphine, $C_{17}H_{19}O_3N$, when the weight becomes constant.

Instead of drying and weighing the alkaloid, the washed precipitate may be placed, together with the filter, in a moderate excess of standard acid, and the excess employed ascertained by titration with litmus or methyl-orange (not phenolphthaleïn). 1 c.c. of decinormal acid neutralises 0.0285 grm. of anhydrous, or 0.0303 grm. of crystallised morphine.

2. If morphine be liberated from the solution of a salt by one of the reagents mentioned above, and the liquid and suspended precipitate be at once shaken with hot amylic alcohol, cold acetic ether, or a mixture of equal measure of ether and acetic ether,[1] the morphine passes into solution, though with some difficulty, and may be obtained in a free state by separating the ethereal liquid, and evaporating it to dryness at a gentle heat. If the liberated morphine be allowed to crystallise before subjecting it to agitation with the solvent, its solution becomes very difficult to effect.

Quantitative Estimation.

For quantitative purposes, hot amyl alcohol should be employed as the solvent. It should be added before the alkaloid is liberated, which should be done by ammonia, magnesia or sodium acid carbonate, and the agitation should be conducted immediately, and the separation and re-agitation effected without delay. On evaporation of the amyl alcohol at 100° the anhydrous morphine will remain as a residue, which can be weighed,[2] or the amyl alcohol containing the alkaloid in solution may be titrated by dilute standard acid and methyl-orange. If desired, the alkaloid may be recovered from its amyl alcohol solution by repeated agitation with dilute hydrochloric acid,[3] or

[1] The acetic ether must be free from acid. This may be ensured by agitating it with some sodium acid carbonate before use

[2] There is some evidence that morphine forms a compound with amyl alcohol not decomposed by evaporation at the ordinary temperature (*Pharm Jour*, 1888 [iii], 18, 161)

[3] A solution of morphine in hydrochloric acid cannot be shaken with amylic alcohol without extraction of some of the alkaloid, probably in the form of hydrochloride

sulphuric acid, and then reprecipitated from the aqueous liquid by ammonia, or an alkaline hydrogen-carbonate. This affords a valuable means of purifying morphine and separating it from other alkaloids.

To effect complete extraction of the morphine liberated by magnesia, ammonia, or an alkaline hydrogen-carbonate, several agitations with amyl alcohol are necessary. If ammonia be employed, sufficient passes into the amylic alcohol to vitiate the subsequent estimation of the morphine by titration; while if the amyl alcohol be freed from ammonia by agitation with water, or even with brine, a portion of the morphine is dissolved out. If the separated amylic alcohol be distilled off, the residual morphine may be titrated, or the difficulty avoided by using magnesia instead of ammonia.

3. A volumetric estimation of morphine may be made by means of Mayer's solution. This method is not of great practical utility, but Heikel (*Midland Drug.*, 1909, **50**, 114, 176, or *Proc. A. Ph. A.*, 1909, 374) has devised a modification whereby its accuracy is distinctly improved.

Further information on the estimation of morphine will be found in the section on the Assay of Opium. (See page 416.)

4. Morphine readily reduces ferricyanides to ferrocyanides, with formation of pseudomorphine (oxydimorphine):

$$4C_{17}H_{19}O_3N,HCl + 4K_3FeCy_6 = 2(C_{34}H_{36}O_6N_2,2HCl) + 3K_4FeCy_6 + H_4FeCy_6.$$

Consequently, on adding to the solution of a salt of morphine, slightly acidified with hydrochloric acid, a mixture of aqueous solutions of ferric chloride and potassium ferricyanide, a blue colouration or precipitate of Prussian blue is produced. This reaction may be conveniently employed for detecting morphine in presence of the cinchona bases.

L. Kieffer (*Annal. Chem. Pharm.*, **103**, 274) has proposed to utilise the reaction with ferricyanide for the quantitative determination of morphine. For this purpose he adds a known weight of solid potassium ferricyanide to the morphine or its salt, and mixes them in a mortar with a minimum quantity of water. The contents of the mortar are rinsed into a flask, potassium iodide and hydrochloric acid added, and the liberated iodine determined by $N/10$ sodium thiosulphate (hyposulphite). The difference between the volume required and that used

in a blank experiment with the same weight of potassium ferricyanide corresponds to the salt reduced by the morphine. 1 c.c. of difference in the $N/10$ thiosulphate used represents 0.0292 of anhydrous morphine.[1]

Venturini (*Gaz. Chim. Ital.*, 1887, 16, 239) reports favorably of Kieffer's process. A. H. Allen's results were discouraging.

5. On mixing a solution of morphine with one of iodine dissolved in hydriodic acid, a crystalline precipitate is formed even in extremely dilute solutions. Under the microscope the crystalline form is characteristic of morphine, which may thus be distinguished from papaverine and codeine, which bases also give crystalline precipitates with the reagent, while narcotine, narceine and thebaine yield amorphous precipitates.

6. Addition of chlorine or bromine water, followed by ammonia, occasions in moderately concentrated solutions of morphine a brown colour or red colouration gradually changing to brown.

7. Morphine and its salts reduce iodic acid with liberation of iodine. This reaction is also produced by albuminoid and various other organic bodies, so that it is not absolute proof of the presence of morphine. The test becomes much improved and increased in delicacy by the following mode of operating:

To the solution to be tested for morphine, as nearly neutral as possible, is added one of iodic acid in 15 parts of water. In presence of 1 part of morphine in 20,000 of liquid a yellow colouration is observed. In moderately strong solutions of morphine addition of starch-liquor gradually changes the yellow colour to blue, but not in solutions containing less than 1 per 1,000. This is important, as with other reducing agents the blue colour is well marked in far more dilute liquids. On adding excess of ammonia to the yellow liquid the colour is discharged if due to foreign matter, but distinctly deepened if due to morphine. If a solution of morphine, which is too dilute to give a blue colour with iodic acid and starch, be mixed with these reagents, and some highly dilute ammonia allowed to flow from a pipette on to the surface of the liquid, two coloured rings make their appearance at the junction of the fluids. A blue ring is seen in the lower acid layer and a brown one in the upper alkaline portion. If a dilute solution of morphine be mixed with one of starch, and evaporated to dryness in

[1] It is possible that Kieffer's process might be applied to the amylic alcohol solution of morphine, by agitating it with potassium ferricyanide solution. In such a case, ammonia, if present, would not interfere.

a porcelain crucible at a gentle heat, and the residue, after cooling, be moistened with iodic acid, a blue colour will be produced in presence of 1–20,000 of a grain of morphine (A. Dupré).

Another way of employing the test is to agitate a solution of iodic acid with an equal measure of carbon disulphide, which should not become coloured even after adding a drop or two of dilute sulphuric acid and again shaking. If the solution to be tested for morphine be now added to the mixture, and the whole again shaken, the carbon disulphide will be found after separation to have a violet colour from dissolved iodine if morphine be present, and the depth of tint will afford an indication of the amount. Morphine can be recognised in this way in a single drop of paregoric or tincture of opium.

Stein and others have described a colourimetric method of estimating morphine, based on the iodic acid reaction. See also Georges and Gascard, *Jour. Pharm. Chim.*, 1906, **23**, 513.

In employing the iodic acid test it is essential that the reagent should not give free iodine on treatment with a drop of dilute sulphuric or acetic acid.

Edlefsen (*Pharm. Ztg.*, 1908, **53**, 289) recommends a 0.5% aqueous solution of iodic acid slightly coloured with malachite green. To 5 c c. of this reagent add a few drops of the solution to be tested and warm gently. A lemon-yellow colour is produced which changes to brown on adding ammonia to alkaline reaction.

8. Morphine and its salts are stated by Aloy (*Bull. Soc. Chim.*, 1903, **29**, 610) to give a characteristic colour reaction with uranium acetate or nitrate whereas most alkaloids give precipitates. To the solution to be tested add a few drops of 5% solution of uranium nitrate. A red colour is given in presence of morphine or if in quantity less than 5 mgrm. an orange colour. Said to detect as little as 0.1 mgrm.

9. Deniges (*Compt. Rend.*, 1910, **151**, 1062) gives the following reaction: To 10 c.c. of a dilute solution of morphine containing as little as 30 mgrm. per litre, add 1 c.c. of 3% hydrogen peroxide, 1 c.c. of ammonia 10% and 1 drop of copper sulphate solution (1 to 4%, according to the amount of morphine), on shaking, a colour varying from rose to intense red is produced. This test may be applied to syrups containing morphine and may be used quantitatively by comparison with standard solutions of morphine hydrochloride in sugar syrup. For the identification of the alkaloid as hydrochloride the dry salt should be mixed with 1 drop of a reagent consisting of

1 c.c. of 3 to 4% copper sulphate solution and 5 c.c. of water. In the presence of morphine a red colour is immediately produced.

No reaction is obtained with codeine, thebaine, papaverine, narceine or narcotine but similar colours are given by direct derivatives of morphine such as oxymorphine and paramorphine and with easily hydrolised esters such as heroin. The copper sulphate appears to have a catalytic action but cannot be replaced by iron or manganese salts.

10. Solutions of morphine salts give no crystalline precipitate with either potassium chromate, thiocyanate (sulphocyanide) or ferrocyanide (distinction from strychnine). With a saturated alcoholic solution of picrolonic acid (dinitrophenylmethylpyrazolone) morphine gives from its solution in ether, chloroform or alcohol a crystalline precipitate of broad flat needles of picrolonate variously stated to melt at 186.5° and 200° to 210° with darkening.

Apomorphine, $C_{17}H_{17}O_2N$. When morphine or its hydrochloride is heated to 140–150° in a sealed tube, with a large excess of strong hydrochloric acid, or with zinc chloride at 110°, it is converted into the hydrochloride of apomorphine, the formula of which base differs from that of the parent alkaloid by the elements of water. Apomorphine may be obtained in a state of purity by dissolving the contents of the tube in water, adding excess of sodium hydrogen carbonate, and agitating with ether or chloroform, in either of which apomorphine is freely soluble (difference from morphine). The ethereal solution is separated and shaken with a very little strong hydrochloric acid, when crystals of the hydrochloride of apomorphine are deposited. These are separated, washed with a little cold water, and purified by recrystallisation. From its aqueous solution of the hydrochloride, sodium acid carbonate precipitates free apomorphine as a snow-white amorphous substance, readily soluble in alcohol, ether, chloroform and benzene, which speedily turns green on exposure to the air. The changed alkaloid is partially soluble in water and alcohol with emerald-green colour, in ether with magnificent rose-purple, and in chloroform with fine violet tint. The colourless solutions of the unchanged substances soon acquire these tints. In its physiological effects, apomorphine differs from morphine in a very marked manner, being a prompt and non-irritant emetic. From 0.001 to 0.010 grm. is the adult medicinal dose by the stomach. Dangerous and even fatal symptoms have followed the hypodermic injection of 0.012 grm.

Apomorphine gives a crimson-red colour with nitric acid, and brown with iodic acid, but (unlike morphine) yields a rose-red or amethystine colour with ferric chloride, changing to violet and black. The most delicate reaction of apomorphine is the production of a green colouration when the solution is rendered faintly alkaline with potassium hydrogen carbonate and exposed to the air. With a solution containing 1 part in 100,000, the green colour appears within 10 minutes. A variation of this reaction is given by Schmidt (*Apoth. Ztg.*, 1908, **23**, 657). By treating a 1 to 10,000 solution of the hydrochloride with 1 c.c. of chloroform, making alkaline with sodium hydroxide and shaking, the chloroform is coloured blue and the aqueous layer red-violet.

If a small quantity of the hydrochloride is shaken with a 0.5% aqueous solution of ferrous sulphate the solution becomes gradually blue and then black, returning to blue on addition of alcohol.

Apomorphine is said to be liable to be formed in old solutions of morphine hydrochloride, which consequently acquire emetic properties; but the statement is disputed by Dott, and requires confirmation (*Pharm. Jour.* 1886 [*iii*], **16**, 287, 299, 604; **17**, 80).

Apomorphine hydrochloride, $C_{17}H_{17}O_2NHCl$, forms anhydrous, minute, shining crystals, which turn greenish on exposure to light and air. It is freely soluble in water and alcohol, forming a neutral solution, which turns green on boiling or standing, and keeps better if very faintly acid. The freshly-made aqueous solution should be colourless, or nearly so. It is generally held that if a 1% solution be emerald-green, the sample should be rejected for medical use; but D. B. Dott (*Pharm. Jour.* 1891 [*iii*], **21**, 916) has pointed out that the colouration is so intense that very little actual change is thereby indicated. Morrell found an old solution which had been exposed to light for 3 months to act quite effectively,[1] but it is nevertheless not desirable to use either the salt or its solutions which have become decidedly green in colour.

Schmidt (*Apoth. Ztg.*, 1908, **23**, 657) and Dott (*Pharm. Journ.*, 1908 [*iv*], **27**, 801) have shown that the hydrochloride crystallises with 2 molecules of water, and Schaefer states that the anhydrous salt absorbs 1 molecule of water from the air.

Recently Frerich and Harnack (*Pharm. Jour.*, 1910, **30**, 293, 454)

[1] Morrell reports that a patient who is made violently ill by 1/6 grain of apomorphine hydrochloride administered hypodermically, can take 4/5 grain thrice daily in the form of pills. Apomorphine acts as a powerful expectorant in cases of chronic bronchitis.

stated that in certain brands of apomorphine hydrochloride they had found an impurity which seemed to be trimorphine, but an investigation by Boehringer and Sœhne identifies this impurity as beta-chloromorphide which they found present in one sample to the extent of 75%. As the physiological action of this is decidedly different from apomorphine it is liable to cause considerable variation in its action.

To detect this impurity a solution of 0.1 grm. of apomorphine hydrochloride in 10 c.c. of water is rendered alkaline with 5 c.c. of saturated solution of sodium carbonate and extracted with 20 c.c. of ether. The ethereal solution is separated and shaken out with 3 successive portions of 20 c.c. of water. The washed ether solution is then evaporated and the residue cooled and treated with 5 c.c. of concentrated nitric acid containing 0.5% of silver nitrate. After standing for 10 minutes the acid liquid is heated for an hour on the water-bath, keeping up the volume with addition of water. The brown, clear, undiluted liquid should then show no appreciable precipitate of silver chloride.

A number of derivatives of morphine resembling codeine have been prepared, and of chief importance we may mention the following:

Heroin, or diacetyl morphine, is an odourless, bitter crystalline powder having an alkaline reaction and melting at 171–172°. It is almost insoluble in water and more readily soluble in cold alcohol and ether and easily soluble in hot alcohol; it is also readily soluble in chloroform and benzine. Its hydrochloride exists as a white powder soluble in alcohol and water.

Its colour reactions resemble morphine; it dissolves in sulphuric acid to a pale yellow colour which becomes pale pink on heating. Some other colour reactions have already been spoken of on page 368. On adding a little heroin to 2 c.c. of a 10% solution of urotropin in sulphuric acid there occurs an immediate golden yellow colour changing to saffron yellow and finally deep blue. Morphine with this reaction gives a purple colour; nerceine gives a saffron yellow and narcotine a golden yellow. Another reaction which may be used for distinguishing it from morphine and codeine is as follows: To a small quantity of the alkaloid add a few drops of concentrated nitric acid and heat gently. The original yellow colour changes to bluish-green becoming almost blue and finally changing altogether to yellow. This test is stated not to be given by any other alkaloid.

Goldman's reaction may also be used for its identification by heating

the alkaloid with concentrated sulphuric acid and a small quantity of alcohol which results in the production of ethyl acetate that may be recognised by its odour.

Dionin is ethyl-morphine and occurs chiefly in commerce as its hydrochloride. This is a white crystalline powder having a m. p. of 123–125°, is soluble in water and very readily soluble in alcohol. Insoluble in ether and chloroform. Dissolved in sulphuric acid it gives no colour and on heating a pale pink. Hesse states that it may be distinguished from codeine by its behaviour with ammonia. To 5 c.c. of a 10% solution of codeine hydrochloride a few drops of ammonia (sp. gr. 0.91) are added and a precipitate occurs which is permanently re-dissolved by the addition of 1 c.c. ammonia water. With dionin it requires the addition of 5 c.c. of ammonia water for complete solution and from this solution crystals, m. p. 93°, are deposited after a short time even from a 1% solution.

Rodinoff states that with 2 c c. of 1% solution of dionin 10 drops of Wagner' reagent gives a reddish-brown precipitate which turns orange-brown with shaking and rises to the surface of the liquid. With codeine the precipitate does not change colour on shaking.

Codeine, $C_{18}H_{21}O_3N$, is described as regards constitution and relation to morphine on page 356. It occurs in opium[1] in proportions ranging from 0.1 to 1.0%.

Codeine crystallises from dry ether or carbon disulphide in small anhydrous prisms. From water it is deposited in well-defined octahedra or orthorhombic prisms containing $1H_2O$ and melting under boiling water to an oily liquid. Anhydrous codeine melts at 155° and solidifies to a crystalline mass on cooling. Codeine is somewhat soluble in water, requiring 75 to 80 parts of cold water, or 17 at the b. p. It is readily soluble in alcohol, ether, amyl alcohol, chloroform and benzene, but is almost insoluble in petroleum spirit (compare page 363). Codeine is as soluble in ammonia as in water, a fact utilised to separate it from morphine, but it is practically insoluble in excess of sodium and potassium hydroxides, and is precipitated by these reagents from its aqueous solution, if not too dilute. Solutions of codeine are optically active, the rotatory power being much af-

[1] Codeine is usually isolated from opium by precipitating the aqueous extract by calcium chloride, evaporating and cooling the filtrate, redissolving the deposited crystals of the hydrochlorides in water, and precipitating the morphine by ammonia From the filtrate, after concentration, the codeine can be recovered by treating by precipitating with alkali hydroxide and purified by crystallisation from ether

fected by the nature of the solvent, and the presence and proportion of free acid.

Hesse gives the following rotation:

In alcohol 97% C = 2 to 8 $[\alpha]_D^{15} = -135.8°$
In chloroform C = 2 $[\alpha]_D^{15} = -111.5°$

while Grimaux gives

In alcohol C = 4.1 $[\alpha]_D = -130.34°$
In alcohol (com'l. codeine) C = 4 1 $[\alpha]_D = -133.18°$.

Codeine has a bitter taste, and resembles morphine in its physiological action. It is official in many pharmacopœias, and is chiefly employed to allay restlessness, cough, and other symptoms for which opium is generally prescribed, and when the latter medicine is not tolerated. In phthisis, it appears to prevent and appease the tickling irritation of the cough, without deranging the digestion. It is an important remedy in diabetes, and is also recommended as an hypnotic in mental diseases. The official dose is from 1/4 to 2 grains. In larger quantities, codeine produces narcotism, often preceded by vomiting and occasionally by purging.

Codeine is a strong base, having a marked alkaline reaction, and forming crystallisable, soluble salts, which are neutral to litmus and methyl-orange. The free base precipitates solutions of lead, iron, copper, and certain other of the heavy metals.

Codeine hydrochloride crystallises in radiated groups of prisms containing $BHCl + 2H_2O$, soluble in about 20 parts of cold water.

Its rotation is given by Hesse as,

In alcohol 80% C = 2 $[\alpha]_D^{22\ 5°} = -108°$.
In water, C = 2 $[\alpha]_D^{22\ 5°} = -108.2°$.

The crystals lose a portion of their water ($1/2H_2O$) readily, but the remainder is only driven off by many days' heating at 100° (Schmidt, *Pharm. Jour.* 1891 [*iii*], **21**, 82), but easily at 120° (Dott). Hence the proportion of water in commercial samples of the salt is variable.

Codeine Phosphates.—The salt $BH_3PO_4 + 2H_2O$ is obtained as a crystalline precipitate by adding codeine to a solution of phosphoric acid till the reaction is only faintly acid, and then adding excess of alcohol. When recrystallised from water the composition is unchanged but the salt deposited from the solution in hot dilute alcohol contains $2BH_3PO_4 + H_2O$. Both forms lose their water at 100°, and are met

with in commerce, as also a preparation containing excess of phosphoric acid. The commercial product practically never contains $2H_2O$ and usually contains approximately $1H_2O$, indeed if exposed to the air the crystals with $2H_2O$ quickly lose part of this water. If the salt turns grey or yellow at 100°, the presence of impurity is indicated. The phosphate is said to be the preferable form of employing codeine for hypodermic injections.

Codeine sulphate may be obtained as $B_2H_2SO_4 + 5H_2O$ by crystallisation from water but the commercial product corresponds more nearly to $3H_2O$ it being very difficult to preserve the crystals with $5H_2O$ without loss of water. It loses its water of crystallisation completely at 100°. It is very soluble in water, difficultly in alcohol and insoluble in ether and chloroform.

In water its rotation is given by Hesse as $[\alpha]_D^{15} = -101.2°$ where $C = 3$.

Detection and Estimation of Codeine.

In its reactions and general characters codeine presents a strong resemblance to morphine, but is sharply distinguished by its ready solubility in ether and chloroform, and its precipitation by excess of alkali hydroxide. Codeine does not reduce iodic acid, and gives no colouration with ferric chloride. In strong nitric acid it dissolves to a yellow liquid which should not become red (difference from and absence of morphine). With pure sulphuric acid, codeine gives no colouration, but on warming, or very prolonged standing (several days) at the ordinary temperature, a blue colour is developed. This colour is produced if a trace of nitric acid, ferric chloride, or other oxidising agent be present, an arsenate being the preferable reagent. The blue colouration on warming with sulphuric acid and ferric chloride is apparently common to all ethers of the codeine class. Frohde's reagent is stated by some observers to produce a dirty green colour, soon becoming deep blue, and changing in 24 hours to yellow; according to others, a cherry-red tint, changing to violet, is produced. L. Raby states that if solid codeine be stirred up with 2 drops of a solution of sodium hypochlorite, 4 drops of strong sulphuric acid added, and the whole mixed together, a splendid and persistent blue colouration results. Esculin was the only other substance (of 30 examined) which gave at all a similar reaction. Lafon uses a solution of 1 grm. of ammonium selenite in 20 c.c. of strong sulphuric acid, which gives a magnificent green colour with traces of

codeine. With picrolonic acid it gives a precipitate of short yellow crystals which melt at 219°.

Commercial codeine has been met with adulterated with *ammonium tartrate* (*Pharm. Jour.*, 1884 [*iii*], **14**, 1035), which salt closely resembles it, but is distinguished from codeine by its insolubility in alcohol.

Claassen has based a method of estimating codeine on the well-known fact that it completely decomposes morphine salts (*N. Y. Pharm. Rundschau*, 1890, 40; *J. Chem. Soc*, 1891, **58**, 1198). The warm aqueous solution of the free base is treated with excess of morphine sulphate with frequent shaking, and allowed to stand in the cold for at least 24 hours, when the deposited morphine is filtered off, dried, and weighed (or titrated). The amount found, multiplied by 0.9868 represents the anhydrous codeine, or by 1.0412, the hydrated codeine ($C_{18}H_{21}O_3N+H_2O$). To separate morphine and codeine, the mixed bases, or their salts, are evaporated to dryness with excess of magnesia, the residue treated with water, and the liquid shaken repeatedly with ether free from alcohol, the ether distilled off, and the residue exhausted with hot water. In the resultant solution the codeine can be estimated as above described.

Claassen (*loc. cit.*) has also pointed out that free codeine completely decomposes ammonium salts when heated with them, and has based on this fact a method of estimating the alkaloid; but as morphine behaves in a similar manner, the fact has little practical value.

The simplest means of estimating codeine and morphine in admixture is to precipitate the solution of the hydrochlorides with sodium acid carbonate, and wash the dried precipitate with chloroform. The residue consists of *morphine*. The aqueous filtrate is treated with sodium hydroxide, agitated several times with chloroform, the various chloroform washings and extracts united, evaporated, and the residual *codeine* dried at 110°, and weighed (D. B. Dott).

Van der Wielen (*Bull. Sci. Pharmacol.*, 1910, **17**, 59; *Pharm. Zeit.*, 1903, 267) gives the following process for the estimation of codeine and narcotine in opium.

Boil 10 grm. of opium for 1 hour under reflux condenser with 100 grm. of alcohol (70%). Restore any lost weight by adding alcohol, filter and in 5 grm. of filtrate determine the dry extract. A sufficient quantity of this filtrate is then used to be equivalent to 3 grm. of opium, which is estimated as follows:

If the solution contains p% of extract then the total quantity of

solution is $\frac{10,000}{100-p}$ and 3 grm. will therefore be contained in $\frac{3,000}{100-p}$ grm. of the solution. Evaporate this quantity to about 3 c.c., transfer to a flask, washing out the evaporating dish 3 times carefully with 2 1/2 c.c. of water. To this solution now add 90 c.c. of ether and after shaking, 5 c.c. of 10% solution sodium hydroxide. Let stand for 3 hours with frequent shaking, then add 3 grm. of gum tragacanth to aid separation of the ether and decant exactly 75 c.c. of the ethereal solution (= 2 5 grm. opium). Evaporate to dryness and dissolve in 4 grm. of 90% alcohol and set aside for 24 hours. Collect the crystals of narcotine which separate, on a filter, wash with 5 c c. of alcohol, dry at 100° and weigh. As narcotine is not entirely insoluble in alcohol a correction of +0.016 grm. must be applied.

Dilute the filtrate containing the codeine with 10 c.c. of water and then evaporate to 10 c.c. After standing 24 hours filter to remove the separated resinous substances, washing the filter thoroughly. To the filtrate add 10 c.c. of $N/100$ hydrochloric acid and titrate the excess acid with $N/100$ sodium hydroxide using *hematoxylin* or *cochineal*. 1 c.c. of $N/100$ acid equals 3.17 mgrm. of codeine. By this method Van der Wielen found 1.08% and 1 29% codeine in 2 samples of Asia Minor opium and 1.51% in a sample of Persian opium.

Caspari criticises this method because of the small quantity of opium taken and it would also seem preferable to use $N/10$ acid and alkali instead of $N/100$.

A somewhat different process for the estimation of codeine is given by Caspari (*Pharm. Review*, 1904, 348). Macerate 50 grm. of opium with 500 c.c. of water for 12 hours with frequent agitation. Filter and wash the residue carefully with sufficient water to make 750 c c. of filtrate. Return the residue to the flask and shake for 15 minutes with 250 c.c. of water. Return to the filter and wash with sufficient water to make 850 c.c. of a second filtrate. Combine the 2 filtrates and evaporate on water-bath to 250 c.c., add 5 grm. of barium acetate and dilute to 700 c.c. This precipitates the meconic acid and part of the resin; filter, wash the precipitate thoroughly with cold water and again concentrate (volume not stated, presumably 250 c.c.), again add 5 grm. of barium acetate or until no further precipitate is produced. Filter and wash as above. Again concentrate and add a slight excess of 10% sodium hydroxide solution which precipitates thebaine, papaverine and narcotine and retains in solution morphine, codeine

and narceine. After standing for a short time filter and wash precipitate thoroughly. Make the filtrate acid with hydrochloric acid, concentrate (volume not stated) add 2% ammonia water in excess and let stand for several hours in the cold after which filter off the precipitated morphine, washing the precipitate with cold water; again concentrate the filtrate and washings and repeat above treatment to remove any additional morphine. After concentrating this last filtrate, previously rendered acid with hydrochloric acid, to about 75 c.c, make alkaline with ammonia and extract several times with benzene which removes codeine but not narceine. Evaporate the benzene solution carefully and either weigh the codeine or better dissolve in excess of $N/10$ acid and titrate the excess with $N/10$ alkali, using *cochineal* as an indicator. By this process Caspari obtained 1.12% and 1.33% codeine in samples of Smyrna opium.

This last process could, it seems, be modified to advantage and parts of the two processes might be combined with good results.

For a more recent process for the development of codeine in opium, see Andrews (*Analyst*, 1911, **36**, 489).

Pseudocodeine, $C_{18}H_{21}O_3N+H_2O$, was discovered by E. Merck in preparing apocodeine (*Arch. Pharm*, 1891, **229**, 161). It is a strong base, crystallising in needles melting at 178–180°. It is levorotatory, $[\alpha]_D = -91\ 1°$ in alcohol where p = 1 91, forms crystallisable salts, gives no reaction with ferric chloride, and has a physiological action similar to, but weaker than, that of codeine.

Apocodeine, $C_{18}H_{19}O_2N$, is said to be produced by heating codeine hydrochloride with a concentrated solution of zinc chloride for fifteen minutes. It is described as gummy, insoluble in water, soluble in alcohol and ether, and yielding amorphous salts. In physiological action it is a valuable expectorant and mild emetic. Apocodeine gives a characteristic blood-red colour with nitric acid. D. B. Dott doubts the existence of apocodeine, and states that commercial *apocodeine hydrochloride* is not of a very definite nature, being probably a mixture of an amorphous modification of codeine, polymerised bases, chlorocodide, and apomorphine. The physiological results appear to harmonise with this view (*Pharm. Jour.*, 1891 [*iii*], **21**, 878, 916, 955, 996).

Basic Associates of Morphine.

As already stated, opium contains a large number of bases, some of which are present in very minute amount, or are altogether absent

from some samples. The names, formulæ, solubilities, and chief colour reactions of these alkaloids have already been given, and morphine has also been described at length. The following are additional facts respecting the less important bases of opium.

Aporeine is reported by Pavesi (*Chem. Centralb.*, 1905, 1, 826) as occurring in the seed capsules of *Papaver Dubium* from which it was extracted by petroleum ether, using a method similar to that of Hesse for extraction of rhœadine. The yield was 0·015%.

Aporeine is a yellowish, amorphous powder which forms a hydrochloride in microscopic crystalline plates from ether, chloroform or petroleum ether; m. p. of the hydrochloride, 230°. It produces a burning, and numbing sensation on the tongue and is a tetanic poison resembling thebaine.

Its aqueous solution is precipitated by silver nitrate and phosphomolybdic acid.

On dissolving the dry alkaloid in sulphuric acid and adding nitric acid (1.30) the solution changes from violet, through brown to yellow and the same colours are given by sulphuric acid and potassium chlorate. Frohde's reagent gives a grey-blue, changing to green then brown and yellow.

Sulphuric acid with formaldehyde gives a green, turning to blue and finally black.

Codamine, $C_{20}H_{25}O_4N$, melts at 126° when crystallised from benzol, and 121° when separated from alcohol or ether. It forms large six-sided prisms, which can be sublimed. It dissolves moderately easily in hot water, giving an alkaline solution. Its salts, which are amorphous, give precipitates with alkali hydroxides and ammonia, soluble in excess of either reagent; with nitric acid codamine gives a dark green colouration; with sulphuric acid, and in presence of a minute quantity of ferric chloride, a greenish-blue. For other colour reactions and solubilities, see page 363 *et seq.*

Cryptopine, $C_{21}H_{23}O_5N$, occurs in but very small quantity in opium, and is precipitated on adding sodium hydroxide to the mother-liquor from which codeine, narceine, thebaine and papaverine have been separated. It crystallises from alcohol in minute six-sided prisms and has a m. p. of 218°. It is optically inactive, sparingly soluble in boiling alcohol, very slightly in benzene or petroleum spirit, but more readily in chloroform. When freshly precipitated it is soluble in ether, but slowly separates from the solution.

Cryptopine and its salts have a bitter taste and pungent cooling after-taste; they are hypnotic and mydriatic.

Cryptopine salts when dissolved in hot water usually produce on cooling a gelatinous mass, which is gradually changed to crystals. The *normal sulphate* does not crystallise; the *acid salt* gelatinises, as the solution cools, and the jelly shows but slight signs of crystallising, even after standing several weeks. The *acid oxalate* and *acid tartrate* are very sparingly soluble. Neutral cryptopine *meconate*, $(C_{21}H_{23}O_3N)_2C_7H_4O_7 + 10H_2O$, is insoluble in cold, and but slightly soluble in boiling water, and is probably the form in which the alkaloid exists in opium (*Pharm. Jour.*, 1888 [*iii*], **18**, 250).

Gnoscopine, $C_{22}H_{23}O_7N$, occurs in the mother-liquors of narceine. When recrystallised from boiling spirit the base forms long, thin, white needles, having a wooly appearance when dried.

It may be prepared from narcotine by heating with acetic acid to 130° and Dobbie and Lauder have shown that it gives the same absorption spectrum as narcotine. For method of preparing see also Rabe and McMillan (*Ber.*, 1910, **43**, 800).

More recently Perkin and Robinson (*Proc. Chem. Soc.*, 1910, **26**, 46, 131) have demonstrated that it is racemic narcotine. They separated the *d*- and *l*-varieties by fractional crystallisation of its *d*-bromocamphorsulphonate from acetic ether. By boiling cotarnine and meconin with potassium carbonate in alcohol *di*-narcotine, identical with gnoscopine, was synthesised. The synthisis of cotarnine by Salway (*J. Chem. Soc.*, 1910, **97**, 1208) therefore makes the synthisis of gynoscopine complete. Gnoscopine probably does not exist in opium as such, but is produced from narcotine by racemisation. It melts at 228°, decomposing at the same time, and burns with a smoky flame, leaving a skeleton of charcoal. In pure sulphuric acid, gnoscopine dissolves with slightly yellow colour, which becomes at once carmine-red upon addition of a trace of nitric acid, the colour being permanent. This reaction distinguishes the base from rhœadine, which becomes red with sulphuric or hydrochloric acid alone (*Pharm. Jour.* [*iii*], **9**, 82). Gnoscopine hydrochloride gives a buff-coloured precipitate with platinic chloride.

Hydrocotarnine, $C_{12}H_{15}O_3N$, is formed from narcotine, together with meconin, by the action of nascent hydrogen, or by the hydrolysis of narcotine with formation of opianic acid as indicated on page 358.

It crystallises in prisms containing 1/2 molecule of water and which

melt at 55°. It is insoluble in water and alkalies but readily soluble in alcohol, chloroform and ether. The base may be distilled with little decomposition at about 100°. It forms easily soluble salts and is more poisonous than cotarnine and narcotine. Its constitution is that of a derivative of methyltetrahydroisoquinoline.

Lanthopine, $C_{23}H_{25}O_4N$, is obtained from the mother-liquors left from the preparation of morphine by the Robertson-Gregory process (see page 372). It is a weak base, forming no acetate, crystallising from chloroform in small prisms, melting at about 200°. It is insoluble in alkalies, slightly soluble in alcohol, ether and benzol, but somewhat more readily soluble in chloroform. It is coloured orange-red by nitric acid, and pale violet by sulphuric acid, the latter colour changing to a dark brown on heating. (See also pages 367, 368.)

Laudanine, $C_{20}H_{25}O_4N$, as indicated by the researches of Hesse is *n*-methyl-trimethylpapaveroline. It is found with lanthopine and has been prepared on a commercial scale by Merck from opium mother-liquors, but the yield is only one-third that of cryptopine. Laudanine crystallises from its solution in boiling alcohol in transparent granules or hexagonal prisms melting at 166°. Laudanine is optically inactive, tasteless, and poisonous, the hydrochloride being bitter and resembling strychnine in its effects. It resembles morphine in dissolving in alkali hydroxide solutions, but the sodium-derivative is reprecipitated in glistening white needles on adding excess of alkali hydroxide. From its solution in alkali hydroxide, laudanine is wholly unremoved by chloroform or amyl alcohol, but is extracted if precipitated by ammonia. Its phenolic character is further evidenced by the green colouration yielded with ferric chloride. Treatment with methyl iodide converts laudanine into a base chemically resembling codeine, and distinct from laudanosine. The solution of laudanine in pure concentrated sulphuric acid has only a very faint pink tint; the same acid containing iron yields a slightly deeper tint; but on heating either solution till the acid begins to volatilise, a violet colouration is obtained. With nitric acid, laudanine gives an orange-red colour. Laudanine is a strong base, having an alkaline reaction, and forms well-crystallised salts of a bitter taste. BHI is sparingly soluble in cold water, and BHCl easily soluble in water, but nearly insoluble in brine. (See also pages 363, 367.)

Laudanidine, $C_{20}H_{25}O_4N$, which was discovered by Hesse in 1894, very closely resembles laudanine and is probably its levo-

modification. It has a m. p. of 177° and is levorotatory, Hesse giving $[\alpha]_D = -87.8°$.

Laudanosine, $C_{21}H_{27}O_4N$, the constitution of which is described on page 361, is homologous with laudanine. It is insoluble in water and alkalies but soluble in alcohol, ether, chloroform and benzol, crystallising from the last solvent in needles which melt at 91°. Its solution is strongly alkaline, and it is dextrorotatory.

In alcohol 97%	C = 2	$[\alpha]_D^{22.5} = +105°$.
In chloroform	C = 2	$[\alpha]_D^{22.5} = +56°$.

Both the free alkaloid and its salts are very bitter in taste and are strong tetanic poisons. Laudanosine is best isolated by conversion into its sparingly soluble hydriodide. It gives no colouration with ferric chloride. (See also page 367.)

Morphine, $C_{17}H_{19}O_3N$, has already been fully described.

Meconidine, $C_{21}H_{23}O_4N$ forms a brownish-yellow amorphous mass, melting at about 58°, insoluble in water, soluble with difficulty in ammonia, but readily in alkali hydroxides. The base cannot be removed from its solution in caustic soda by agitation with ether, but is extracted from its ammoniacal and lime-water solutions. Meconidine is alkaline in reaction and nearly destitute of taste, but yields very bitter, unstable salts. It is very easily decomposed by mineral acids, with production of a rose colouration. It is dissolved by strong sulphuric acid with an olive-green, and by nitric acid with an orange-red colour.

Narceine, $C_{23}H_{29}O_9N$, is described as regards its constitution on page 360. This base was originally discovered by Pelletier, who attributed to it m. p 92°, but Hesse found it to melt at 145°. This latter figure, although subsequently corrected by Hesse himself, has been generally adopted by compilers, though Claus and Meixner found 162°; but E. Merck has shown (*Chem. Zeit.*, 1889, 525) that the ordinary commercial alkaloid of English manufacture melts between 150 and 160°, and the pure base at 170–171°.[1] Narceine crystallises from water in long white prisms or delicate needles, containing $3H_2O$, which is driven off at 100°. It has a bitter taste, with styptic after-taste, and the researches of Von Schroder (*Arch. f. Expt. Pathol.*, 1883, 132) indicate that it is the most strongly narcotic of all the opium alkaloids. It is optically inactive. It is very sparingly

[1] Dott states that the m p. is indefinite, as partial decomposition occurs.

soluble in cold water or alcohol, but dissolves very easily on heating. It is but slightly soluble in chloroform, and insoluble in ether, benzene and petroleum ether. Narceine is precipitated on adding ammonia or potassium hydroxide to solutions of its salts, but dissolves in excess of either reagent, and on addition of a large excess of alkali hydroxide is reprecipitated as any oily liquid.

Narceine is a very weak base, the free alkaloid having a very feeble alkaline reaction to delicate litmus; the solutions of its salts may be titrated with litmus just as if the alkaloid were absent. The acetate is decomposed by water, and the base is said to be extracted by chloroform (but not by amylic alcohol) from liquids containing even free mineral acids. BHCl forms needles or short stout prisms very easily soluble in water and alcohol, and melting with decomposition at 163°. Narceine liberated from the hydrochloride or other salts by ammonia retains hydrochloric acid with great persistency, and cannot be purified by recrystallisation from water or dilute alcohol. According to E. Merck (*Chem. Zeit.*, 1889, **13**, 525; *Pharm. Jour.*, 1889 [*iii*], **19**, 1034; **20**, 481) narceine can best be obtained pure by crystallisation from water containing some ammonia or alkali hydroxide, but a considerable quantity remains in permanent solution. For therapeutic purposes, the presence of a small proportion of hydrochloride is of no consequence, and Merck considers that a preparation free from meconin, and so far freed from basic salt as not to melt below 165°, is sufficiently pure.

Chlorine-water, followed by ammonia, gives a blood-red colour with narceine, but many other substances (*e. g.*, tannin) behave similarly. Potassium dichromate gives a crystalline precipitate after some time. Iodine gives a brown precipitate in narceine solutions, but if ammonia be added to remove excess of iodine the precipitate is seen to be blue. Weak iodine solution colours narceine black-blue; in boiling water a colourless solution is obtained, but the crystals formed on cooling have a violet or blue colour. Sulphuric acid containing iodic acid gives with narceine a black colouration changing to red (see also page 365 *et seq.*).

A substance called meconarceine has been described by Merck but it is a mixture of variable composition. (See *Pharm. Zeit.*, 1889, **34**, 90.)

Narcotine,[1] $C_{22}H_{23}O_7N$, occurs in opium in very variable quantity, the usual range being from 1.3 to nearly 11%, but some samples con-

[1] The constitution and decomposition-products of narcotine are described on page 358.

tain traces too minute to be recognised by the usual methods. Narcotine may be extracted from dried opium by ether or benzene, or by the same solvents from the precipitate produced by ammonia in the aqueous solution of opium.[1] It may be separated from narceine by precipitating the solution with excess of ammonia, when the narceine remains in solution.

Narcotine crystallises from alcohol or ether in colourless, transparent, glittering prisms or groups of needles, which melt at 176°, and resolidify at 130°, crystallising if cooled slowly. Above 200° narcotine is decomposed into meconin and cotarnine. It is feebly narcotic, exhibiting poisonous effects only in somewhat large doses (1 5 to 3.0 grm.). The solid base is nearly tasteless, but the solutions are bitter. In the free state narcotine is levorotatory, but the salts exhibit dextrorotation Hesse found the following rotation in different solvents:

In alcohol 97%	C = 0.74	$[\alpha]_D^{22\,5} = -185°$.
In chloroform	C = 2–5	$[\alpha]_D^{22\,5} = -191.5°$.
2 mol. HCl + water	C = 2	$[\alpha]_D^{22\,5} = +47.0°$.
10 mol. HCl + water	C = 2	$[\alpha]_D^{22\,5} = +50.0°$.
2 mol. HCl + alcohol 80%	C = 2	$[\alpha]_D^{22\,5} = +145.5°$.

For a solution in benzene Dott and Peddie found $S_D = -229°$ (when C was 1.5), and for a solution in dilute oxalic acid, $S_D = +62°$.

D. B. Dott has obtained the acetate, sulphate and hydrochloride of narcotine in a crystalline state; but the first of these salts is almost completely decomposed by solution, the base being precipitated and free acetic acid formed. The same reaction occurs when sodium acetate is added to a solution of narcotine hydrochloride (compare page 370). The hydrochloride and sulphate of narcotine are somewhat more stable, their solutions remaining clear even when largely diluted; but they react with litmus just as if the acid were uncombined,[2] and yield the narcotine to chloroform and similar solvents. These facts prove the basic properties of narcotine to be very feebly marked.

Narcotine was at one time used to some extent in India as an antiperiodic in place of quinine because of its lower cost at that time. For an extended paper on its pharmacology see Crawford and Dohme (*Proc. A. Ph. A.*, 1902, 472).

[1] Opium from which the narcotine has been removed in this manner is an article of commerce; it constitutes the so-called deodorised opium of the pharmacopœias.
[2] Narcotine hydrochloride is neutral to methyl-orange (Dott).

Narcotine meconate forms a syrupy solution, which on evaporation dries to a varnish which redissolves perfectly in water.

The alkali hydroxides, alkali carbonates, and ammonia throw down narcotine as a white crystalline precipitate, almost insoluble in cold water and in excess of the precipitants. It may be extracted from the alkaline liquid by chloroform or benzene, or less readily by ether or amyl aclohol. It is practically unaffected by petroleum spirit.

Narcotine is precipitated by the usual alkaloidal reagents, but the reactions are not very characteristic. With potassium thiocyanate it yields a crystalline precipitate readily soluble in acids, even in acetic acid. Iodised potassium iodide precipitates narcotine from extremely dilute solutions. Narcotine may be precipitated and titrated by Mayer's solution.

If a solution of narcotine in dilute hydrochloric acid be treated with bromine, a yellow precipitate is obtained, which dissolves on boiling; by gradually adding bromine-water, and boiling, a fine rose colour is produced, but is readily destroyed by excess of bromine. The reaction is characteristic. With chlorine-water, narcotine gives a yellowish-green colour, turned orange by ammonia. Iodic acid gives no colouration with narcotine. If narcotine be mixed with twice its weight of cane-sugar, and the mixture moistened with strong sulphuric acid, a fine and persistent mahogany-brown colouration is produced, said by M. Robin to be highly characteristic. (See also page 381.)

According to Labat if 0.1 c.c. of a 1% solution of narcotine in 10% sulphuric acid is added to 2 c.c. sulphuric acid (1.84) and then solutions of various phenolic substances added, on warming intense colour reactions are given. Use 0.1 c.c. of the reagent.

Gallic acid (1:20), emerald green turning to blue.

Guaiacol or catechol (1:20), red changing to violet.

Morphine (1:50), violet.

Very similar reactions are obtained by oxidation of narcotine with potassium permanganate which forms opianic acid, then adding alcohol and subsequently treating as above. The colour reactions are due to opianic acid.

For a process to assay opium for narcotine see under Codeine page 393.

Oxynarcotine, $C_{22}H_{23}O_8N$, is contained in the mother-liquors of

narcotine.[1] It forms minute crystals, somewhat soluble in hot water, but little soluble in hot alcohol, and insoluble in ether, chloroform or benzol. By oxidation with ferric chloride it yields cotarnine and hemipinic acid. $BHCl + 2H_2O$ forms crystals.

Papaverine, $C_{20}H_{21}O_4N$, is a weak base of feeble narcotic properties. It is separated from narcotine by crystallisation from a strong solution in oxalic acid, the *acid oxalate* of papaverine (m. p. 196°) being very sparingly soluble. Papaverine crystallises in rhombic prisms or needles, or sometimes in scales, melting at 147°. It is practically inactive; Goldschmidt gives $[\alpha]_D^{15} = +0.11°$ in chloroform where C = 17.8.

The neutral *succinate* forms large tabular crystals melting at 171°, and soluble in hot water; the *benzoate*, triclinic crystals melting at 145°, and soluble in alcohol but insoluble in water; and the *salicylate*, monoclinic crystals melting at 130°. Sulphuric acid containing iodic acid gives with papaverine a purple colour, turning black and green. Dilute solutions of papaverine salts are not precipitated by phosphomolybdic acid. Tincture of iodine, added to an alcoholic solution of papaverine, gives gradually a precipitate of crystalline needles. With potassio-iodide of cadmium, papaverine yields a dense white precipitate.

Several colour reactions of papaverine are described on page 365, but Pictet and Kramers (*Ber.*, 1910, **43**, 1329) have recently shown that many of the colour reactions given by papaverine have been due to cryptopine present as an impurity.

Papaverosine, found by Deschamps (1864) in the dried seed capsules of the poppy, crystallised in prisms, was soluble in alcohol, ether, chloroform and benzene, and formed a gummy hydrochloride. With sulphuric acid it gave a violet colouration.

Porphyroxine, described by Merck in 1837 as the red colouring matter of opium, according to Hesse is a mixture of several bases, one of which is meconidine, and another probably rhœadine, which latter alkaloid also occurs in the capsules and other parts of the red poppy. Kanny Lall Dey (*Pharm. Jour.*, 1882 [*iii*], **12**, 397) states that by treating the aqueous extract of Indian opium with ammonia, or sodium carbonate, and immediately agitating with ether, the ethereal solution always leaves on evaporation a substance (rhœadine?) which,

[1] Oxynarcotine was first isolated in an impure condition by D Brown, from crude narceine This product was purified and analysed by Alder Wright and Beckett

when warmed with dilute hydrochloric acid, gives a rich purple colouration, and he recommends the reaction as a test for Indian opium.[1] With Turkey and Smyrna opium no such reaction is obtained.

Protopine, $C_{20}H_{19}O_5N$, appears to be the most widely distributed of all the opium alkaloids. It is found in very minute quantity in opium, but has been met with also in *Macleya cordata*, *Stylophorum diphylluim*, *Sanguinaria canadensis*, *Chelidonium majus* and in *Corydalis veruyi* to the extent of 0.13%. Protopine resembles cryptopine, but the solutions of its salts have a bitter taste, and do not gelatinise on cooling. In small doses, protopine acts on frogs as a narcotic, and in stronger doses paralyses the muscle-substance and the peripheral ends of the nerves. Upon mammals it has a poisonous action like that of camphor, but differs from it in paralysing the circulating organs. (See also pages 354, 367.)

Pseudomorphine. Oxydimorphine, $C_{34}H_{36}O_6N_2$.[2] This alkaloid is best purified by solution in ammonia, from which it crystallises in colourless leaflets or delicate silky needles containing $3H_2O$ which on heating decompose without melting. It is a very weak base, forming no acetate, and is without action on vegetable colours. It is tasteless and not poisonous. It dissolves readily in alkali hydroxides and milk of lime, but is insoluble in all the ordinary alcoholic and ethereal solvents, as also in water, in dilute sulphuric acid and alkaline carbonates. Its optical rotation is given by Hesse as follows:

1 mol. HCl + water, C = 0.8 to 1.6 $[\alpha]_D^{22.5} = -114.76° + 4.96\ C$
5 1/2 mol. Na_2O + water, C = 2 $[\alpha]_D^{22.5} = -198.9°$.

Its most soluble salt is the hydrochloride, which requires 70 parts of cold water for solution. On adding ammonia, avoiding excess, the alkaloid is precipitated in a crystalline state from the hot, and in a gelatinous state from the cold solution. Hesse finds that when pseudomorphine is mixed with an equal weight of cane-sugar, and strong sulphuric acid (pure) added, a characteristic dark green colouration is obtained, which gradually turns brown (compare test 5, page 381). If the acid contain a minute quantity of iron, a blue colouration changing to green is produced.

[1] Merck repeatedly dips a slip of filter paper in the ethereal solution, allowing it to dry spontaneously after each immersion. The paper is then moistened with hydrochloric acid and exposed to steam, when it will acquire, especially after drying, a more or less distinct rose-red colour.

[2] Pseudomorphine occurs very rarely, having been observed by Hesse in good Smyrna opium only once in 4 years. It may be prepared by treating morphine with oxidising agents of moderate power, such as potassium ferricyanide or dilute permanganate.

In forensic examinations it may be met with as a transformation product of morphine in the body. (See Reichardt, *Ph. Centralh.*, 1908, **49**, 951.)

Rhœadine, $C_{21}H_{21}O_6N$, exists in all parts of the red poppy (*Papaver Rhœas*), and in the ripe seed-capsules of the white poppy. It forms small white prisms, which are tasteless and not poisonous. It melts at 232° and is but slightly soluble in water, ammonia, alcohol, ether, chloroform and benzol. Its solutions in weak acids, avoiding excess, are colourless, but on adding excess of sulphuric or strong hydrochloric acid a purple-red colour is produced. This is destroyed by alkalies and restored by acids, and is so intense that 1 part of rhœadine will colour 10,000 parts of water purple-red, 200,000 deep rose-red, and 800,000 distinctly red, although only a fraction of the base is converted into colouring matter. The colourless solution of rhœadine in acids is precipitated by tannin. On adding potassium iodide to a solution of the acetate, the hydriodide is precipitated as a dense crystalline mass, consisting of microscopic prisms. An aqueous solution of rhœadine becomes red by prolonged boiling, part of the alkaloid being converted into the isomeric base rhœagenine (soluble without colour in acids), and on adding a drop of hydrochloric or sulphuric acid the whole base is decomposed, the solution acquiring a purple-red colour. Cold dilute sulphuric acid converts solid rhœadine into a colourless resinous mass, which soon dissolves with splendid purple colour, changing to dark purple on boiling, and depositing on cooling small prisms which are brownish-red by transmitted and green by reflected light; while the liquid retains rhœagenine equal to 99% of the rhœadine present, together with the colouring matter.

Opium sometimes contains a base which gives the above colour-reactions with sulphuric acid, but it is somewhat doubtful if it is actually rhœadine. (Compare Porphyroxine, page 403.)

Thebaine, $C_{19}H_{21}O_3N$, is described as to its structure and relation to other opium alkaloids on page 356. Thebaine occurs in opium in proportions ranging from 0.15 to 1.0%. It crystallises in silvery scales from dilute alcohol, and in needles or hard quadatic prisms from strong alcohol. It melts at 193° and some observers state that it sublimes at 135°, but Hesse and Dott agree that this is not the case. It is almost insoluble in water and alkalies, soluble in 10 parts alcohol and in 140 parts ether at 10°; readily soluble in chloroform and benzol. It is levorotatory.

In alcohol 97%, C=1 $[\alpha]_D^{22.5} = -216.4°$.
In chloroform C=5 $[\alpha]_D^{22.5} = -229.5°$.

It has a sharp and styptic taste, and is a powerful tetanic poison, producing symptoms resembling those due to strychnine. The fatal dose is smaller than that of morphine. Thebaine gives a reddish-brown colouration with chlorine-water and ammonia. Its other colour-reactions (and its solubilities) have already been described. (See page 363 *et seq.*) For some additional colour tests see Reichard (*Pharm. Centralh.*, 1906, **47**, 623).

Thebaine is stated to be extracted (with some difficulty) by chloroform from its acid solutions; but the statement requires confirmation, as it is inconsistent with the strongly-marked basic characters of thebaine.[1] From narcotine, thebaine may be separated by treating the concentrated acetic solution with excess of basic lead acetate, which precipitates the narcotine only. It may be removed from the aqueous opium extract in the course of a morphine assay, as the almost insoluble thebaine salicylate by the use of sodium salicylate.

Dilute acids readily alter thebaine, converting it into the amorphous base *thebenine*, $C_{18}H_{19}O_3N$, which is sparingly soluble in hot alcohol and insoluble in other simple solvents. When heated to 90°, under pressure, with fuming hydrochloric acid, thebaine yields *morphothebaine*, $C_{18}H_{19}NO_3$, which crystallises from benzol in plates, melting at 190–191°; insoluble in alcohol and ether, slightly soluble in water and easily in alkalies. (See Howard, *Ber.*, 1884, **17**, 527; 1886, **19**, 1596, and Freund, *Ber.*, 1899, **32**, 168.)

Tritopine, $C_{42}H_{54}O_7N_2$, was isolated by Kauder in minute quantity from the mother-liquors of the opium-alkaloid manufacture It resembles morphine and laudanine in being soluble in NaOH solution, but is reprecipitated in the form of an oil by a large excess of the reagent. Tritopine crystallises in characteristic anhydrous, transparent, needle-like plates melting at 182°, easily soluble in chloroform and alkalies, but only slightly in ether or petroleum ether. With sulphuric acid it behaves like laudanine. It appears to be a di-acid base (*Arch. Pharm.* **228**, 419) and closely resembles laudanine, laudanidine and laudanosine

Xanthaline, $C_{20}H_{19}O_5N$, was isolated from opium by T. and H. Smith (*Pharm. Jour.*, 1893 [*iii*], **23**, 793) and by them given the

[1] It is possible that certain thebaine salts are soluble in chloroform (as are those of codeine) and are dissolved as such by agitating their aqueous solutions with chloroform

formula $C_{37}H_{36}N_2O_9$, but Dobson and Perkin have recently investigated it (*J. Chem. Soc.*, 1911, **99**, 135) and give the first mentioned formula. Their work shows its identity with papaveraldine, a product of the oxidation of papaverine by permanganate.

It is a crystalline powder, insoluble in water and alkalies, sparingly soluble in hot alcohol, more easily in benzol and readily in chloroform. It melts at 208° (Dobson and Perkin), 206° (T. and H. Smith), and by reduction with zinc and hydrochloric acid gives *hydroxanthaline*, melting at 137°. Xanthaline forms salts having a yellow colour, hence the name given it. It is not certain whether it is present in opium or is formed from papaverine during the process of extraction.

Opium.

Opium is a gummy mass, consisting of the inspissated juice from the incised unripe fruit-capsules of *Papaver somniferum*, hardened in the air.

Opium is produced in Turkey, Asia Minor, Persia, India, China, and other countries, but Smyrna, Constantinople, or Turkey opium is the only variety recognised by the majority of the pharmacopœias. Persian and East Indian opiums are imported chiefly as sources of the opium alkaloids.[1] Chinese opium is wholly consumed locally.

Opium varies considerably in appearance, composition, and quality, according to its origin and mode of preparation.[2]

Opium is remarkable for the large number of definite, highly complex, crystalline principles contained in it. Of these the majority are alkaloids, a list of which is given on page 354. In addition, opium contains acetic, lactic, sulphuric and meconic acids, the last substance being peculiar to opium. Besides these bodies and the inorganic constituents, opium also contains the indifferent bodies meconin, meconoiosin, and opionin, and a variety of sugar, together with gummy and pectous matters, albumin, wax, fat, caoutchouc, resin, and a humoïd

[1] The variety of poppy cultivated in Asia Minor is said to be the *black*, which usually has purple flowers, and black, though occasionally white, seeds It is said to be usually richer in morphine than that from the *white*-flowering and white-seeded poppy, which is rich in narcotine, and appears to be the only kind cultivated in Egypt, Persia, India, China, and Japan. (For a chemical distinction between Turkey and Indian opium, see pages 403, 404).

[2] The product of Asia Minor entering commerce largely as Smyrna opium, occurs in rounded, irregularly formed, or flattened masses, varying in weight but commonly about 8 ounces to 2 pounds, usually covered with portions of poppy leaves, and scattered over with the reddish-brown chaffy fruits of a species of Rumex. When fresh, plastic and internally somewhat moist, coarsely granular, and reddish- or chestnut-brown, but becoming harder by keeping, and darkening to blackish-brown. Odour strong, peculiar, narcotic; taste nauseously bitter.

acid. Woody fibre and other extraneous matters are also frequently present; but genuine opium is wholly free from both starch and tannin.

The following may be taken as the *general* composition of opium:

	%		%
Morphine	6 to 15, average 8	Fat	1 to 4
Narcotine	4 to 8	Gum and soluble humoïd acid substances	40 to 56
Other alkaloids	0 5 to 2	Insoluble matters and mucus.	18 to 20
Meconin	under 1	Ash	4 to 8
Meconic acid	3 to 8, average 4	Water	8 to 30 average 20.
Peculiar resin and caoutchouc	5 to 10		

Alkaloids.—*Morphine* is the most abundant of the bases of opium, and the most valuable of the constituents. It exists in opium in combination with sulphuric and meconic acid. The various pharmacopœias differ as to their standards for the morphine content of opium, but good gum opium should contain from 9 to 11% of morphine and if dried should range from 12 to 15%. *Good* Smyrna opium deprived of water usually contains from 12 to 15% of morphine, though cakes from the same case are apt to vary considerably; but if the proportion be below 10% on the dry substance, adulteration may be suspected. Egyptian opium is poorer in morphine than that from Asia Minor, the proportion ranging from 6 to 12%, but it contains a larger proportion of narcotine. Persian opium is extremely variable in quality, probably partly in consequence of the practice of mixing it with sugar and other adulterants, though much of it is equal to ordinary Turkish opium. East Indian opium is, as a rule, remarkably weak in morphine, the proportion being sometimes as low as 2 5%, more commonly between 3.5 and 5, and occasionally as high as 8 or 9%. This inferiority is probably partly due to climate and partly to defective methods of collection and preparation.[1] The variety known as "Patna garden opium" is prepared specially for medical use, and contains from 7 to 8% of morphine. In Chinese opium, the proportion of morphine is generally low. French opium yielded Guibourt from 14.4 to 22.8% of morphine, and German from 16.5 to 20%; that from the white poppy containing, according to Biltz, 6.8%(?). Algerian opium from red poppies yielded 10.4 to 17.8% of morphine, and from white poppies 1.5 to 8.5%(?). In the United States opium, the proportions of morphine observed have ranged from 6 to 15%.

The morphine in opium is usually stated to exist in combination

[1] Aubergier states that in one case the product contained 18% of morphine, while the opium from a neighbouring farm, where the collection was made somewhat later, contained only 11%.

with meconic acid, but Dott has shown that morphine ordinarily exists in opium partly as meconate and partly as sulphate.[1] Dohme's investigation (*Am. Jour. Pharm.*, 1891, **73**, 164) led him to conclude that morphine and also codeine and narceine are combined with sulphuric acid in opium, while the meconic acid is partly free and partly combined with narcotine. In some cases traces of acetate and lactate are present.

Narcotine exists in opium in widely varying proportions and often in considerable abundance. Upward of 10% has been occasionally met with. East Indian opium always contains more narcotine than morphine, while French opium sometimes affords neither narcotine narceine, nor thebaine.

The narcotine in opium is generally assumed to be uncombined, as it is readily extracted by treating the original (dried) substance with ether or benzin; but as narcotine is readily removed from the acidified solutions of most of its salts by agitation with a suitable solvent, such as chloroform or benzin, it does not follow that its extraction from opium is due to its presence in a free state. It most probably usually exists as meconate. Occasionally the narcotine resists the action of solvents, unless the sample of opium has been previously treated with ammonia.[2]

[1] *Pharm Journ*, 1884 [iii], 14, 389 This conclusion is based on the following observations: 1 An alcoholic extract of opium contains sulphuric acid, which cannot be in combination with alkaloids, as metallic sulphates are insoluble in alcohol. 2 An aqueous extract of opium contains sulphuric acid in quantity sufficient to combine with the whole of the morphine 3. The same extract contains meconic acid in quantity insufficient to convert all the morphine into meconate 4 The same extract contains inorganic and organic bases with which the sulphuric acid will unite in preference to the morphine, and the remainder of the sulphuric acid will not suffice to combine with all the morphine (See also *Proc Roy Soc Edin*, 1882–83, page 189)

[2] Twelve samples of opium analysed by Flückiger (*Pharm Journ*, 1875 [iii], 5, 845) gave the following analytical results. The proportions of morphine are most probably sensibly below the truth

Description of opium	Ethereal extract, consisting of		Pure narcotine	Morphine	
	Wax	Crude narcotine		Crude	Pure
1 Patna	14 2	10 0	4 0	11 2	8.6
2 Indian (1852–53)	12 7	9 0	6.1	11 2	4 3
3. Akbari	13 5	8 5	5.5	14.2	3.5
4. Behar.	13 0	7 6	4.5	10.6	4.6
5 Malwa	6 5	7 6	4.7	14.4	6.1
6 Synd	9 4	8 0	3 1	.....	3 8
7. Hyderabad	10 7	9 7	5 4	....	3 2
8 Candeish	.....	.....	7 7	.....	6 1
9. Persian	14 8	10.2	6.4	. .	7 1
10. Egyptian	11.5	12.2	8.7	.. ...	5 8
11. Playford, Suffolk (1823)	8 8	9 3	6.0	.....	4.3
12. English (1859)	12 0	11 6	8.1	.. . .	8 3

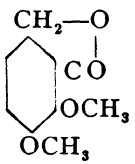
Assays of 38 samples of opium, published by M. Adrian, showed a proportion of mor-

Porphyroxine, according to Kanny Lall Dey (*Pharm. Jour.*, 1882 [*iii*], **12**, 397), is a definite basic substance, always present in Indian opium, but absent from Turkey or Smyrna opium. Dey regards its presence as so constant and characteristic of Indian opium that he utilises it in toxicological investigations.

The *other alkaloids* of opium have been observed in the following proportions:

Codeine, 0.2 to 0.4%.	Narceine, 0.02 to 0 1 (0.7)%.[1]
Codamine, 0.003%.	Papaverine, 1.0%.
Cryptopine, very small.	Pseudomorphine, 0.02%.
Lanthopine, 0.005%.	Rhœadine, minute.
Laudanine, 0.005%.	Thebaine, 0.15 to 1 0% [1]

Meconin, $C_{10}H_{10}O_4$, is produced by the treatment of narcotine with zinc and hydrochloric acid and is a reduction product of opianic acid having the formula



It is found in opium in small quantities and was found also by Freund (*Ber.*, **22**, 456) in *Hydrastis canadensis*. It has been synthesised from guaiacol by Fritsch (*Ann.*, 1898, **301**, 351).

Meconin is an indifferent substance, crystallising in colourless, shining six-sided prisms, which melt under water at 77°, or alone at 102.5° and distil at 155°. It is odourless, bitter, and readily soluble in alcohol and chloroform, but only sparingly in ether. Meconin may be readily crystallised from boiling water, in which it is moderately soluble.

The meconin contained in opium, in which it exists in the proportion of less than 1%, is probably a decomposition-product of narcotine.

Meconin is extracted from its acidified aqueous solution by agitation with benzene, chloroform, or amyl alcohol, the first-named solvent

phine exceeding 7% in all but 2 cases, the average being 10%. The narcotine averaged 2.5%, but bore little relation to the proportion of morphine A sample showing only 3.87% of morphine contained 3 45 of narcotine, while other samples contained over 10% of morphine and only the same percentage of narcotine This variation is doubtless the reason why some samples of opium cause little or no headache and others occasion very disagreeable symptoms

[1] Narceine often occurs more abundantly than thebaine.

being preferable. Meconin dissolves in concentrated sulphuric acid, without at first producing any colouration; but the solution gradually assumes a greenish tint, changing to reddish in the course of 24 hours. If the liquid be then warmed, the colour changes to emerald-green, blue, and purple, finally becoming red. The shades and order of the colours obtained depend much on the proportion of acid used, the tints being bluer and the reaction more delicate with a small quantity. Evaporated with slightly diluted sulphuric acid, meconin gives a green colouration. In concentrated hydrochloric acid it dissolves without change of colour, even on heating. If meconin be dissolved in strong sulphuric acid and a minute fragment of potassium nitrate added, a yellow colouration is obtained, rapidly changing to a fine scarlet, which fades slowly and is changed to yellow on heating. The reaction is delicate.

An aqueous solution of meconin gives precipitates of characteristic microscopic appearance with iodised potassium iodide and a solution of bromine in hydrobromic acid (T. G. Wormley).

Meconoisin, $C_8H_{10}O_2$, was obtained by T. and H. Smith in brown, leaf-like crystalline masses from the mother-liquors left on the isolation of meconin. When pure it is colourless, freely soluble in alcohol, ether, and hot water, fuses at 88°, and on evaporation with somewhat diluted sulphuric acid yields a red colour, changing to purple.

Opionin, according to Hesse, is contained in small quantities in Smyrna opium. It forms white needles which melt at 227° and contain no nitrogen. It is insoluble in water, but dissolves in alkalies, alcohol, and ether. When boiled with milk of lime, opionin is decomposed, an acid being formed which is freely soluble in water and ether, and gives a bulky precipitate with lead acetate in alkaline solution.

Meconic Acid, $C_7H_4O_7 = C_5HO_2(OH):(CO.OH)_2$. This substance is characteristic of opium, in which it exists chiefly in combination with the alkaloids, but sometimes a portion of it appears to be present in a free state.

Meconic acid may be prepared from opium by precipitating the neutralised aqueous solution of the drug with calcium chloride, filtering, and decomposing the precipitate of calcium meconate by repeated treatment with warm diluted hydrochloric acid. A preferable plan is to precipitate the aqueous solution of opium with neutral lead acetate, filter, suspend the precipitate in water, and decompose it with a stream of sulphuretted hydrogen. The filtered and concentrated solution

deposits meconic acid on addition of hydrochloric acid. The product may be purified by re-solution in hot water, cooling, and adding hydrochloric acid. Meconic acid may also be conveniently prepared by precipitating it as the calcium salt, decomposing this with a slight excess of oxalic acid, filtering, and concentrating. See also Valenti (*Boll. Chim. Fann.*, 1905, 44, 373 or *J. Chem. Soc.*, 1905, 88, 788) for method of preparation and a few colour tests.

Meconic acid crystallises in micaceous scales or small rhombic prisms containing $3H_2O$. On being heated to 100°, it loses its water of crystallisation and leaves a white effloresced mass. At 120° it splits up into carbon dioxide and comenic acid, $C_6H_4O_5$, which at a higher temperature again loses carbon dioxide, and forms pyromeconic acid, $C_5H_4O_3$.[1] Comenic acid is but sparingly soluble in hot, and is almost insoluble in cold water. In absolute alcohol it is quite insoluble. Meconic acid dissolves in 115 parts of cold, or 4 parts of boiling water; its solubility in the cold is diminished by addition of hydrochloric acid, which therefore causes a precipitate in strong solutions. When the solution of meconic acid is boiled for some time, especially if hydrochloric acid be present, comenic acid is formed, and crystallises out as the liquid cools. The aqueous solution of meconic acid has a sour, astringent taste, and strongly acid reaction.

Meconic acid is freely soluble in alcohol (distinction from comenic acid) and is deposited in fine crystals on spontaneous evaporation of the solution. It is much less readily soluble in ether, slightly soluable in amyl alcohol, petroleum ether, or carbon disulphide and is almost wholly insoluble in chloroform.

Nitric acid readily acts on meconic acid, much oxalic acid being formed.

Meconic acid derives its chief analytical interest from the fact that it is *strictly peculiar to opium* and its preparations, and hence its positive detection is a decided proof of the presence of a preparation of opium. It is not poisonous.

The microscopic appearance of the precipitates produced in not

[1] The relationship between these 3 bodies appears to be as follows:

$C_5HO_2\begin{cases}OH\\CO.OH\\CO\,OH\end{cases}$ Meconic acid. $C_5HO_2\begin{cases}OH\\H\\CO\,OH\end{cases}$ Comenic acid. $C_5HO_2\begin{cases}OH\\H\\H\end{cases}$ Pyromeconic acid.

Comenic acid forms prisms, minute or granules, insoluble in alcohol, soluble in 16 parts of boiling water, but deposited on cooling

Pyromeconic or pyrocomenic acid contains no carboxyl-group, and its acid characters are very feebly marked It crystallises in prisms, is readily soluble in water and alcohol, melts at 117° and boils at 227°, but sublimes slowly at the ordinary temperature and readily at 100°.

too dilute solutions of meconic acid or soluble meconates by barium chloride, calcium chloride, potassium ferrocyanide, and hydrochloric acid are highly characteristic.

The most characteristic reaction of meconic acid is the formation of a deep purplish-red colouration on adding ferric chloride to the solution of meconic acid or a meconate. The shade of colour is distinctly different from that of the ferric *acetate* or *formate*, and the ferric meconate also differs from these in not being readily destroyed by boiling, or by adding cold dilute hydrochloric acid, and from the ferric *thiocyanate* in being unaffected on addition of mercuric chloride or auric chloride.[1] If any doubt exists as to the presence of an acetate, it is desirable to precipitate the neutralised solution with nitrate or neutral acetate of lead, wash the precipitated lead meconate thoroughly, suspend it in water, and decompose it with sulphuretted hydrogen. After evaporating the filtered liquid at a gentle heat to drive off the excess of sulphuretted hydrogen, the test with ferric chloride may be safely applied. Instead of adding ferric chloride to the solution of meconic acid, the reagent may be applied to the solid substance, as obtained by the evaporation of its aqueous or ethereal solution.

The red colouration produced by meconic acid and a ferric salt is much weakened by oxalic and phosphoric acids, and still more so by metaphosphoric acid.

Comenic and pyromeconic acids also strike a red colouration with ferric chloride, but with the latter acid the colour is less deep.

Meconic acid may be extracted from its acidified solutions by agitation with ether, a property which enables it to be readily separated from morphine, acetic acid, tannin, and other substances liable to interfere with the observance of its reaction with ferric chloride. The extraction is not perfect, even when several times repeated, and hence the method cannot be employed for quantitative purposes.

Meconic acid may be estimated by converting it into a lead salt, or colorimetrically by ferric chloride, by comparing the depths of tint produced by the sample with that obtained by treatment with a known quantity of opium. Very fair approximate estimations of meconic acid, and less accurately of opium, may be made in this way, even when the quantity of material at disposal is very insignificant.

Three of the atoms of hydrogen in meconic acid are replaceable

[1] Thiocyanates (sulphocyanides) exist in sensible quantity in the saliva (and hence in the contents of the stomach) and also in white mustard

by metals, but it is, properly speaking, dibasic, only 2 carboxyl groups CO.OH, being present. The third atom of hydrogen belongs to hydroxyl, and when this is replaced by metals basic salts of a yellow colour result. Astrue reports it as dibasic to most indicators such as methyl-orange but tribasic to Poirrier's Blue.

The metallic meconates are mostly insoluble in water, except the meconates of the alkali-metals. They are nearly all insoluble in alcohol, and are but slightly affected by acetic acid. The salts having 2 atoms of basic hydrogen replaced by metals are neutral to litmus paper.

Acid calcium meconate, $CaH_2[C_7H(OH)O_6]_2$, is precipitated as a sparingly soluble salt of characteristic microscopic appearance on adding calcium chloride to not too dilute a solution of meconic acid or a soluble meconate. In presence of free ammonia, less soluble, yellow, *dicalcium meconate*, $Ca_2[C_7H(OH)O_6]_2$, is precipitated. On treating either of these salts with hot dilute hydrochloric acid, meconic acid crystallises out on cooling.

Iron Meconates.—Ferrous meconate is a colourless, very soluble salt, which turns red on exposure to air. Ferric meconate exists in the purple-red liquid produced on adding a ferric salt to a soluble meconate.

Lead meconate is obtained by precipitating meconic acid or a meconate (or an aqueous solution of opium) with neutral acetate of lead. The triplumbic meconate is stated to be formed even in presence of excess of meconic acid, but it is more probably a mixture or compound of the normal meconate, $PbC_7H_2O_7$, with lead hydroxide. The precipitate is quite insoluble in cold and boiling water, and is not affected by acetic acid.

Morphine meconate has already been described (page 378).

Action of Solvents on Opium.

The action of different solvents and reagents on opium and its constituents is briefly as follows:

Water dissolves meconic acid readily, as also sulphate, meconate, and acetate of morphine. The morphine is very sparingly soluble in cold water, and narcotine still less so. Narceine is much more soluble than morphine, while the resins, caoutchouc, etc., are insoluble, though certain gummy matters pass into solution.

Alcohol dissolves free morphine as well as the acetate, meconate, and sparingly the sulphate. The other alkaloids of opium, as also the resin and caoutchouc, are dissolved by alcohol.

Amylic alcohol dissolves all the alkaloids of opium, if in a free state. The resin also is slightly soluble in amylic alcohol.

Ether, benzene, and carbon disulphide dissolve only about .05% of free morphine, but the other free alkaloids of opium more readily. These solvents also dissolve the caoutchouc, but not the resin.

Acids dissolve all the alkaloids from opium, together with a resinoid substance.

Fixed alkalies, used in excess, dissolve morphine freely, while narcotine remains insoluble. Lime water dissolves morphine, but is a solvent for narcotine only in presence of morphine. The resin of opium is partly soluble in alkalies.

Ammonia dissolves morphine sparingly, narceine and codeine readily, while the other alkaloids and the resin of opium are insoluble.

From the foregoing statements, the arrangement of which is mostly due to E. L. Cleaver (*Year-book Pharm*, 1876, page 502), it follows that an *aqueous solution* of opium will contain sulphate and meconate of morphine and other alkaloids, calcium salts, meconic acid, extractives, and resinous matter.

An alcoholic solution will contain, in addition to the above, free narcotine, caoutchouc, fat, and resin.

Opium which has been exhausted with water still retains a bitter taste, but this is probably due to narcotine, as it is removed by carbon disulphide, benzene, or ether, in which morphine and its salts are insoluble. Water, even when cold, may be depended upon to dissolve the whole of the morphine from opium, if the resultant solution be distinctly acid. In some processes of assaying opium, the sample is subjected to a preliminary treatment with *benzin*, *chloroform* or *ether* to remove narcotine, caoutchouc, and colouring matter. By this means the subsequent exhaustion with water is much facilitated, and a purer solution of morphine is obtained. In presence of much narcotine, morphine is soluble in benzin, but this is not true of the sulphate, meconate, or other *salts* of morphine. Hence there is no loss of morphine on extracting opium with benzin. Meconate of morphine is, however, freely soluble in a mixture of alcohol and chloro-

form; but the simultaneous presence of ether prevents its solution more or less completely.

Adulterations and Assay of Opium.

Opium is liable to a variety of adulterations, some of which are of a very gross kind. Sand, clay, ashes, stones, shot, bullets, lead turnings and other make-weights are occasionally met with. Sugar, gum tragacanth, pulp of apricots and figs, pounded poppy-capsules, and other vegetable substances of a saccharine, mucilaginous, and resinous nature are also employed. Aqueous extracts of poppies and of *Glaucium luteum* are said to be sometimes added in Turkey, though rarely if ever seen in the opium imported into England. Such adulterants are indicated by the darker colour and hygroscopic character of the product, by the difficulty in filtering the solution, and by the continuous streak which the sample leaves when drawn across a sheet of paper, whereas good opium makes an interrupted mark.

The proportion of *ash* yielded by opium should not exceed 8%. The proportion of *water* in opium averages about 20%, the usual range being from 15 to 28%. It is best determined by taking a known weight of the opium in thin slices, and noting the loss in weight on drying at 100°.

The *extract* of opium is determined by exhausting the dried sample with cold water, and collecting, drying, and weighing the residue; or evaporating the whole or an aliquot part of the solution to dryness, and weighing the extractive matter left. Should the insoluble residue exceed 40 to 45% of the *dried* sample (equal to a minimum of 55% of extract), the presence of sand, clay, or other insoluble (mineral) adulterants is probable; while if the residue is below this proportion the presence of sugar, gum, or other soluble impurity is indicated.[1]

[1] According to Hanbury and Flückiger, *dried* opium from Asia Minor should yield from 55 to 66%—generally more than 60—of extractive matter soluble in cold water, the proportion of extract from Indian opium being from 60 to 68%

The following are determinations by D B Dott (*Year-book Pharm*, 1876, page 498) of the leading constituents of 18 samples of opium, purchased from druggists of good standing in London, Dublin and Edinburg. The aqueous extract was determined by subtracting the sum of the water and insoluble matter from 100 00. The proportion of morphine calculated on the dried opium averaged 11.06%. The proportion of morphine in the dry extract was 18.3%. (Compare page 418.)

Hager recommends the following additional tests for the purity of opium: 25 grains weight of the previously dried sample is triturated with half an ounce of boiling water, when the formation of a stiff paste will indicate the presence of starch, flour, gum, etc. 2 ounces of water should next be added and the liquid filtered. If the filtrate be brown or of a deeper colour than "wine-yellow," the presence of liquorice or other vegetable extracts is probable. The liquid should have an acid reaction, or admixture with chalk, litharge, or ashes may be suspected. The liquid should give no reaction with potassium ferrocyanide (heavy metals), and if evaporated to 1 ounce and treated with twice its measure of alcohol no precipitate should be produced (indicative of adulteration with gum or certain salts).

On agitating powdered opium with chloroform, any starch or mineral adulterants will settle out, and may be weighed and further examined microscopically and chemically.

When moist, opium is very liable to become mouldy, and hence should be dried at a moderate temperature and carefully preserved from the air. If kept in a damp condition, fungoid growths soon make their appearance, and gradually diminish and destroy the aroma of the opium, besides materially reducing its alkaloidal value.

Estimation of Morphine in Opium.

By far the most important item in the examination of opium is the estimation of the morphine present. The proportion of this con-

Description of opium	Percentage composition			% of morphine (hydrated)
	Water	Insol residue	Aqueous extract	
1 Turkey	19 6	32 60	47 80	10 75
2 Turkey	20 0	28 85	51 15	12 30
3 Turkey	26 0	25.95	48 05	10 20
4 Turkey	21 2	23 70	55 10	7 57
5 Turkey	22 0	30 95	47 05	9 60
6 Turkey	18 4	25 45	56 15	11 69
7 Turkey	19 2	25 90	54 90	12 30
8 Turkey	20 4	34.20	45 40	12 30
9 Turkey	27.2	35.80	37.00	6 76
10. Turkey	21.2	38 80	40 00	9 80
11 Turkey	22 8	29.70	47 50	8 85
12. Turkey	31.2	47 90	20 90	6 93
13 Persian	14 0	26 80	59 20	6 00
14. Persian	12.0	27 40	60 60	8 50
15. Persian	16 0	25 90	58 10	2 10
16. Malwa	15.2	*24.10	60.70	7 30
17. Malwa	13.6	25.20	61.20	5 88
18. Egyptian	14 8	28.30	56 90	7 00
Average	19 70	29 86	50 44	8 88

stituent varies considerably, as already stated; but dried and powdered opium intended for medicinal use should assay not *less* than 10%.

The assay of opium for morphine has received much attention, the investigators being very numerous and the bibliography very extensive. The accurate estimation of morphine in opium is attended with peculiar difficulties, and many of the processes which have been published give little better than rough approximations to the truth, especially when employed for the assay of abnormal samples. Because of the extraordinary extent of the literature on the assay of opium it is impossible to do more here than give the details of a few of the most prominent methods and refer in brief to some other of the most important work on the subject. Even then many things of interest will of necessity be omitted.

The sampling of opium for the purpose of analysis is doubtless often responsible for varying reports which may be made on the same lot and for the purpose of analysis this is not always an easy operation nor is it conducted on a uniform plan. J. B. Nagelvort recommends that small slices be cut by a knife from the interior of each lump and a lot of these pieces mixed together and 10 grm. given for the estimation of moisture. The remainder is dried, pulverised and the morphine estimated in it.

Squibb recommends a more desirable form of sampling. Every fifth lump from a case of opium, except the very small lumps, and every tenth lump of these, is separated for sampling. A cone-shaped piece is cut from each of these lumps, the apex to come from near the center of the lump. From the side of each cone a narrow strip is cut, taking about an equal proportion from its whole length, so as to give a proper relation of quantity from the dry exterior and the moist center. These strips are collected together in a cone so as to lose but little moisture by drying and the cones returned to the lumps. This sample which need not exceed 25 to 30 grm for a case of opium is then used for the determination of moisture and morphine.

The various pharmacopœias differ somewhat in their standards for opium, that of the *United States Pharmacopœia* being not less than 9% crystallised morphine in the gum opium and 12 to 12.5% in dry powdered opium. The *British Pharmacopœia* requires 9 5 to 10.5% anhydrous morphine in powdered opium, while the *German Pharmacopœia*, 1910, and the last *Austrian Pharmacopœia* require 10% anhydrous morphine in powdered opium. The French Codex of 1908

gives a standard of 10 to 11% morphine in powdered opium and the *Japanese Pharmacopœia* adopts the same standard. The Brussels Conference adopted a standard of 10% morphine for opium dried at 60°.

The methods of assay differ considerably and the process of the *United States Pharmacopœia*, *British Pharmacopœia* and the *German Pharmacopœia* are given in detail on the following pages. The Austrian process is nearly identical with the German, and the official French and Japanese processes are likewise nearly identical, both being lime and ammonium chloride precipitation, which are not given in detail as a similar and rather preferable process is referred to in detail under the title of the Stevens method.

The *United States Pharmacopœia*, Eighth Revision, uses the following method of assay which is practically identical with Squibb's revised process (Ephemeris **3**, 1152), this being based upon the earlier method of Fluckiger (*Archiv. Pharm.* [*iii*], **27**, 721, 769; *Pharm. Jour.* [*iii*], **20**, 588) and which is somewhat similar to that proposed by Teschemacher and Smith (*Chem. News*, **57**, 93, 103), the outline of which process may be still further traced to one originally devised by Prollius.

"Introduce the opium (which, if fresh, should be in very small pieces, and if dry, in very fine powder) into an Erlenmeyer flask having a capacity of about 300 c.c., add 100 c.c. of distilled water, stopper the flask, and agitate it every 10 minutes (or continuously in a mechanical shaker) during 3 hours. Then pour the contents as evenly as possible upon a wetted filter having a diameter of 12 cm., and, when the liquid has drained off, wash the residue with distilled water, carefully dropped upon the edges of the filter and its contents, until 150 c.c. of filtrate have been obtained. Then carefully transfer the moist opium back to the flask by means of a spatula, add 50 c.c. of distilled water, agitate it thoroughly and repeatedly during 15 minutes, and return the whole to the filter. When the liquid has drained off, wash the residue, as before, until the second filtrate measures 150 c c, and finally collect about 20 c.c. more of a third filtrate. Evaporate carefully in a tared dish, first, the second filtrate to a small volume, then add the first filtrate, rinsing the vessels with the third filtrate, and continue the evaporation until the residue weighs 14 grm. Rotate the concentrated solution about in the dish until the rings of extract are redissolved, pour the liquid into a tared Erlenmeyer flask having a capacity of about

100 c.c., and rinse the dish with a few drops of water at a time until the entire solution, after rinsings have been added to the flask, weighs 20 grm. Then add 10 grm. (or 12.2 c.c.) of alcohol, shake the flask well, add 25 c.c. of ether, and repeat the shaking. Now add 3.5 c.c. ammonia water (10%) from a graduated pipette or burette, stopper the flask with a sound cork, shake it thoroughly during 10 minutes, and then set it aside, in a moderately cool place, for at least 6 hours, or over night. Remove the stopper carefully, and should any crystals adhere to it, brush them into the flask. Decant the ethereal solution as completely as possible through 2 counterpoised 7 cm. filters. Add 10 c.c. of ether to the contents of the flask, rotate and again decant the ethereal layer upon the filter. Repeat this operation with another 10 c.c. of ether, then pour the aqueous liquid together with the crystals on the filter, and when the liquid has passed through transfer the remaining crystals to the filter by washing the flask with several portions of water, using not more than 15 c.c. in all. A feather or rubber-tipped glass rod may be used to remove adhering crystals from the glass. Allow the filter to drain, then wash with water drop by drop until practically free from mother-liquor, afterward with alcohol previously saturated with powdered morphine, applied drop by drop. When this has passed through displace the alcohol by ether, using about 10 c.c. Allow the filter to dry in a moderately warm place at a temperature not exceeding 60° until its weight is constant. Transfer the crystals into a tared watch glass and weigh. Treat the impure crystals in a flask with lime water (10 c.c. for each 0.1 grm. of morphine) and shake at intervals during half an hour. Filter through a counterpoised filter, rinsing the flask with more lime water and washing the filter until the filtrate, after acidifying, gives no precipitate with Mayers' reagent. Press the filters until nearly dry between bibulous paper and dry to constant weight, then weigh contents, using the outer filter as a counterpoise. Deduct the weight of insoluble matter on the filter from the weight of the impure morphine previously found. The difference multiplied by 10 represents the percentage of crystallized morphine in the opium."

Various minor modifications of this method have, from time to time, been proposed, and special emphasis is laid by different analysts on certain points in the manipulation. On the whole, the process is a fairly good one though somewhat tedious, its chief defect being that results obtained by it are not always concordant.

The morphine obtained by the precipitation is of very variable purity but the lime water purification largely corrects this. The quantity of morphine precipitated is affected by the temperature, time of standing, and length and violence of shaking. The time is specified, but the temperature is not defined with any certainty and the thoroughness of shaking probably accounts in considerable measure for discrepant results of different analysts.

J. U. Lloyd (*Proc. A. Ph. A.*, 1906, 454) states that the method is reported to him by chemists in the Orient, as not giving results sufficiently high, but these opinions are not borne out by American investigators.

Teschemacher and Smith digested the precipitated, dried morphine with benzin to remove narcotine and such other opium alkaloids as might be present and this was also used by Dott (*Pharm. Jour.* [*iii*], **22,** 746). In view of the fact that they both ultimately determined the morphine by acid titration with *litmus* indicator, and as narcotine, narceine and papaverine have no action on litmus and codeine is not only soluble in water, but readily in alcohol and ether, this treatment seems superfluous. The purification with lime water will leave any narcotine undissolved. Bergstrom states that in the presence of narcotine, high results are obtained by titration with *iodeosin*, as this indicator is not wholly indifferent to narcotine. Longer standing has been recommended as giving more morphine, Bergstrom (*Pharm. Centralh* , 1906, **47,** 632) stating that the precipitation of morphine is not complete even after 24 hours, but the higher results are undoubtedly attributable to impurities. Fromme (Caesar and Loretz, *Rep.*, 1904, 55) found that in precipitating morphine by ammonia in presence of ether after 24 hours' standing, calcium meconate was present and in one case a lithium salt was also found. Later (*Pharm. Zeit.*, 1907, **52,** 778) he reported that the difference between weight and titration of precipitated morphine was 0.05% to 0.15% after 10 minutes' standing and about 0.4% after 24 hours' standing

Mallinckrodt and Dunlap (*J. Am. Chem. Soc.*, 1895, **27,** 946) determined the composition of meconates contaminating morphine obtained by the *United States Pharmacopœia* 1890 process which differs but little from that of the *United States Pharmacopœia*, Eighth Revision, except in the final purification, and found that it was largely *calcium-ammonium meconate*, $CaNH_4C_7H_4O_7$. This salt requires more acid for neutralisation than does morphine, so titration by $N/10$

acid does not show correctly the purity of the precipitated morphine. For this reason the lime water purification is preferable.

Dohme (*Bull.* 99, *Bureau Chem., U. S. Dept. Agric.*, page 162) recommends the following modifications of the *United States Pharmacopœia* assay:

Proceed as directed by the pharmacopœia to precipitation of morphine. To the 20 grm. of aqueous extract add 60 grm. of alcohol, cork flask, shake well for 1 minute, and set aside for 30 minutes, during which time the precipitated material should have completely subsided. Decant the clear supernatant liquid into a tared 250 c.c. evaporating dish, transfer the precipitate to a 7 cm. filter previously moistened with a mixture of alcohol (3 parts) and water (1 part). The last portions of the residue are transferred to the filter by using small portions of the above hydro-alcoholic solution. The filtrate is to be collected in the tared evaporating dish. Continue washing the residue and filter by dropping the alcoholic solution on the filter and the residue until the filtrate is no longer bitter. Add 35 c.c. of water to the contents of the evaporating dish and evaporate on water-bath to 14 grm., then proceed as directed by the *United States Pharmacopœia.* Lamar (*ibid.*) prefers the following:

Place 10 grm. of the opium in a flask provided with a condensing tube and heat with 50 c.c. of 75% alcohol for about 10 minutes. Decant the fluid through a filter into a porcelain dish and extract the opium once more with 50 c.c. of the same alcohol. Filter, put filter back in the flask, heat the contents of the flask with 30 c c. of the alcohol, filter again, and wash filter and residue with about 20 c.c. of the alcohol.

The combined alcoholic solutions are evaporated to a thin syrup, and then water is added until no more resin is precipitated. The aqueous fluid is filtered and after thorough washing evaporated to about 15 grm. Then proceed as given under opium in the *United States Pharmacopœia.*

The results obtained by 9 analysts and reported by Kebler (*ibid.*) indicate that the Lamar modification, though being somewhat more complicated, gives the best results and is to be preferred. A quite similar method is recommended by A. B. Lyons (*Bull. A. Ph. A.*, 1909, **4**, 412). He also calls attention to the need of care in the treatment of the morphine with lime water to avoid absorption of carbon dioxide (*Proc. A. Ph. A.*, 1906, 465).

Parker (*ibid.*, 1907, 490, 497) suggests a method using lead subacetate to remove colouring matter and extractive.

British Pharmacopœia Process.[1]—This method of assay is based on: The conversion of the resinous matters of opium into insoluble lime compounds; the decomposition of the morphine meconate with formation of insoluble calcium meconate, the solubility of the resultant free morphine in lime-water; the decomposition of the solution by ammonium chloride, with formation of calcium chloride, ammonia, and free morphine; the use of alcohol to dissolve impurities, and of ether to promote the crystallisation of the alkaloid; and the collection, washing, and weighing of the morphine thus obtained. The following are the details of the process as laid down in the *British Pharmacopœia* of 1898:

"Take of powdered opium, dried at 100°, 14 grm.; lime, freshly slaked, 6 grm.; chloride of ammonium, 4 grm.; alcohol, ether, distilled water, of each a sufficiency. Triturate together the opium, lime, and 40 c.c. of distilled water in a mortar until a uniform mixture results; then add 100 c c. of distilled water, and stir occasionally during half an hour. Filter the mixture through a plaited filter about 10 cm. in diameter into a wide-mouthed bottle (having the capacity of about 300 c c., and marked at exactly 104 c.c.) until the filtrate reaches this mark.[2] To the filtered liquid (representing 10 grm. of opium) add 10 c.c. of alcohol, and 50 c.c. of ether, and shake the mixture; then add the chloride of ammonium, shake well and frequently during half an hour, and set it aside for 12 hours.[3] Counterbalance 2 small filters; place 1 within the other in a small funnel, and decant the ethereal layer as completely as practicable upon the inner filter. Add 20 c.c. of ether to the contents of the bottle and rotate it; again decant the ethereal layer upon the filter, and afterward wash the latter with 10 c.c. of ether added slowly and in portions. Now let the filter dry in the air, and pour upon it the liquid in the bottle in portions, in such a way as to transfer the greater portion of the crystals to the filter.

[1] This method was originally devised by Portes and Langlois (*Chem News*, 45, 67), and with slight alterations was adopted by the Société de Pharmacie of Paris, and made official in the *United States Pharmacopœia* of 1880. It was further improved by M. Conroy (*Pharm. Jour* [iii], 15, 473), and adopted as the official test in the *British Pharmacopœia* of 1885. With some further modifications, especially in the increase of the quantity of opium used and the titration of the morphine obtained for purity, the method as given here was adopted in the 1898 edition.

[2] The additional 4 c c is intended as an allowance for the average increase in the volume of the liquid caused by the extractive matter of the opium.

[3] "The use of an excess of ether, much beyond ether-saturation, so as to cause an ethereal layer to rise above the crystallising liquid, along with the frequent shaking up of the ether with the aqueous liquid in the closed flask during crystallisations, marks an important advance in opium assay"—(A B Prescott.) The practice has been adopted in all recent methods of assaying opium.

"When the fluid has passed through the filter wash the bottle and transfer the remaining crystals to the filter with morphineated water.[1] Wash crystals with morphinated water until washings are free from colour. Allow the filter to drain and dry it first by pressing between sheets of bibulous paper and afterward at a temperature between 55 and 60° and finally at 110° for 2 hours. Weigh the crystals in the inner filter, counterbalancing by the outer filter. Take 0.5 grm. of the crystals and titrate with $N/10$ sulphuric acid until the liquid after boiling slightly reddens blue litmus paper. 1 c.c. $N/10$ sulphuric equals 0.0283 grm. anhydrous morphine. The weight of the pure anhydrous morphine indicated by the titration plus 0.104 grm. (representing the average loss of morphine during the process) should amount in total to 1 grm.; that is to say to a total of not less than 0.95 grm. and not more than 1.05 grm., corresponding to about 10% of anhydrous morphine in the dry powdered opium."

The method of measuring the filtrate in a wide-mouthed bottle is decidedly objectionable and could well be improved. The method of titration would also be preferable if a definite weight of crystals were dissolved in excess of $N/10$ sulphuric acid and the excess titrated with $N/10$ alkali using methyl orange or cochineal as an indicator.

This process is tolerably simple and rapid and when carefully executed gives fairly constant results.

As suggested by Conroy and proved by Braithwaite and Farr the time allowed for precipitation of morphine may be reduced from 12 to 2 hours without affecting the accuracy of the results, but it is safer to allow 6 or 8 hours to elapse before filtering. The titration of the precipitated morphine was not practised in earlier editions of the *British Pharmacopœia*, though directed by Portes and Langlois, the original proposers of the method (*Jour. Pharm. et Chemie*, November, 1881).

For comments on the correction factor given see Braithwaite and Farr (*Pharm. Jour.* [*iii*], **17**, 398) and Smith (*Chem. News*, **57**, 93, 103).

Farr and Wright (*Pharm. Jour.* [*iv*], **27**, 164) comment extensively on this process and commend it in general, but criticise chiefly the large quantity of filtrate used for the assay. Dott (*Pharm. Jour.* [*iv*], **27**, 78, 356) discusses various details of the process and recommends some changes though approving the process as a whole.

[1] Prepared by digesting pure morphine in chloroform water for 7 days at 15° with occasional agitation to obtain a saturated solution and filtering off any undissolved morphine.

Dowzard (*Pharm. Jour.* [*iv*], **17**, 909, and **18**, 397) uses hot digestion of opium with water for 1 hour at 80–90° and then after adding the slaked lime shakes for 2 hours. He increases the speed of the process by titrating the washed crystals of morphine without previous drying. The amount of opium used is less than in the *British Pharmacopœia* process and the necessity of using different corrections than those of the *British Pharmacopœia* in case of the tincture and liquid extract is pointed out, as the *British Pharmacopœia* gives low results.

A closely related method is that of A. B. Stevens which was considered for adoption in the *United States Pharmacopœia*, Eighth Revision, either in its original or slightly modified form, as being a very desirable form of the lime process. The method as modified by doubling the quantities throughout is as follows:

Take 8 grm. of dried and finely powdered opium and triturate in a mortar with 4 grm. of fresh oxide of lime (not air slaked) and 20 c c. of water until a uniform mixture results. Add 38 c c. of water and stir frequently for half an hour. Filter through a dry 10 cm. filter and transfer exactly 30 c.c. to a 120 c.c. flask. To this add 8 c c. of alcohol and 20 c c. of ether and shake the mixture. Add 1 0 grm. ammonium chloride, shake well and frequently for half an hour and set aside in a cool place for 12 hours.

Remove the stopper carefully and preserve, with any adhering crystals, for future use. Pour the ethereal layer into a small funnel, the neck of which has been previously closed with a piece of absorbent cotton. Rinse the bottle with 20 c c. of ether, and when this has passed through, pour the contents of the bottle into the funnel. Without trying to remove all the crystals from the bottle, wash the bottle and contents of the funnel with morphinated water until the washings are colourless. When the crystals have drained, place the funnel in the bottle containing adhering crystals, and with a small glass rod drawn out to a curved point, lift the cotton and rinse the crystals into the bottle with 25 c.c. of $N/10$ sulphuric acid, using the cotton on the end of the rod to detach any adhering crystals. Place the cotton in the bottle, replace the cork and agitate until the crystals are all dissolved. Rinse the cork and funnel with water and titrate the excess of acid with $N/50$ potassium hydroxide.

The number of c.c. of $N/10$ acid consumed by the morphine, multiplied by 1.516, will give the percentage of crystallised morphine obtained. To this add 1.12 for the morphine remaining in the solution.

This method is rapid, easy and gives concordant results. To avoid evaporation it is well to use a flask and glass rod instead of a mortar and pestle and keep the flask closed as much as possible. Objections have been made to it because of the use of an aliquot part of the solution, but if carefully handled this is not a serious defect. See Geisler (*Proc. A. Ph. A.*, 1888, 153), Conroy (*Pharm. Jour.* [*iii*], **15**, 473), Dowzard (*Pharm. Jour.* [*iv*], **18**, 397) and Stevens (*Pharm. Archives*, 1903, **5**, 87).

Ascher (*Am. Jour. Pharm.*, 1906, **78**, 262) claims that ammonium salts in opium may interfere and recommends treatment of opium with a little alkali hydroxide and drying before treating with lime and water.

For additional comments see Stevens (*Pharm. Archives*, 1901, **4**, 81, and 1903, **5**, 41) and Dohme (*ibid.*, **5**, 81).

The process of the French Codex, 1908, is based upon the same general principle and resembles the above process in many respects. Its most important differences are in the use of freshly slaked lime instead of unslaked lime, the peculiar method of facilitating precipitation by rubbing with a glass rod instead of violent shaking and the washing of the morphine precipitate after drying at 100° with benzin. A. and A. Petit (*J. Pharm. Chim.* [*vi*], **21**, 107) recommend practically the same method but use chloroform for washing the morphine instead of benzine. Marcelet and Marcelet in using this process endeavoured to dry the morphine at 60° and found that after 12 hours the greater portion of the water was lost, but after 60 hours there was still a slight loss and the weight remained constant after 122 hours' drying.

A different form of analysis, though resembling it in some particulars, is that suggested by Leger (*J. Pharm. Chim.* [*vi*], **17**, 553). He extracts the morphine with a 2% aqueous solution of sodium salicylate which will eliminate the *thebaine* as an insoluble salicylate and precipitates with ammonia in the presence of ether. He also washes the precipitate of morphine finally with benzin. Very similar is the process adopted by Arkin (*Proc. A. Ph. A.*, 1909, 205) who uses, however, a 10% solution of sodium salicylate for extracting the opium.

Schidrowitz (*Analyst*, 1904, **29**, 144) describes a process which he states gives results very closely approximating those obtained by unpublished processes in use by some of the suppliers of opium. This process invclves the extraction of opium by watei and the subsequent addition of a solution of sodium salicylate to the filtrate. An aliquot

portion of this is subsequently taken for precipitation with ammonia in the presence of ether and the morphine finally separated is dissolved in an excess of $N/10$ sulphuric acid and the excess titrated with $N/10$ alkali, using methyl-orange. He states that the use of *methyl-orange* as indicator is desirable, but Kippenberger does not agree with him and this really seems to be a weak part of the process, as *cochineal* or *iodeosin* would seem preferable.

The *German Pharmacopœia* of 1910 gives the following process for the assay of opium:

Rub 7 grm of powdered opium with 7 grm. of water and then put into a flask, adding sufficient water to make a total weight of 63 grm. Let stand 1 hour with frequent shaking, then filter through a 10 cm. dry filter obtaining 42 grm. of filtrate (=4.88 grm. of opium). To the filtrate add 2 c.c. of a mixture of 17 grm. 10% ammonia water and 83 grm. of water, avoiding strong shaking. Filter at once through a 10 c.c. dry filter. Place 36 grm. of this filtrate (=4 grm. of opium) in a flask and add while rotating 10 c.c. acetic ether and then 5 c.c. ammonia mixture as above. Shake well for 10 minutes, add 20 c.c. acetic ether, let stand 15 minutes with occasional rotation and then pour the acetic ether through an 8 cm smooth filter as completely as possible. Add to the remaining aqueous liquid 10 c.c. more of acetic ether, rotate for a few minutes and pour this acetic ether through the filter. When it has entirely drained through pour on to the filter the aqueous solution and wash flask and filter 3 times with 5 c.c. of water saturated with acetic ether, disregarding any crystals of morphine which may adhere to the sides of the flask. Drain flask and filter and dry both at 100°. Dissolve the morphine crystals on the filter, in the flask and on the cork by means of 25 c.c. $N/10$ hydrochloric acid. Pour solution into 100 c.c. flask, washing filter, flask and stopper carefully with water and make up solution to exactly 100 c.c. Place 50 c.c. of this solution (=2 grm. of opium) in a 200 c.c. flask, add 50 c.c. of water and sufficient ether to make a layer 1 cm. thick. Add 10 drops *iodeosin* solution and titrate with $N/10$ potassium hydroxide, shaking vigorously after each addition. A faint red colour in the aqeuous solution indicates the end point. 1 c.c. $N/10$ hydrochloric acid =0.02852 grm. anhydrous morphine.

The great objection to the process is the use of acetic ether in place of ether, as in the previous edition of the *German Pharmacopœia.* Acetic ether is a fairly good solvent of morphine, and Stevens (*Pharm.*

Archives, **5**, 89) shows the deficiencies of this process in some work upon the extraction of morphine from solutions by acetic ether. See also page 375.

For comparison and criticism of some of the above methods see also Kebler (*Bull.* 90, *Bu. Chem. U. S. Dept. Ag*, page 141) and C. Pape (*Apoth. Zeit.*, **24**, 70, 81, 88 and 99), who there records examination of 11 methods.

Other processes of different character have been devised for the estimation of morphine, such as that of Tickle (*Pharm. Journ.* [IV], **24**, 162) who employs a mixture of 2 parts freshly distilled cresol and 1 part amyl alcohol for extracting morphine from an aqueous solution made alkaline with sodium bicarbonate. The cresol is a better solvent for morphine than amyl alcohol and is of special value in toxicological examinations, as it fixes and eliminates albuminoid extractive matter that otherwise often causes trouble. In the course of his experiments he found a mixture of phenol 2 parts and amyl alcohol 1 part to be the best solvent for extracting morphine from an aqueous solution. Attention is also called to the work of Emery (*U. S. Dept. of Agr.; Bureau of Chem., Bull.*, **137**, 183, and also Eaton, *ibid*, 188.

Reichard (*Chem. Zeit.*, 1901, **25**, 816) endeavored to estimate morphine by means of ammoniacal silver chloride solution, taking advantage of the fact that morphine alone of all the opium alkaloids is said to reduce this to metallic silver. Schidrowitz, however (*Analyst*, 1902, 117), reports the results very unreliable.

An iodometric method based on the formation of morphine tetraiodide, $C_{17}H_{19}O_3N.HI\ I_3$, has been devised by Prescott and Gordin (*J. Am. Chem. Soc.*, 1898, **20**, 724) which has come to be known as the Gordin method. They proceed upon the following plan: The opium in fine powder is treated with a mixture of 5 c c. ammonia water 28%, 5 c c. alcohol, 20 c.c. ether and 10 c.c. chloroform to liberate the alkaloids and dried down with excess of finely powdered salt. The free narcotine, papaverine, codeine and thebaine are then removed by repercolation with benzin after which the morphine is taken out by percolation with acetone in which the morphine is sufficiently soluble. Instead of acetone there may be used pure amyl alcohol boiling between 128° and 132° and leaving no residue on evaporation below the b. p. The acetone or amyl alcohol is then evaporated and the residue taken up with lime water, which completely dissolves and purifies the morphine. This solution is filtered, acidified with hydrochloric acid

and the morphine is estimated as periodide by precipitating with a definite quantity of $N/10$ iodine in potassium iodide, filtering off an aliquot part through a dry filter and titrating the excess of iodine with $N/10$ thiosulphate. Each c.c. $N/10$ I = 0.0095 grm. anhydrous morphine or 0.0101 grm. of crystals. For details it is well to see the original paper.

L. Kieffer, in 1857, described a volumetric process of assaying opium, based on the reactions of the morphine with potassium ferricyanide, reaction of the excess of this salt with potassium iodide, and titration of the liberated iodine with standard thiosulphate (*Ann. Chem. Pharm.*, **103**, 280). Experiments in A. H. Allen's laboratory on this process did not yield encouraging results.

A. D. Thorburn has recently devised a process for estimating morphine, which is as yet unpublished, and is designed for use with powders, tablets, etc. The processes depends on the fact that phenylethyl alcohol dissolves about 5% of its weight of crystallized morphine, and as this alcohol is only slightly soluble in water the alkaloid can be extracted from an aqueous solution of a salt of morphine after rendering it alkaline. The details of this method will shortly appear in the *J. of Ind. and Eng. Chem.*

Extract of opium is now nearly the same strength in the more important pharmacopœias, namely, 20% morphine, but in some cases this is the crystalline and in others the anhydrous alkaloid.

It may be assayed by the same process as for opium, using one-half the amount of sample.

Tincture of opium or laudanum likewise is not identical in various countries, but is usually of 10% strength. The same assay methods as for opium are applicable with such modifications as the difference in strength and need of first removing alcohol by evaporation makes necessary. Hinsdale (*Chem. News*, **62**, 77) has described a simple method of determining the morphine in tincture of opium by observing the depth of the blue or green colouration produced on treating the sample with a freshly prepared mixture of ferric chloride and potassium ferricyanide solutions.

Camphorated tincture of opium, compound tincture of camphor, or paregoric presents a more difficult problem in analysis because of the presence of a very small quantity of morphine and much larger amounts of camphor, benzoic acid, oil of anise and in the *United States Pharmacopœia* preparation of glycerin.

Paregoric is more liable to be deficient in one or more of its constituents than any other preparation of opium and great care is necessary in its analysis to prove any deficiency in the quantity of opium present. The alcohol being the most costly ingredient, there is a strong inducement to the vendor to reduce its amount, a practice which is objectionable because the prescribed proportion of oil of anise cannot be kept in solution in a very weak spirit. Sometimes only traces of oil of anise are present, in which case the tincture remains clear when diluted with 3 or 4 measures of water. The benzoic acid is sometimes deficient in quantity, and occasionally wholly absent, even in the case of tinctures purchased from registered pharmacists. The opium is the most important constituent of *paregoric*, and is apt to be deficient in amount or quality, besides being sometimes wholly omitted. In England a preparation destitute of opium is largely substituted by general shop-keepers for the genuine "paregoric" or "compound tincture of camphor" sold by the druggists. In an instance within the personal experience of A. H. Allen, the opium of paregoric elixir was replaced by henbane. Potassium and ammonium bromides are used in factitious paregoric.

If a measured quantity (25 c.c.) of paregoric be rendered distinctly alkaline with sodium hydroxide, and evaporated to about 10 c.c., the alcohol and a portion of the camphor and oil of anise will be volatilised. On then shaking the liquid with ether, the remaining camphor and oil of anise will be extracted. If the ether be separated, and the aqueous liquid acidified with hydrochloric acid, benzoic acid will in some cases be precipitated; but whether it separates or remains in solution, it should be dissolved out by agitating the acidified liquid with ether. On allowing the separated ethereal solution to evaporate spontaneously in a small beaker, the benzoic acid is obtained in a state fit to weigh;[1] but a better and more rapid plan is to repeatedly agitate the ethereal liquid with water until the washings no longer redden litmus, add a little more water and a few drops of phenolphthaleïn olution, and titrate the liquid with $N/10$ alkali hydroxide, which should be added until the aqueous layer acquires a pink colour, not destroyed by agitation with the ether. Each 1 c.c. of $N/10$ alkali required represents 0 0061 grm of benzoic acid. The meconic acid extracted together with the benzoic acid is too small in quantity to affect

[1] Allen has occasionally observed the benzoic acid thus extracted to have a distinct urinous odor.

the result, but its presence may be detected and the amount roughly estimated by separating the ethereal layer after the titration is complete, and destroying the pink colour of the aqueous liquid by a drop of dilute hydrochloric acid. On now adding a drop of ferric chloride solution, the deep purple-red colouration characteristic of meconic acid will be produced.

To identify the presence of meconic acid Bourquelot (*J. Pharm. Chim.*, 1902, **15**, 342) gives the following reaction: Mix 2 c.c. of tincture with 4 c.c. of water. Add a few drops of hydrochloric acid and shake the mixture with ether. Separate and shake the ethereal extract with 2 or 3 c c. of water and a drop of ferric chloride solution. A red colour is produced in the aqueous layer if meconic acid is present.

The detection of meconic acid in the manner first described of course proves the presence of opium in the tincture. When this information alone is sought, the paregoric may be diluted in a test-tube with proof-spirit till it is of a light yellow colour, and a drop or two of solution of ferric chloride then added If opium be present, more or less deep red colouration will be produced, owing to the formation of meconate of iron. By comparing the depth of red colour with that given by a standard tincture, a rough indication of the proportion of opium present can be obtained; but the amount of meconic acid in opium is too variable to allow of much stress being placed on the result obtained. It sometimes happens that paregoric is coloured with cochineal or contains a variety of tannin, in which case the colouration with ferric chloride becomes obscured. On cautiously adding hydrochloric acid, drop by drop, the colour produced by tannate of iron is destroyed, while that due to the meconate persists till considerably more acid has been added.

The proportion of opium in paregoric is too small to allow of the ordinary method of determining morphine being conveniently used; but fair results, sufficiently accurate for most purposes, may be obtained by volumetric or colorimetric application of the reactions with potassium ferricyanide and iodic acid.

F. C. J. Bird (*Year-book Pharm.*, 1905, 459) gives the following method for the detection and approximate estimation of morphine in a small quantity of paregoric.

"The following process answers well with as little as 2.5 c c. *Tr. Camph. Comp.* (containing rather more than 1 mgrm. morphine), although 10 c c is recommended as the most suitable quantity:

"Compound tincture of camphor, 10 c.c. Evaporate to dryness on a water-bath, take up with dilute alcohol and a minute drop of acetic acid, evaporate again to dryness, and dissolve the residue in 2 c.c. distilled water. 1 drop of this solution tested with Mayer's solution should give a copious precipitate.

"Filter the aqueous solution and wash filter with distilled water. Transfer to a small separator and extract with hot amyl[1] alcohol and a few drops of a saturated solution of potassium carbonate. Separate the amyl alcohol and wash the same with 1/2 c.c. distilled water. Repeat the amyl alcohol extraction twice and evaporate the mixed amylic extracts on a water-bath to dryness.

"The amyl alcohol residue from a genuine tincture is at this stage brownish-yellow, but if no opium is present, nearly colourless.

"Dissolve the amyl alcohol residue in 2 c.c. distilled water and 4 drops of diluted hydrochloric acid. Filter the solution through a tiny filter with a little talc, to remove colour, until perfectly bright, and wash filter with distilled water. Extract the clear aqueous solution in a separator with 4 c.c. hot amyl alcohol and sufficient powdered ammonium bicarbonate to make alkaline and repeat the extraction twice with successive 2 c.c. quantities of hot amyl alcohol. The mixed amylic extracts should be quite colourless and measure 8 c.c. Evaporate 2 c.c. of the amylic extract to dryness in a very small glass basin, concentrating the residue on to one spot; place on a white surface and moisten the residue with a very dilute solution of neutral ferric chloride. A perfectly distinct dirty blue colouration characteristic of morphine should appear. Another 2 c.c. evaporated should afford an orange yellow colour with nitric acid.

"The reactions may be compared with those obtained from 10 c.c. of a known sample of *Tr. Camph. Comp.* carried through the process at the same time, when there should be no difficulty in coming to a conclusion as to the approximate correctness or otherwise of any sample in question. The reactions are also given quite distinctly with the residue from 2.5 c.c. tincture, but when that amount is taken one-fourth only of the quantities of solvent, etc., mentioned in the process must be used throughout."

Allen and Scott-Smith (*Analyst*, 1902, **27**, 345 and 350) call attention to the similarity in colour reactions of the ipecac alkaloids and mor-

[1] It is very important that the amyl alcohol be specially redistilled; 20 or 30 c.c. evaporated in a glass capsule on the water-bath should not leave the slightest residue.

phine, especially when extraction has been made with amyl alcohol and show that in some cases such as cough remedies of unknown constitution suspected to contain morphine, special care must be observed to avoid any confusion. In cough remedies squill and senega may also be present and the most hopeful plan for identifying squill is by the isolation of scillain, while senega is remarkable for the magnificent purple colour which it yields with strong sulphuric acid. The principle that gives the colouration is not extracted by amyl alcohol from either acid or alkaline solutions.

For the estimation of morphine in medicinal preparations, methods applicable to the particular case must usually be devised. If only minute quantities are present some of the colour reactions of morphine may at times be used to advantage.

The colourimetric estimation of morphine is accomplished by Georges and Gascard (*J. Pharm. Chim.*, 1906, **23**, 513) by treating a neutral or faintly acid aqueous solution with iodic acid, and, subsequently, ammonia and the comparison of the colour with that given by a standard solution of morphine hydrochloride. Mai and Rath (*Archiv. d. Pharm.*, 1906, **244**, 300) use iodic acid, and Frödhe's reagent but prefer formaldehyde and sulphuric acid, which reagent will detect 0.03 mgrm. in 1 c.c. of liquid.

From a solution containing glycerin, morphine may be separated by precipitation as periodide, according to Gordin (*Proc. A. Ph. A.*, 1907, 374). This precipitate, after filtering off and washing, can be decomposed by a stream of sulphur dioxide or sodium sulphite and sulphuric acid and the alkaloid then precipitated by potassium carbonate.

A method for estimating morphine, codeine and cotarnine as picrolonates is described by Mathes and Rammstedt (*Z. anal. Chem.*, 1907, **46**, 565) and as it is applicable in presence of sugar it is useful for work on tablets and in some solutions. An $N/10$ alcoholic solution of picrolonic acid is used to precipitate the aqueous solution. For complete precipitation 15 hours' standing is derisable and after filtering off, washing with water and drying at 110° the crystals may be weighed. The form of the crystals is characteristic.

The chemistry of opium smoke is entered into at some length by Moissan (*Compt. Rend.*, **115**, 988) and Gautier (*ibid.*, 992), who carried out interesting researches on it and proved that the quantity of morphine in it is extremely small.

Toxicology of Opium and Morphine.

In whatever form or manner it may be administered, opium is found to act as a typical and powerful narcotic, and in excessive doses is fatally poisonous.

The poisonous effects of opium are essentially due to the morphine contained in it, and the symptoms it produces differ but little from those consequent on the administration of pure morphine, except that there is a greater tendency to convulsions, and in the latter case the effects are usually manifested more rapidly than in the former, generally commencing in from 5 to 20 minutes if the poison has been taken in solution.

After poisoning by morphine or opium, dimness of sight and relaxation of the muscles, with drowsiness and stupor, are usually the first symptoms observed. At first the patient may be aroused without much difficulty, but as time goes on this becomes impossible, the drowsiness passing into complete coma, often accompanied by slow and stertorous breathing, ending in death. In the large majority of cases the pupils are strongly contracted in tne earlier stages; but later, and when a fatal termination is approaching, they are often dilated.[1] They are usually insensible to light. Occasionally, especially with excessive doses of opium, these is vomiting, or even purging. The pulse is at first weak, quick, and irregular, but afterward slow and full.

Poisoning by morphine or opium often closely simulates alcoholic drunkenness, and, in the absence of a smell of opium in the breath or vomit, it is often very difficult to distinguish between them. Coma, due to uremia, apoplexy, or violence, may also be mistaken for poisoning by opium or its preparations.

The dose of morphine necessary to destroy life is extremely variable. Infants and young persons are peculiarly susceptible to opium and its preparations. Death has been caused to infants by 1/8, 1/10, 1/15, and even 1/19 of a grain of opium, as also by a few drops, and even a single drop, of tincture of opium. On the other hand, children have recovered after doses of 1 grain, 5 grains, and 7 1/2 grains of opium, and after 2 teaspoonfuls of laudanum. Half a grain of morphine acetate has proved fatal to an adult; but as a rule, the usual

[1] A. Swaine Taylor mentions a case of opium poisoning in which one pupil was contracted and the other dilated.

inimum fatal dose for an adult may be stated as 1 grain of a salt of norphine, or 7 grains of opium. Personal habit, as in the case of pium-eaters, and idiosyncrasy will of course largely modify the above onclusion.

The *post-mortem* appearances of poisoning by morphine are by no neans well marked. The stomach and intestines usually appear ealthy. If opium itself has been taken, its peculiar and characteristic dour may often be recognised when the stomach is first opened.[1] Congestion of the lungs and brain are most commonly met with; but hese appearances are not invariable and, when they exist, afford no lefinite evidence of opium poisoning. The blood is usually very fluid.

Besides opium itself, morphine and its salts, and the various official reparations of opium (*e. g.*, the tincture and extract), there are various ostrums containing opium, which have not unfrequently been the ause of death; especially in the case of infants, for whom opiates may e regarded as generally dangerous and unsuitable.

Detection of Morphine and Opium.—In cases of suspected poisoning the detection of opium is based, in addition to the recognition of ts smell, on the extraction of morphine and meconic acid in a sufficiently pure form to allow of the production of their characteristic eactions. The following is the usual mode of procedure:

Observe if any smell of opium is apparent. If not, it may become vident on gently warming some of the contents of the stomach. Test small quantity of the strained or filtered liquid with ferric chloride, nd note if any red colouration (characteristic of meconic acid) is roduced.

Next cut up the stomach and any solid contents into small pieces, nd reduce the whole to pulp by beating in a mortar. Mix the roduct with the liquid contents of the stomach, and treat the whole vith alcohol acidified with acetic acid, in sufficient quantity to coagulate the albumin.[2] Keep the mixture warm for some time, with occasional agitation. Then filter or strain from the solid matter.

The filtrate is treated with basic acetate of lead as long as a precipitate is produced, when the liquid is boiled and allowed to cool. When old it is again filtered, and the precipitate washed with cold water. The precipitate contains the meconic acid of any opium present. It

[1] Allen has observed an unmistakable smell of opium in the contents of the bladder 60 ours after death by taking laudanum

[2] Meconic acid adheres very tenaciously to albuminous matters, and hence the precipitate hould be digested with strong alcohol, and the liquid strained and added to the main olution.

should be washed off the filter with water, and completely decomposed by passing a rapid stream of hydrogen sulphide gas. The liquid is next filtered, and concentrated to a small bulk by evaporation at as low a temperature as possible. It should then be placed in a porcelain dish and tested with ferric chloride, which will produce a purplish-red colouration if meconic acid be present. It is necessary to distinguish carefully between the colouration produced by meconic acid and the somewhat similar reactions given by thiocyanates and acetates. This may be affected with certainty as described on page 413.

A very useful indication of the amount of opium present may be obtained by comparing the depth of tint produced by ferric chloride with that obtained on treating a known quantity of opium in a similar way.

The filtrate from the lead precipitate will contain any morphine which may have been present. Separate the excess of lead by passing sulphuretted hydrogen for some time, filter, evaporate cautiously nearly to dryness, add a little water and filter. The filtrate will probably have a bitter taste if morphine (or other alkaloid) be present. Transfer the solution to a stoppered separator, render the liquid alkaline with ammonia or (preferably) an alkaline bicarbonate, and shake with hot amyl alcohol without delay. The amyl alcohol solution is then separated, passed through a dry filter, and either at once evaporated to dryness, and the residue examined by the colour-tests described on page 379, or it is shaken with a little dilute hydrochloric acid, which is then separated and examined for morphine. An estimate of the quantity of morphine present may be obtained from the intensity of colour produced by the iodic acid and ferricyanide tests.

Instead of treating the alcoholic extract of the material under examination with basic acetate of lead, as described in the foregoing process, the method may in some cases be shortened and rendered more delicate by evaporating off the alcohol at a low temperature, taking up the residue with water, filtering, acidifying the filtrate with dilute sulphuric or hydrochloric acid, and agitating with ether.[1] This removes meconic acid, though not perfectly, while phosphates and other interfering matters remain in the aqueous liquid, and if the ethereal layer be separated, evaporated, and the residue treated with hot water, a solution is obtained, which after filtration may be very

[1] After this treatment the aqueous liquid may be rendered alkaline with sodium bicarbonate, and agitated with hot amyl alcohol for the extraction of the morphine.

advantageously used for the application of the ferric chloride test. If preferred, the solution may be treated with lead acetate, and the meconic acid recovered from the filtered and washed precipitate by decomposing it with sulphuretted hydrogen.

The positive detection of meconic acid affords as perfect a proof of the presence of opium as does the recognition of morphine itself; and as the tests for and methods of separating meconic acid from foreign matters are somewhat more satisfactory than those for morphine, and the acid is more stable than the alkaloid, it occasionally happens that the acid may be isolated and positively identified, when morphine cannot be recognised with certainty (especially where ptomaines may be present).[1] The detection of meconic acid of course indicates the pre-existence of actual opium or some galenical preparation thereof, and not morphine or one of its salts. Hence it sometimes enables a useful distinction to be drawn as to the form in which the poison was taken.

Edlefsen uses the following procedure to detect morphine in the stomach contents. Digest the stomach contents on a water-bath with equal volumes of 4% solution of tartaric acid in alcohol, filter and evaporate filtrate carefully to dryness. Dissolve the residue in a little water slightly acid with hydrochloric acid. To 5 c.c. of a reagent made by colouring a 0.5% solution of iodic acid slightly with Malachite Green, add a few drops of this solution and warm on a water-bath. If morphine is present it changes to a lemon-yellow colour and on adding ammonia to an alkaline reaction, to a brown.

Attention is called by Reichardt (*Pharm. Centralh.*, 1908, **49**, 951) to the possibility of morphine being transformed into dehydromorphine (pseudomorphine) in the human organism, which would give somewhat different reactions than morphine. Separation of the two may be effected by their different solubility and the whole of the morphine may be converted into pseudomorphine by H_2O_2 but this reaction requires further study. To distinguish between morphine and pseudomorphine Hashida (*Pharm. Zeit.*, 1908, **53**, 702) employs a mixture of 0.15 grm. sodium molybdate, 10 drops 35% formaldehyde and 30 c.c. concentrated sulphuric acid. Morphine changes from violet to dirty green while pseudomorphine gives a violet and then a stable blue-green, disappearing on adding water.

[1] A H Allen obtained satisfactory proof of the presence of meconic acid in the stomachs of two children exhumed 5 months after death, whereas no positive conclusion could be formed as to the presence of morphine.

The possibility of morphine condensing with the formaldehyde of embalming solutions in forensic examinations is mentioned by Venturoli and Ciacci.

Jörgensen (*Zeit. Anal. Chem.*, 1910, **49**, 484), for extracting morphine from animal organs prefers ether containing 1 to 1.5% of alcohol, but at least 10 extractions are necessary and this does not seem to be a desirable method.

For isolating morphine from animal matter, the following process was used by Rübsamen (*Archiv. Expt. Path. Pharm.*, 1908, **59**, 225) and though criticised by Winterstein (*ibid.*, 1910, **62**, 139) it was shown by Gottlieb and Steppuhn (*ibid.*, 1911, **64**, 54) to be reliable.

The following general process may be outlined as derived from the method which they used. In their experiments they used both aqueous solutions of morphine hydrochloride and bodies of mice to which a similar solution had been added.

In the case of animal matter, extract it with several portions of alcohol and render the combined alcoholic extracts slightly acid with 5 c.c. $N/10$ hydrochloric acid and evaporate. Take up the residue with warm water and coagulate the albuminous matter by adding a little acetic acid and heating. Filter and use the filtrate diluted to about 200 c.c. for the further work. To this solution add $N/10$ sodium hydroxide until it is very faintly alkaline to phenolphthaleïn and then extract with 600 c.c. of chloroform freshly washed with distilled water. After thorough stirring for about 10 minutes, with the constant addition of $N/10$ sodium hydroxide, drop by drop so as to maintain faint alkalinity, as indicated by slight pink colour, the solution is allowed to separate and the chloroform removed. A similar amount of chloroform is used to repeat the operation 4 or 5 times. No more than a faint pink colour must be present at any time, as stronger alkalinity decomposes the chloroform vitiating the results. It is also necessary, from time to time, to add fresh indicator to the aqueous layer as it is gradually taken up by the chloroform. Evaporate the combined chloroform solutions to a small volume in a flask and then transfer it to a dish and evaporate it carefully to dryness. Treat the residue with a measured amount of $N/10$ sulphuric acid, warm slightly and add absolute alcohol, filter the solution if necessary and evaporate to 5 to 10 c.c. This solution is now used for the estimation of the morphine by the Gordin method.

By this process Gottlieb and Steppuhn were able to recover 94 to

99% of the morphine present in aqueous solutions and 91 to 96% from the bodies of mice. They showed that the criticism of Winterstein regarding the accuracy of the Gordin method of estimation did not hold with the small quantities of morphine present.

As a means of identification of morphine and other opium alkaloids, characteristic crystalline forms of some of their salts may sometimes be used and reference may be made here to the very extensive work on the microscopic appearance of such crystals by Howard and Stephenson (*Bul.* 122, *Bu. Chem. U. S. Dept. Ag.*, page 97). For this purpose the characteristic crystals produced by picrolonic acid previously referred to are of value.

A very interesting and comprehensive bibliography of morphine from 1875 to 1896 was compiled by H. E. Brown for a research committee of the American Pharmaceutical Association. (*Pharm. Rev.*, Oct. and Nov., 1897, and *Pharm. Archives*, Jan. and Feb., 1898.)

STRYCHNOS ALKALOIDS.

By CHARLES E. VANDERKLEED.

The seeds of *Strychnos Nux-vomica*, *Strychnos Ignatia*, and some other species of *Strychnos*, a genus of plants belonging to the family *Loganiaceæ*, contain certain alkaloids remarkable for their intensely poisonous properties. Of these, the two which have been most thoroughly investigated are strychnine and brucine, the latter base being the dimethoxy derivative of the former.

Strychnine and brucine occur also in the bark of *Strychnos Nux-vomica*, the so-called "false angustura bark," in the root-bark of *Strychnos-Tieuté* or "deadly upas tree" of Java, and in the wood of *Strychnos colubrina*. The leaves of *Strychnos Nux-vomica* contain brucine but no strychnine, while the root of the West African *Strychnos Ica a* contains strychnine, but no brucine. On the other hand, the seeds of *S. potatorum*, *S. Brachia*, *S. innocua*, *S. Pseudo-quina*, *S. spinosa*, *S. laurina*, and *S. monosperma* contain neither strychnine nor brucine.[1]

The seeds of *Strychnos Ignatia* or "St. Ignatius' beans," which contain on the average about 1.5% of strychnine and about 0.5 of brucine, are sometimes employed for the manufacture of the alkaloids, but the seeds of *Strychnos Nux-vomica*, which contain on the average about 2.5% of alkaloids, the brucine being generally slightly in excess, are more commonly used for this purpose. Both are used for the manufacture of medicinal extracts and tinctures.

STRYCHNINE, STRYCHNIA, $C_{21}H_{22}N_2O_2$.

Strychnine was discovered in the year 1818 by Pelletier and Caventou in the seeds of *Strychnos Ignatia*, and later by the same investigators in the seeds and bark of *Strychnos Nux-vomica*, in which, together with brucine, it occurs in combination with caffetannic acid.

[1] See F. A. Flückiger, "Ueber die Verbreitung der Alkaloïde in der Strychnosarten," *Arch. der Pharm*, 1892, 230, 343.

The chemical constitution of strychnine has not yet been completely disclosed.

For the preparation of strychnine, the ground seeds of *Strychnos Nux-vomica* are commonly employed. These are moistened with hot water to soften them, extracted with hot diluted alcohol, and evaporated to recover the solvent. The resulting aqueous extract is treated with lead acetate solution and filtered, excess of lead in the filtrate precipitated with sodium sulphate, and the filtered concentrated solution precipitated with sodium hydroxide. The so-obtained crude strychnine, mixed with considerable brucine, is dried, extracted with 80% alcohol and evaporated to crystallisation. Most of the strychnine is thus separated from the more readily soluble brucine which remains in solution. The strychnine is purified by boiling with charcoal in 90% alcohol, solution and recrystallising. The mother-liquors serve for the preparation of brucine (see page 464).

Strychnine occurs as a white crystalline powder, or in colourless, anhydrous, four-sided prisms of the rhombic system. Their production on a microscopic scale is of importance in recognising the alkaloid in toxicologic cases. Well-formed crystals of strychnine may be obtained by the gradual addition of water to the alcoholic solution of the free base. Strychnine separates from its solution in benzene in octahedral crystals.

Strychnine crystallised from alcohol has a sp. gr. of 1.359 (E. Schmidt).

Strychnine is odourless and is permanent in the air. On being heated in very small amounts, it melts[1] at 268° without decomposition and imperfectly subliming. In larger amounts it melts with decomposition. On ignition it is consumed, leaving no residue. Its solutions are lævogyrate and have a marked alkaline reaction. It has an intensely bitter taste, perceptible even in solutions of 0.1 of a grain per gallon. Strychnine should be tasted with great caution as it is an exceedingly violent tetanic poison.

Strychnine is sparingly soluble in cold water, requiring about 7,000 parts for its solution, but it dissolves in about 2,500 parts of boiling water. In commercial ether it is very sparingly soluble; in absolute ether, practically insoluble. In cold alcohol of 0.834 sp. gr. it is soluble in about 170 parts; in boiling alcohol of 0.834 sp. gr. it is

[1] Several investigators have placed the m. p. of strychnine at 265–6°. Loebisch and Schoop (*Monatsh f Chemie*, 1888, 9, 858) found its m p. to be 268°, and the author verified this figure in 1902 for inclusion in the *United States Pharmacopœia*.

soluble in 12 parts. The limited solubility of strychnine in cold alcohol is utilised for its separation from brucine, which is readily soluble in the same liquid. Strychnine is soluble in about 6 parts of chloroform, about 160 parts of benzene, and about 180 parts of amylic alcohol. It is readily soluble in a mixture of equal measures of chloroform and ether. It is insoluble in petroleum ether and in acetone.

Strychnine is not sensibly soluble in solutions of the fixed alkali hydroxides, but dissolves somewhat more readily in ammonia. In dilute acids it is readily soluble.

Pure strychnine dissolves without colouration in cold hydrochloric and sulphuric acid and may be heated to 100° with concentrated sulphuric acids without visible change.

The latter treatment converts it, however, into strychnine-sulphonic acid, $C_{21}H_{21}O_2N_2.SO_3H$, a colourless, amorphous mass difficultly soluble in water or alcohol. When heated with sulphuric acid to 150° a water-soluble disulphonic acid is formed (Stoehr, *Ber.*, 1885, **18**, 3429). Strong nitric acid dissolves strychnine with the formation of a yellowish nitrostrychnine; the production of a pink or reddish colouration indicates contamination with brucine.

Bromostrychnine, $C_{21}H_{21}O_2N_2Br$, is obtained on adding bromine water in theoretical quantity to a diluted aqueous solution of strychnine hydrobromide or hydrochloride, and then precipitating with ammonia. Bromostrychnine melts at 222° (*Arch. der Pharm.*, 1890, **228**, 313).

Strychnine Periodide, $C_{21}H_{22}O_2N_2.I_2.HI$, is obtained in violet-coloured crystals on evaporating the alcoholic solution of the precipitate produced by treating an aqueous solution of strychnine sulphate with iodine-potassium iodide solution.

Salts of Strychnine.

Strychnine is a strong monoacid base and readily forms crystallisable, water-soluble salts which are intensely bitter and exceedingly poisonous. The salts of strychnine are mostly soluble in alcohol, but are insoluble in ether, chloroform, benzene, petroleum ether, amylic alcohol, and carbon disulphide. Strychnine may be removed completely from aqueous solutions of its salts by rendering the solutions alkaline with an excess of potassium or sodium hydroxide and" shaking out" with chloroform.

The basic strychnine thus obtained may be estimated either gravi-

metrically or volumetrically by titration with a standard mineral acid, using a suitable indicator (see page 181 *et seq*).

The chromate, ferrocyanide, mercuroiodide, periodide, phosphotungstate, phosphomolybdate and picrolonate are among the most insoluble salts of strychnine. All these forms are occasionally used for the isolation or estimation of the alkaloid. The high insolubility of the ferrocyanide serves to separate the alkaloid from brucine.

Of the several salts of strychnine, the hydrochloride is official in the *British Pharmacopœia;* the *nitrate* and the *sulphate* in the *United States;* the *nitrate* in the *German*, and the *sulphate* in the *French Codex.* The following table indicates the leading characteristics of the principal salts of strychnine:

Salt	Formula	Appearance	Proportion of strychnine	Solubility	
				Water	Alcohol
Hydrobromide ...	$BHBr + H_2O$	Prismatic needles	77%	Difficultly	
Hydrochloride	$BHCl + 2H_2O$	Trimetric prisms or silky needles	82%	1 part in 35 5[1]	1 part in 73[1]
Hydriodide	$BHI + H_2O$	Quadrangular needles	70%	Sparingly	Very sparingly
Arsenate	$BH_2AsO_4 + \frac{1}{2} H_2O$	Acicular crystals or crystalline powder	68 8%	1 part in about 14[1]	
Nitrate	$BHNO_3$	Silky needles	84%	1part in 63[1]	1 part in 120[1]
Sulphate	$B_2H_2SO_4 + 5H_2O$	Prismatic needles	78%	1 part in 48[1]	1 part in 135[1]
Acid sulphate . .	$BH_2SO_4 + 2H_2O$	Long thin needles	71 4%		

Analytical Reactions of Strychnine.

1. On adding to a solution of a soluble salt of strychnine a solution of an alkaline carbonate, ammonia, potassium or sodium hydroxide, or lime-water, strychnine is thrown down as a white precipitate insoluble, or but very slightly soluble, in excess of the precipitant. The precipitate rapidly becomes crystalline. The crystals have a characteristic microscopic appearance, being usually long, rectangular, well-defined prisms. They are well developed if a drop of a dilute

[1] *British Pharmaceutical Codex.*

solution of a strychnine salt (*e. g.*, the nitrate or sulphate) be placed on a slip of glass, and covered with a small beaker rinsed with strong ammonia. After half an hour the beaker may be removed, the drop of liquid covered with a circle of thin glass, and examined under the microscope. If the solution contain extraneous matter, it may be found difficult or impossible to obtain crystals from it.

Solutions of the bicarbonates free from normal carbonates do not precipitate strychnine from solutions of its salts.

2. If strychnine be liberated from the solution of one of its salts by one of the reagents mentioned above, and the liquid (with the suspended precipitate) be at once shaken with an equal measure of chloroform, the alkaloid is readily dissolved by the latter and may be obtained in a solid state by separating the chloroform and evaporating it to dryness on the water-bath. (The agitation of the aqueous liquid with the chloroform should be repeated several times if quantitative results are desired.) From the aqueous liquids containing little solid matter, chloroform separates readily, but if, as is generally the case in practice, there be much extractive matter present, violent agitation is liable to cause the formation of an emulsion which may require many hours to break up into distinctive layers. The use of a mixture of equal volumes of chloroform and ether, in which strychnine is sufficiently soluble to insure its extraction, will oftentimes obviate the formation of such emulsions. The residue left on the evaporation of the chloroform or ether-chloroform solution may be employed for the application of colour-tests and other methods of identification.

3. Most of the general alkaloidal reagents (see page 185 *et seq.*), including platinic chloride, auric chloride, picric acid (Hager's reagent), tannic acid, phosphomolybdic acid (Sonnenschein's reagent), phosphotungstic acid (Scheibler's reagent), potassio-bismuth iodide (Dragendorff's reagent), potassio-mercuric iodide (Mayer's reagent), and iodine-potassium iodide (Wagner's reagent), precipitate even very dilute solutions of strychnine salts. All of the above reagents, with the exception of tannic acid which must be used in neutral solution, should be applied to acidified solutions for detecting the presence of strychnine. Since many other alkaloids give similar precipitates with these reagents, their value lies in demonstrating the absence rather than the presence of strychnine. The usefulness of certain of the reagents mentioned above for isolating, purifying, identifying, or estimating strychnine will be described in subsequent paragraphs.

4. On adding phosphomolybdic acid (Sonnenschein's reagent, to a neutral or a slightly acid solution of the alkaloid, the strychnine is thrown down as a yellowish-white amorphous precipitate. Since the precipitation is practically complete in dilutions as great as 1 part in 10,000, this reagent is very useful in removing strychnine in concentrated form from complex organic liquids and purifying it from extraneous matters. Many alkaloids besides strychnine, however, give similar precipitates. The precipitate should be filtered off, washed with water containing the reagent, then suspended in water and the alkaloid liberated again in free state by decomposing the phosphomolybdate compound with excess of ammonia. The alkaloid may then be extracted with ether-chloroform as in test 2. The precipitate can, however, be directly examined by the colour tests described in test 9.

5. Phosphotungstic acid (Scheibler's reagent, may be substituted for the phosphomolybdic reagent, as it precipitates strychnine from even more highly diluted solutions than does the latter.

6. When potassium ferrocyanide is added to a solution of a salt of strychnine, the ferrocyanide of the base is precipitated as a yellowish-white crystalline powder, very sparingly soluble in cold acidified water. Since the ferrocyanide of brucine is readily soluble, the above-mentioned reagent has been employed for the separation of these alkaloids in the assay of *nux-vomica* and *ignatia* and their preparations. Dunstan and Short first proposed to precipitate the strychnine from a 5% sulphuric acid solution, in which strychnine ferrocyanide is quite insoluble, but Schweissinger found that brucine ferrocyanide is partly precipitated from such a solution. Beckurts and Holtz (*Pharm. Centralh.*, 1887, **28**, 119, and 1889, **30**, 574) substituted hydrochloric acid for sulphuric, and titrated a solution containing about 1% of the mixed alkaloids with a standard solution of potassium ferrocyanide, adding the latter until a portion of the filtered liquid gave a blue stain with paper moistened with ferric chloride solution. 224 parts of the crystallised potassium ferrocyanide represent 334 parts of strychnine. This method of estimating strychnine in the presence of brucine can only be applied to a solution of the pure alkaloids.

7. Strychnine may also be separated from its tolerably concentrated neutral solutions by precipitation with potassium chromate. The test is best applied to a chloroform residue obtained as described in 2.

This should be dissolved in dilute acetic acid, the liquid filtered, if necessary, and evaporated to dryness at 100°. The resultant acetate of strychnine is dissolved in a little cold water and neutral potassium chromate is added to the solution. Strychnine chromate, $(C_{21}H_{22}O_2N_2)_2,H_2CrO_4$, is thrown down as a reddish or yellowish-brown precipitate, soluble in boiling water (1 in 171) and redeposited on cooling in orange-yellow needles. The precipitate is very slightly soluble in cold water (1 in 470), a fact which enables strychnine to be separated from brucine, the chromate of which is more soluble. Potassium dichromate throws down from solutions of strychnine, not too dilute, an *anhydrochromate* of the formula $B_2H_2Cr_2O_7$ as a yellowish-brown crystalline precipitate. The precipitate is not soluble in excess of the reagent or in very dilute acids, and its formation is much facilitated by stirring. It dissolves in 1,800 parts of cold and about 240 parts of boiling water. When recrystallised from boiling water, it forms orange-yellow needles, from hot acetic acid, reddish-yellow octahedra. The chromates of strychnine give the characteristic violet oxidation-product directly on treatment with strong sulphuric acid as described in test 9; or the alkaloid may be obtained in a free state by suspending the precipitate in water, adding ammonia, and agitating with ether-chloroform as in 2.

8. A valuable reagent for the identification of strychnine is a solution of potassium or ammonium thiocyanate. A concentrated solution of a strychnine salt which may be prepared from a chloroform residue as described in 7, is precipitated on addition of this reagent as strychnine thiocyanate, forming beautiful, crystalline, four-sided columns. The reaction may be applied on a microscopic scale by placing a drop of the strychnine solution on a slide, adding a drop of the thiocyanate solution, and examining the separated crystals, which are characterised by their remarkably sharp edges, under the low power of a microscope.

9. When the precipitate obtained by treating an acidified solution of a strychnine salt with iodine-potassium iodide (Wagner's reagent) is dissolved in alcohol and the alcoholic solution is concentrated, strychnine periodide, $C_{21}H_{22}O_2N_2.I_2HI$, crystallises out in the form of violet coloured columns. Under the polarisation microscope these crystals appear analogous to, and have similar optical properties with, *herapathite*. The following is the best method of utilising the reaction for the detection of strychnine. On a microscope-slide place a very small

drop of an alcoholic solution of iodine, and allow it to evaporate. As soon as it is dry add a drop of a solution of strychnine, made by dissolving the alkaloid in dilute acetic acid and adding a drop of sulphuric acid. Add also a drop of alcohol, and allow the mixture to evaporate spontaneously. On examining the residue under the microscope with a Nicol's prism and selenite, but using no analyser, characteristic crystalline structures will be observed. These may take the form of small circular tufts of very fine black needles; of minute dots of a more or less triangular form, exhibiting yellow, pink, and green tints; large triangular crystals of a yellow or green colour, composed of 3 parts radiating from a centre; numerous solid macled prisms, occasionally showing complementary tints; or solid rosettes of 4-, 5-, and 6-sided prisms. In all cases it is desirable to compare the results with those obtained from a minute quantity of strychnine treated in precisely the same manner. The test is said to be sensitive to 1/2,500 of a grain of strychnine.

10. On treating a cold solution of strychnine in concentrated sulphuric acid with an oxidising agent, a deep violet-blue colouration is developed. This changes more or less rapidly through purple and crimson to a bright cherry-red tint, which is somewhat persistent. The colour finally changes to orange or yellow. The rapidity of the change is largely dependent on the nature and quantity of the oxidising agent employed. Various substances have been recommended for the purpose. The following are the most notable:

a. Potassium Dichromate.—This is the favourite oxidising agent with most operators, and the test is perhaps the most widely known of all alkaloidal colour reactions. The change of colour is very rapid with potassium dichromate, and very little of the latter should be used in order not only to retard the reactions as greatly as possible, but to minimise the amount of chromium salt resulting from the reaction, too much of which interferes with the display of colours due to the oxidation of the strychnine.

A useful way of employing dichromate is to precipitate the strychnine by means of it (as in 7), and apply sulphuric acid to the precipitate. This plan has the great advantage of separating brucine, the presence of which interferes with the play of colours.

b. Potassium permanganate may be used in place of potassium dichromate. It gives the reaction with great distinctness, and possesses the advantage over the latter of giving the violet-blue colouration with

strychnine even in the presence of an equal quantity of brucine. On the other hand, care must be taken in employing this reagent not to become confused by the crimson colour which it is liable to give with the sulphuric acid itself.

c. Lead Dioxide (PbO_2).—This oxidising agent acts very well, but the reddish colour of the reagent is liable to be confusing to the operator.

d. Manganese Dioxide (MnO_2).—This reagent, employed in small quantity and finely powdered, is preferred by some operators. It possesses the advantage of producing a very gradual change of colour, and its black colour does not interfere if it be used in very moderate quantity.

e. Sulphuric acid containing 1% of *ammonium vanadate* (*Mandelin's reagent*) produces with strychnine a deep violet-blue colour, changing to a deep purple, and finally to a cherry-red. On diluting the red-coloured mixture with water, there results a rose-coloured solution which remains unchanged for a considerable time.

f. Cerosoceric Oxide (Ce_3O_4).—This oxidising agent, which must be used in somewhat larger quantity than those previously mentioned, possesses the advantage over all of them of being light in colour and giving a colourless reduction product.

The oxidation-test for strychnine is usually performed on the residue left by evaporating to dryness the ether-chloroform with which an alkaline solution of the alkaloid has been extracted. The test may, however, be directly applied to the chromate or phosphomolybdate of strychnine (see reactions 4 and 7). The following mode of operating is best calculated to ensure delicacy and accuracy:

The solution of the strychnine in ether-chloroform may be evaporated in a porcelain dish, or a small portion of the residue may be transferred to the inverted lid of a porcelain crucible. If the quantity of strychnine to be sought for is likely to be very small, the dish should be immersed in hot water, and the solution of the alkaloid allowed to fall slowly into it from a burette or pipette, so that each drop may almost completely evaporate before another arrives. In this manner the strychnine-residue may readily be confined to a very small area, and the after-reactions thus rendered proportionately delicate. When quite dry and cold the residue should be treated with 2 or 3 drops of pure concentrated sulphuric acid, which should be thoroughly incorporated with it by means of a glass rod. The mixture should then be

allowed to stand for 5 minutes in order to note if any colour is produced. Salicin and certain other bodies will cause a red colouration, while some may be more or less charred. If any marked colouration is produced, the dish should be gently heated (not to the b. p. of water) for half an hour, the contents diluted with water, filtered, made alkaline with ammonia, agitated with a mixture of ether and chloroform (as in test 2), and the strychnine recovered by evaporating the solvent. The residue is then again treated with a drop or two of sulphuric acid.

The oxidising agent is then added to the sulphuric acid by dipping a glass rod moistened with the latter into the powdered solid. A moderate quantity only should be used, so as not to obscure the reaction by the colour of the oxidising agent or of its reduction products. On stirring the drop of strychnine solution with the rod dipped in the oxide the blue colouration will become developed. In a minute or so it will be distinctly purple, passing in a few minutes to crimson, and ultimately to a cherry-red, the last tint being persistent for some minutes. Finally the colour will fade to an orange or yellow. The test is exceedingly satisfactory, delicate, and characteristic, but the order of colours is as important as their shades. The reaction is said to be capable of detecting 1/20,000 of a grain of strychnine.

There are but very few substances which at all simulate the reaction of strychnine when treated with sulphuric acid and an oxidising agent, and few indeed of these that are dissolved together with strychnine on agitating the alkaline solution with ether-chloroform. *Salicin*, *santonin*, *piperine*, *solanine*, certain *opium bases*, *cod-liver oil*, and certain *resins* give colours with sulphuric acid alone, but they are extracted from acid solutions by ether and chloroform, and certain of them may also be got rid of by gently heating the liquid as already described. *Aniline* gives no colour with sulphuric acid alone, but coloured products are formed on treating the solution with an oxidising agent. These cannot be mistaken for the oxidation-products from strychnine, for the order of tints is entirely different, commencing, in the case of aniline, with a green, changing to a very persistent blue, and ultimately becoming black. *Colocynth* resin gives a very similar reaction to strychnine, but is readily extracted by agitating the acidified solution with benzene or ether.

It is always *desirable*, therefore, not only to purify the strychnine by extracting it from an alkaline liquid by agitation with ether-chloroform

(see page 445), but to precede this extraction by shaking its acid solution with ether-chloroform for the removal of possibly present substances which would interfere with the oxidation-test. On the other hand, the oxidation-reaction is readily obtained even in the presence of considerable quantities of certain foreign substances. Thus oat-meal, tartar-emetic, and dextrin do not materially interfere with the reaction when the quantity of strychnine is considerable, and sugar of milk does not prevent the reaction at all. Hence the presence of strychnine in hypodermic tablets may be shown directly in the case of tablets which have been prepared with a pure milk sugar vehicle, by crushing the tablet and applying the oxidation-test to a fragment. Cane-sugar, however, is sometimes used in the manufacture of hypodermic tablets and this substance wholly prevents the application of the colour-test. Some drug extractive matters and nitrates act like cane-sugar in this respect, and hence the *absence* of strychnine must never be assumed before the test has been applied to an ether-chloroform residue.

The alkaloids of cinchona, hydrastis, the mydriatic drugs, veratrum, and many other drugs may be found with strychnine in the ether-chloroform residue, but do not interfere with the application of the test if not present in excessive amounts. Morphine in small proportions does not interfere, and the presence of any quantity larger than traces is excluded by its limited solubility in the ether-chloroform.

J. U. Lloyd has pointed out a colour-change which a mixture of morphine and hydrastine undergoes when subjected to the oxidation-test for strychnine. Mixtures of these alkaloids varying in proportion from equal parts to 1 part of hydrastine to 9 of morphine give with concentrated sulphuric acid and potassium dichromate a greenish to violet-blue colour slowly changing to purple, which colour lasts for a half hour or more. The colour does not then pass over into cherry-red and finally into orange or yellow as it does in the case of strychnine, but slowly changes to a dirty greenish-brown. Moreover, as pointed out in the last paragraph, the solubility of morphine in ether-chloroform is quite limited, and it would not be possible, therefore, to extract from a solution containing both hydrastine and morphine, a mixture of there alkaloids in the proper proportions to produce the colour-reaction described above. Hydrastine alone, or with traces of morphine, gives only a red colouration and no blue or violet.

In small proportions brucine exercises no injurious influence on

the oxidation-test for strychnine, but when much is present it interferes in a marked manner. Hence it is safest to separate the strychnine first of all as ferrocyanide or chromate, as described in reactions 6 and 7, or a strong solution of a salt of the alkaloid can be treated with decided excess of ammonia, when the strychnine will be precipitated and the brucine will remain in solution. If a mixture of brucine and strychnine be treated with chlorine-water, the former base dissolves as *dichlorobrucine*, and the residue then gives the colour-reaction perfectly (Beckurts). Brucine can be sought for in the filtrate as described on page 464. In toxicological investigations its presence together with strychnine points to an administration of one of the natural sources of the alkaloids rather than to the use of a purified salt of strychnine. Commercial strychnine and its salts may contain traces of brucine, but not in sufficient amount to interfere at all with the application of the oxidation-test.

Curarine, one of the alkaloids found in Indian arrow poison, gives rise to a series of colour reactions somewhat similar to the oxidation-reactions of strychnine. This alkaloid is readily distinguished from the latter, however, by the fact that a blue colouration is produced on treating it with sulphuric acid alone. Moreover, it is practically insoluble in ether-chloroform, and hence could not be present in the ether-chloroform extraction residues (see page 464).

Certain ptomaines have been reported to produce a blue colour with the oxidation-test. Those which have most closely resembled strychnine were reported by Amthor (*Chem. Ztg.*, 1887, **11**, 228) and by Mecke and Wimmer (*Pharm. Zeit.*, 1898, **43**, 300). The former differed from strychnine in that it did not form a crystalline chromate, picrate, ferricyanide, or thiocyanate, while the latter differed in that it gave a yellowish-red colouration with sulphuric acid alone. Both differed from strychnine in that they were much less bitter in taste and did not produce tetanic convulsions when subjected to the physiological test (see page 454).

Acetanilide produces a crimson to purplish-red colour changing to green, on subjection to the sulphuric acid-chromate test. This, as well as many organic synthetic substances, would be removed by shaking out from *acid* solutions with ether-chloroform.

Many of the above sources of confusion may be avoided by performing the oxidation-test in a manner suggested by H. Letheby, which consists in employing electrolytic oxygen instead of either of the

oxidising agents mentioned on pages 448, 449. The solution of the ether-chloroform residue in a drop or two of strong sulphuric acid is placed in a cup-shaped depression in a piece of platinum foil. The foil is connected with the platinum plate of a single Grove's cell, and a platinum wire connected with the zinc plate of the battery. As soon as the end of this platinum wire is dipped into the drop of acid, the violet colour of the oxidation-product will flash out, and on removing the wire from the liquid the tint will remain. The test may be rendered still more delicate by placing the drop of liquid at the bottom of a porcelain crucible, and momentarily immersing in the liquid 2 platinum wires connected respectively with the zinc and platinum plates of the battery.

11. A dilute alcoholic solution of *picrolonic acid* (dinitrophenyl-methylpyrazolone) quantitatively precipitates strychnine as crystalline strychnine picrolonate, from dilute aqueous, alcoholic, or ethereal solutions of either the free base or one of its salts. Picrolonic acid precipitates also other alkaloids, but the precipitates are characterised by their great insolubility and high m. p. The latter are useful as confirmatory tests of identity. Strychnine picrolonate melts with decomposition at 286°.

12. Bloxam (*Chem. News*, 1887, **55**, 155) is authority for the following series of colour reactions said to be characteristic of strychnine. If strychnine be dissolved in a drop of dilute nitric acid, the liquid gently heated, and a small particle of potassium chromate be added, an intense scarlet colouration is produced. This is changed to brown on the addition of ammonia, and on evaporation to dryness leaves a dark green residue, soluble in water to a green coloured solution which is changed to orange-brown by potassium hydroxide. The solution again becomes green on addition of nitric acid.

13. Malaquin (*J. Pharm. et Chim.*, 1909 [*vi*], **30**, 546) has reported a reaction for strychnine that is said to be characteristic. 1 c.c. of a very dilute solution of a strychnine salt (not stronger than 1:1,000) is mixed with 2 c c. of hydrochloric acid and 1 grm. of granulated zinc. After 2 or 3 minutes at ordinary temperature, it is heated quickly to boiling, cooled and poured carefully, so as to form a separate layer, on to 2 c.c. concentrated sulphuric acid contained in a test-tube. If strychnine be present a rose-red ring will be formed at the surface of contact of the 2 liquids and gradually the whole mixture will become rose-red in colour. On heating to boiling the colour is not changed

but the mixture is rendered colourless by potassium thiocyanate ammonia, or sodium hydrogen sulphate in excess.

The test is sufficiently delicate to detect strychnine in a dilution of 1:100,000. It is stated that the only known alkaloid which might be mistaken for strychnine by this test is veratrine, which gives a beautiful red colouration with the sulphuric acid, but on boiling the mixture it is changed to a dirty yellow, whereas the red colour produced by strychnine remains unchanged.

Many other colour reactions for strychnine have been proposed and described from time to time, but it must be remembered that the particular shades of colour developed are influenced not only by traces of impurities that may be present in the material being tested, but by the relative proportions of reagents and alkaloid employed for the test, by the temperature, by the influence of air and moisture, and even by the salt form of the alkaloid, if the test be applied to a salt instead of the free base. It is essential, therefore, in applying the above-mentioned colour reactions for strychnine, to employ only ether-chloroform extractions prepared as described in test 2, and to repeat the purification steps described on page 442 until a residue is obtained which remains colourless on treatment with concentrated sulphuric acid.

14. An exceedingly important confirmatory test for strychnine, and one that should invariably be made in toxicologic cases, is the physiologic one first proposed by Marshall Hall. Small frogs or white mice, being remarkably susceptible to strychnine poisoning, afford the best subjects for the test, but ordinary barn or field mice, or even rabbits and guinea-pigs may be employed.

The test is made by taking a small quantity of the suspected liquid, or a portion of an ether-chloroform residue dissolved in water very slightly acidified with hydrochloric acid, and injecting it under the skin of the animal by means of a hypodermic syringe. In the case of a frog, it may be injected, by means of a small pipette, into a small slit made with a small pair of scissors in the lifted skin of the animal. The first symptom usually observed is a difficulty in breathing gradually increasing until the animal appears to gasp for breath. A slight tremor will then be observed extending over the whole body but especially noticeable in the hind legs. In some cases a frog will remain perfectly quiet; in other cases it will leap about convulsively. Finally, after a few minutes, the characteristic tetanic convulsions

make their appearance. These occur intermittently, with intervals of rest, and, if the dose has been sufficiently large, end in the death of the animal. The convulsions may be induced by touching the animal, or by making a sudden noise as by clapping the hands or knocking on the table. In the case of white mice and small frogs, as little as 0.06 mg. of strychnine will produce the tetanic convulsions.

Toxicology of Strychnine.

Owing to the violently poisonous character of strychnine and the fact that it enters into the composition of a number of commercial "vermin-killers," its toxicology assumes a considerable degree of importance to the analyst. In all cases of poisoning the symptoms manifested by the subject are of importance to the chemist in furnishing him a clue as to the nature of the poison. In the case of no other substance, perhaps, are the symptoms of poisoning more characteristic than with strychnine. These are usually manifested at first by a bitter taste followed by a feeling of suffocation. Gradually the characteristic tetanic convulsions come on, often accompanied by an extreme rigidity of the entire body which tends to bend it backward into the shape of a bow. Lockjaw is a constant symptom, but vomiting, such as occurs with the metallic poisons and arsenic, is very rare. Consciousness, as a rule, is retained till the last, accompanied by a terror of the rapidly recurring and agonising convulsions.

Death has been known to ensue within as short a time as 12 minutes, but usually occurs in from 1/2 to 2 hours. In rare cases with fatal ending, life has been prolonged for 1 or more days.

The *usual medicinal dose* of strychnine is from 1/12 to 1/100 of a grain. 1/6 of a grain is usually distinctly dangerous. 1 grain may be regarded as the *average fatal dose* for an adult, but death has been known to occur from 1/4 grain. Much, or course, depends upon the method of administration—a smaller dose, injected subcutaneously, being more toxic than a larger dose taken by mouth. The absorption of strychnine through the stomach is not very rapid, especially when introduced in solid form and mixed with foodstuffs not quickly digested, such as fats.

The *elimination of strychnine* occurs through the saliva, milk, and particularly the urine. This elimination, however, is never complete; with large non-fatal doses, 50 to 75%, according to Gadamer, appears in the urine; with small doses, much less.

It is fortunate that the tests for this not uncommon poisoning agent should be both delicate and distinctive. Not only may the small fatal dose taken have been widely distributed throughout the organs of the body and have been partly eliminated, but that portion which was actually the cause of death, been oxidised or changed in such a manner as to cause it no longer to respond to the reactions for strychnine. The full amount taken can therefore never be recovered by the toxicologist, and if the amount taken has been only slightly in excess of that required to produce death, it follows that the amount found may be much less than that represented by the average fatal dose. When, however, strychnine has been recovered in sufficient amount for positive identification, and the symptoms were typical of poisoning by this agent, little doubt will remain as to the cause of death, even though less than a normally fatal dose be recovered.

The *post-mortem appearances* of poisoning by strychnine are not very striking or characteristic. Rigidity of the muscles is usually prolonged, but if death occur in one of the intervals between the convulsions, no rigidity will be observed. The heart is usually, but not always, full of blood, especially on the right side. The stomach usually appears normal, but sometimes intensely congested. In a case reported by Allen, the stomach presented such an appearance as to suggest the presence of arsenic or other irritant poison; but no mineral poison could be detected. That death was due to administration of a vermin-killer containing strychnine was subsequently fully proved by analysis and admitted by the murderer. The most characteristic appearance is the intense congestion of the brain and spinal cord, often accompanied by a considerable effusion of blood.

For the *detection of strychnine* in the dead body, the portions of the body operated upon should be chosen according to the manner in which the poison is likely to have been administered. Thus it is of no use to search in the stomach or intestines for strychnine injected hypodermically, although in cases where the poison has been administered by mouth these organs will usually be found to furnish the larger proportion of the amount recovered. Next in importance for the toxicologist are the liver, kidneys, brain, and spinal cord.[1] The blood and urine should always be examined also when this is possible. In extreme cases it is desirable to operate on very considerable quan-

[1] In the case of a body examined by the reviser in Indiana in 1900, 0.23 grain of strychnine was recovered from the kidneys

tities of materials as death may be caused by so small a quantity of strychnine that the poison may be altogether missed if this precaution be not taken.

The portions of the body to be tested for strychnine should be cut into small fragments with a pair of scissors, and then further reduced by bruising in a mortar. The product should be made *slightly* alkaline by the cautious addition of sodium hydroxide solution, then distinctly acid by means of acetic acid,[1] and mixed thoroughly with 3 or 4 volumes of 90 to 95% alcohol. After macerating for 3 or 4 hours with occasional stirring, the mixture is strained through muslin, and the strained liquid filtered through paper. This treatment serves thoroughly to extract any alkaloids that may be present, and at the same time leaves behind the greater part of the albuminous matter which has been coagulated by the alcohol. The clear filtrate is evaporated to a thin syrup on the water-bath, diluted somewhat with water, and, if turbid, again filtered. The clear liquid is next transferred to a separatory funnel, a few drops of hydrochloric acid added to ensure its acid character, and shaken out with an equal volume of ether. The extraction of the acid solution with ether serves to remove many ether-soluble substances such as glucosides, fats, oils, etc., which would otherwise contaminate the strychnine. The lower aqueous layer, which retains the strychnine in the form of a salt, is carefully run off into a second separatory funnel and shaken out with an equal volume of chloroform. This serves to further purify the solution, taking out the impurities left by the ether.[2] The chloroform layer is carefully run off and rejected, and a fresh portion of chloroform added to the contents of the separator. Ammonia water is now added until the liquid has become distinctly alkaline, and the whole immediately and thoroughly shaken for a period of about 1 minute. On coming to rest, the aqueous liquid will separate from the chloroform layer, which can be run off into a separate vessel. If quantitative results are required, the alkaline aqueous liquid should be extracted twice more with chloroform, the chloroform extracts united, filtered through paper, and evaporated to dryness on the water-bath.

[1] Some toxicologists prefer to employ tartaric acid, permitting of the subsequent maceration with alcohol at an elevated temperature

[2] After the preliminary extraction with ether, there is not so great a tendency to form an emulsion on shaking out with chloroform Liquids charged with sugar or other extractive matter may, however, render impossible the shaking out with chloroform without the formation of troublesome emulsions In this case, the emulsified solution should be transferred to a shallow dish, the chloroform evaporated by exposure to a gentle heat, and thereafter all shakings out from acid solution carried out with ether alone.

The further treatment of the residue thus obtained depends upon its state of purity at this point. It may be sufficiently pure to respond to the reactions for strychnine given on page 444 *et seq;* on the other hand, impurities which interfere with the characteristic colour reactions for strychnine and prevent its crystallisation may be present, thus requiring further purification. This may be effected by dissolving the entire residue in dilute acid, filtering, adding chloroform or a mixture of chloroform and ether, rendering alkaline with ammonia, shaking out the precipitated alkaloid, and again evaporating the chloroformic solution on the water-bath, in a tared beaker.

Dialysis through parchment-paper is an efficient and occasionally a convenient means of separating strychnine from organic matter. The finely divided tissue should be suspended in water to which some alcohol and acetic acid have been added. Distilled water should be used on the other side of the membrane, and changed at intervals of 12 hours. After 36 to 48 hours the dialysate may be evaporated to dryness and treated with alcohol, etc., as described on page 442.

For the carrying out of the several identification tests, the *weighed* strychnine residue should be redissolved in chloroform and evaporated in portions on watch glasses or inverted porcelain crucible lids, some of which should be preserved for demonstration of strychnine reactions before the court.

In addition to responding to the several colour and crystallisation tests as well as the physiological test on animals, the strychnine residue should be sufficiently pure to produce no marked colouration when a small portion is treated with a few drops of cold sulphuric acid. This is essential as proof that the residue is sufficiently pure to be weighed for quantitative estimation.

The most delicate and characteristic chemical reaction of strychnine is the oxidation-test described on page 448. Reactions 7 and 8, and the production of crystals of strychnine as described in 1, are also valuable as confirmatory tests, and should never be omitted if the material at disposal be sufficient for their performance. The intensely bitter taste is also of great importance, and in conjunction with a distinct reaction by the characteristic oxidation-test, may usually be regarded as ample proof of the presence of strychnine, provided the absence of interfering substances has been insured by the previous treatment. The ptomaines mentioned on page 452 as giving colour-

reactions simulating those of strychnine, can only be present when putrefaction has taken place, and their formation must be very rare, or they would have been met with in the numerous cases in which no alkaloidal substance has been detected. Moreover, no ptomaine has ever been found to agree with strychnine in all its properties, including colour reactions, taste, and power to crystallise. The precautions mentioned therefore should be ample to guard against the mistaking of a ptomaine for strychnine.

Fortunately for the toxicologist, strychnine possesses a high power of resistance to decomposition in the dead body. Allen reports the detection of strychnine in a stomach preserved in alcohol for 6 years. A portion of the untreated stomach and liver was kept in a jar, the mouth of which was closed by a bag containing wood-charcoal. On opening the jar after six years, the whole of the contents were found to have disappeared, with the exception of a small quantity of dust, in which abundance of strychnine was detected. Nevertheless, it has not infrequently happened that a postmortem analysis has failed to detect strychnine in corpses almost certainly containing it. This result has probably been due in most cases to the use of defective methods of analysis, or to the search being restricted to too small quantities of material or to wrong parts of the body. Occasionally, failure has probably been due to an elimination of the poison during life, especially in cases in which death has resulted from a minimum dose. If elimination has not occurred prior to death, strychnine ought to be found by the toxicologist.

Blood should be examined for strychnine by diluting it with an equal bulk of water, adding a little acetic acid, boiling for a short time, filtering, and evaporating the filtrate nearly to dryness. The residue is taken up with alcohol, and the solution treated as already described.

From urine, strychnine may be directly extracted by agitating the fluid with ammonia and ether-chloroform.

Preparations of Strychnine.

The official preparations of strychnine are as follows:

Pharmacopœia	Preparation	Strychnine per fluid-dram
British . .	Solution of strychnine hydrochloride	45/100 grain
	Syrup of phosphate of iron with quinine and strychnine (Easton's syrup)	1/32 grain.
United States . .	Compound laxative pills .	1/130 grain (per pill)
	Compound syrup of hypophosphites	1/150 grain
	Elixir of iron, quinine and strychnine phosphates	1/64 grain
	Glycerite of the phosphates of iron, quinine and strychnine	1/22 grain
	Iron and strychnine citrate	0 9 to 1 (%)
	Syrup of the phosphates of iron, quinine and strychnine	1/88 grain
French .	Granules of sulphate of strychnine .	1/83 grain (per granule)

The above preparations may be divided naturally into 2 classes—those consisting of simple solutions or mixtures of strychnine or one of its salts, which require no special directions for determining their strength, and those consisting of rather complex mixtures, the analysis of which is fraught with many difficulties, chief among which is the presence of large amounts of another alkaloid.

Most simple among these is the analysis of *iron and strychnine citrate, United States Pharmacopœia*, which occurs in garnet-red to yellowish-brown transparent scales, soluble in water. The official process for its assay for strychnine consists in shaking out an aqueous ammoniacal solution of the scales 3 times with chloroform, evaporating the combined chloroform liquids, and drying the alkaloidal residue to constant weight at 100°.

On account of the extensive use of *syrup of the phosphates of iron, quinine and strychnine* (an out-growth of the old formula for Easton's syrup), and the corresponding *elixir*, as reconstructive tonics, both by direction of practising physicians, and as household remedies, their analysis becomes of importance to the analyst and occasionally to the toxicologist. The other complex preparations such as the compound laxative pills and compound syrup of hypophosphites contain such minute quantities of strychnine as to render their consideration by the toxicologist almost negligable, while the glycerite, although containing 4 times as much strychnine as the syrup, is used only for the preparation of the latter.

In analysing the syrup or elixir, the *iron* may be estimated by evaporating 5 c.c. of the preparation, igniting the residue, dissolving the ash in hydrochloric acid, and titrating the iron with standard dichromate solution after reducing it to the ferrous state.

The free phosphoric acid may be estimated by titration of 10 c.c. with methyl-orange and $N/2$ sodium hydroxide. The neutral point is attained when NaH_2PO_4 is formed.

The alkaloids may be estimated by diluting 100 c.c. of the syrup or elixir with twice its volume of water, adding some citric acid and excess of ammonia, and shaking out twice again with ether-chloroform to remove the alkaloids.[1] The ether-chloroform solution is then evaporated to dryness and the residue of mixed quinine and strychnine weighed.

The actual separation of the strychnine from the quinine is quite difficult both on account of the relatively small amount of the former in proportion to the latter and on account of the possible traces of impurities in the mixed alkaloids. Theoretically, the residue from 100 c.c. of the syrup of the *British Pharmacopœia* should amount to 1.15 grm.; that from the syrup of the *United States Pharmacopœia*, 2.62 grm.; and that from the elixir, 0.90 grm. The ratio of strychnine to quinine in the 3 preparations should be, respectively, 1 to 19, 1 to 130, and 1 to 32.

The ferrocyanide method of separating the strychnine cannot be successfully used because much of the quinine is precipitated along with the strychnine.

Another method which has been suggested consists in dissolving the ether-chloroform residue from 100 c.c. in 20 c.c. of water acidified with a few drops of sulphuric acid. The solution is neutralised with ammonia and mixed with excess of ammonium oxalate solution. After 24 hours, the precipitated quinine oxalate is filtered off, the mother-liquor removed by gentle pressure, and the precipitate washed once with a little cold water. It is then dried at 100° and weighed. The weight multiplied by 0.878 gives the amount of anhydrous quinine alkaloid in the 100 c.c. of sample. The filtrate and wash water are then placed in a separator, made alkaline with ammonia and shaken out with ether-chloroform. The residue obtained by evaporating the ether-chloroform contains the strychnine slightly contaminated with

[1] From the aqueous liquid the total phosphoric acid may be thrown down by magnesia mixture.

quinine. This should be washed twice with 5 c.c. of ether to remove the quinine after which the residue of strychnine should be weighed.[1] The residue may be redissolved and tested for quinine by means of the thalleioquin test.

A method proposed by the reviser consists in dissolving the mixed alkaloids from 100 c.c. of the syrup or elixir in a small quantity of 0.5% hydrochloric acid, making the solution exactly neutral with potassium hydroxide, and adding with constant stirring a solution of 1.5 grm. of potassium sodium tartrate in 5 c.c. of water. After 24 hours, the crystals of quinine tartrate may be filtered off, washed with a few drops of cold water, dried at 105° and weighed. The weight multiplied by 0.794 gives the amount of anhydrous quinine alkaloid in the 100 c.c. of sample, or the precipitate may be transferred to a separator, with a little water, rendered alkaline with ammonia, shaken out with chloroform, evaporated to dryness and weighed as quinine alkaloid. The filtrate and washings are then treated as described in the last paragraph, for the estimation of the strychnine.[1]

Among the unofficial preparations containing strychnine none are so widely used as the *tablets* both of simple composition and complex. Because of this extensive use, the ease with which they may be administered, and the extreme danger of taking them by mistake for other widely used tablets, they have been prolific in the causation of strychnine poisoning. For the assay, the tablets containing strychnine or one of its salts should be crushed and dissolved or extracted with water acidified with hydrochloric or sulphuric acid. The filtered acid solution should then be shaken out 2 or 3 times with ether-chloroform to remove non-alkaloidal substances soluble in these solvents, after which the aqueous layer may be rendered alkaline with ammonia and shaken out again with ether-chloroform. On evaporating the latter extractions to dryness, the strychnine (together with any other alkaloids that may be present in a complex tablet) will be left in condition for weighing. The further separation of the strychnine from other alkaloids will then have to be devised according to the nature of the contaminating substances. (See page 511 for the separation of strychnine and quinine.)

A rapid and accurate method for estimating strychnine in *hypo-*

[1] Bernegau and E'We report these methods to be liable to give high results for strychnine in the case of syrups, and low results in the case of elixirs By the oxalate method they found in 100 c c of the elixir only 0 015 grm. of strychnine instead of 0.03 grm , and in 100 c c. of the syrup 0 0255 instead of 0 02 grm. By the tartrate method they found respectively in the elixir and the syrup, 0.021 grm. and 0.0258 grm.

dermic tablets, which consist of a soluble strychnine salt in admixture with sugar of milk as a vehicle, is a modification of Prescott and Gordin's iodometric method (*J. Amer. Chem. Soc.*, 1898, **20**, 722). As commonly found on the market, these tablets vary in strength from 1/100 to 1/20 grain of a strychnine salt (nitrate or sulphate) per tablet. The procedure employed by the reviser is as follows.

A number of tablets containing from 1/2 to 1 grain of strychnine are dissolved in 30 c.c. of water, and 10 to 20 drops of hydrochloric acid are added. The solution is then poured with constant agitation into 15 c.c. of $N/10$ iodine solution contained in a 50 c.c. cylinder. The solution is then diluted to exactly 50 c.c. with distilled water, and the whole shaken vigorously for several minutes until the periodide of strychnine separates in the form of a curdy precipitate, leaving the supernatant liquid a clear deep red in colour. (If the supernatant liquid be light yellowish-red in colour, an insufficient excess of iodine has been employed, and the assay must be repeated, using a smaller number of the tablets.) The solution is then filtered, the first few c.c. of filtrate rejected, an aliquot part of the filtrate then collected, and titrated back with $N/10$ sodium thiosulphate solution. From the amount of $N/10$ iodine solution used to precipitate the strychnine as periodide, $C_{21}H_{22}O_2N_2.HI.I_6$, the amount of strychnine in the tablets is calculated. 1 part of iodine precipitates 0.439 parts of strychnine, or 1 c.c. of $N/10$ iodine corresponds to 0.005555 grm. of alkaloid.

Vermin-killers.—Strychnine has been largely used, particularly in Great Britain,[1] for the extermination of rodents and other pests, and on account of the ease with which it may be purchased in the form of commercial vermin-killers, the latter have often been the cause of poisoning. In order to disguise its bitter taste and make the mixtures attractive to vermin, these preparations usually contain considerable starchy matter such as rice-starch, wheat-flour, or oatmeal, and sometimes sugar. They are generally coloured to attract attention to their poisonous character. Ultramarine, prussian blue, carmine, and charcoal have been employed for this prupose and in cases of poisoning resulting from vermin-killers, the presence of these colouring matters often indicate the source of the poison.

Strychnine can be determined in vermin-killers by exhausting a known weight of the dry powder with chloroform or benzene, and

[1] In the United States, vermin-killers containing strychnine are not at all common, arsenical and phosphorous preparations being more often employed for this purpose.

weighing the alkaloidal residue left on evaporating the solvent. The insoluble portion must be examined by the taste and oxidation-test, to insure complete extraction and the absence of a salt of strychnine insoluble in the solvent used. An alternative, and in many respects preferable, method is to treat the vermin-killer with cold water acidified with acetic acid, until the residual powder has no bitter taste, and gives no colouration by the oxidation-test. The solution is then treated with excess of ammonia and the strychnine extracted by ether-chloroform, which is separated, evaporated to dryness, and the residue weighed.

BRUCINE, BRUCIA, $C_{23}H_{26}O_4N_2$.

Brucine was discovered by Pelletier and Caventou in 1819, the next year after their discovery of strychnine, in the bark of *Strychnos Nux-vomica*. It occurs associated with strychnine in many species of strychnos. The seeds of *Strychnos Nux-vomica* contain on the average about 1.25% of brucine; the seeds of *Strychnos Ignatia* about 0.5%. The leaves of *Strychnos Nux-vomica* are stated to contain brucine but no strychnine.

In chemical constitution, brucine is a dimethoxystrychnine.

Brucine is prepared from the mother-liquors obtained in the manufacture of strychnine (see page 442). These are concentrated to a small volume, the brucine precipitated as oxalate by neutralisation with oxalic acid, washed with cold strong alcohol, dissolved in hot water, decolourised with animal charcoal, and evaporated to dryness with magnesia. The brucine is extracted from the residue with acetone, and purified after recovery of the solvent, by recrystallisation from dilute alcohol. An alternative method consists in treating the dry mixture of precipitated alkaloids obtained in the manufacture of strychnine, with cold acetone, whereby the brucine is extracted, leaving behind the strychnine.

Brucine occurs as a bitter, white, odourless, crystalline powder, or as colourless, transparent, monoclinic prisms, containing 4 molecules of water of crystallisation. The crystals are somewhat efflorescent in dry air, and readily melt and lose all their water at 115°. The anhydrous base then solidifies, and melts again at 178.° On ignition it is consumed, leaving no residue. Its solutions are lævogyrate, intensely bitter, and have a marked alkaline reaction.

Brucine is much more soluble than strychnine in water, the crystal-

line base dissolving in about 320 parts of cold water and in about 150 parts of boiling water (E. Schmidt). In alcohol and in acetone it is very soluble, a fact which is employed to separate it from strychnine. It is also very soluble in chloroform (about 7 parts, Schmidt), but is almost insoluble in absolute ether. Brucine is insoluble in the fixed alkalies and only sparingly soluble in excess of ammonia.

Brucine resembles strychnine closely in its general characters, but is many times less poisonous. It is excreted far more rapidly than strychnine, so that when given by the stomach it produces little effect, though it is fatal when injected hypodermically (T. Lauder Brunton, *Jour. Chem. Soc.*, 1885, **47**, 143). T. J. Mays (*J. Physiol.*, 1887, **8**, 391) finds that, when frogs are poisoned, brucine primarily affects the posterior and strychnine the anterior extremities; convulsions occur very early and invariably before death in strychnine poisoning, and very late or frequently not at all in brucine poisoning. Like strychnine, it is not acted on readily by cold sulphuric acid, or by alkali hydroxides. It dissolves without decomposition in strong hydrochloric acid, and forms readily crystallisable and soluble salts.

Analytical Reactions of Brucine.

1. Brucine is precipitated in a free state on adding an alkali to the solution of one of its salts, and may then be taken up by agitating the alkaline liquid with ether-chloroform in the same way as strychnine (see page 442).

2. Brucine is precipitated from dilute solutions of its salts by the general alkaloidal reagents in much the same way as strychnine (see paragraph 3, page 445).

3. Brucine is not precipitated at once from its hydrochloric acid solution by potassium ferrocyanide, a fact which is used to separate it from strychnine (see paragraph 6, page 446).

4. Potassium dichromate throws down from solutions of brucine salts, even when very dilute, a yellow precipitate of brucine chromate, insoluble in acetic acid, but soluble with deep red colour in strong nitric acid. The microscopic appearance of brucine chromate is characteristic, and, together with its behaviour with nitric acid, distinguishes the precipitate from all others produced by the reagent.

5. The microscopic appearances of the precipitates produced in brucine solutions by platinic chloride and potassium ferricyanide are also highly peculiar.

6. When treated with concentrated sulphuric acid and an oxidising agent, brucine does not give the coloured products so characteristic of strychnine.

7. The most satisfactory reaction of brucine is that with nitric acid. On adding a drop or two of cold nitric acid to an ether-chloroform residue, or other solid product containing brucine, a scarlet or blood-red colouration is produced, which on heating changes to yellowish-red, and finally to yellow.[1] If the mixture be then cooled and treated very cautiously with stannous chloride (or other reducing agent, such as sodium thiosulphate), a purple colouration is produced, which is destroyed by excess of either nitric acid or the tin salt.[2]

The red colouration of brucine caused by nitric acid may likewise be developed by dissolving the alkaloid in strong sulphuric acid in a test-tube, and allowing nitric acid to run on to the surface of the heavier liquid. A red zone, passing to yellow, will be produced at the junction of the two liquids. If cold nitric acid be added to solid brucine, so as to develop the red colour, and the mixture be then largely diluted with water, a substance called kakotelin, $C_{20}H_{22}(NO_2)_2N_2O_6$, separates in yellow flocks. The filtered liquid, after neutralisation by ammonia, gives a precipitate of calcium oxalate on being treated with calcium chloride.

The production of a red colour with nitric acid, accompanied by a formation of oxalic acid and yellow scales or crystals, insoluble in water but soluble in dilute acids, constitutes a combined reaction which is peculiar to brucine.

8. Brucine dissolves in chlorine-water with red colour. On evaporation, dichlorobrucine, $C_{23}H_{24}Cl_2O_4N_2$, remains as a reddish-brown, amorphous mass.

9. Picrolonic acid precipitates brucine from its dilute solutions in the form of cubical or rhombohedric crystals which darken at 214° and melt at 256° (Gadamer).

Toxicology of Brucine.

Like strychnine, brucine resists decomposition for a long time, and hence may be recovered along with strychnine, when the cause of the poisoning has been due to a preparation of nux vomica or ignatia. In

[1] Strychnine, on the contrary, gives no colouration with cold nitric acid, but develops a yellow colour on warming

[2] The orange colour produced by adding nitric acid to morphine remains unchanged on addition of stannous chloride.

fact, its special significance in toxicological cases is to indicate the probable employment of such a preparation rather than one of the pure alkaloids or salts. On account of its limited use, as such, in medicine, cases of poisoning by brucine alone are scarcely known. The presence of brucine in an ether-chloroform residue may be readily detected by the nitric acid test. This may be made by treating the residue first with concentrated sulphuric acid, followed by a trace of nitric acid. After the red colouration due to brucine has faded to a yellow, a small fragment of potassium permanganate may be drawn through the mixture, whereupon, if strychnine also be present, the characteristic blue colouration will appear.

The quantitative separation of brucine and strychnine in toxicological work has already been considered. For the separation in assays of nux vomica and its preparations, see pages 469 *et seq.*

Nux Vomica.

Under this name, the dried, ripe seeds of *Strychnos Nux-vomica* Linné (Fam. *Loganiaceæ*), a small tree growing in the East Indies, are official in the *British* and the *United States Pharmacopœias*. English synonyms are poison-nuts and Quaker buttons; French, noix vomique; German, Krahenaugen and Brechnusse. The tree produces a fruit resembling the orange, which contains many seeds imbedded in a juicy bitter pulp. The seeds are removed from the ripe fruit, cleaned, dried, and sorted. The principal ports of shipment are Bombay, Cochin, and Madras, which lend their names to the 3 commercial varieties.

The appearance of the whole seeds is highly characteristic. They have the shape of flattened disks about three-quarters of an inch in diameter, flat or slightly convex on one side and concave on the other. They are densely covered with a coat of satiny ash-coloured or yellowish-grey hairs radiating from the centres of the sides, giving them a highly characteristic appearance. They are extremely hard and difficult to grind or powder. In practice they are often steamed in order to soften them and render them more easily comminuted. They have no odour but taste intensely bitter.

On the other hand, the powdered seeds are light grey to greyish-buff in colour and might readily be mistaken for many other powdered drugs, such as jalap, or liquorice. If moistened with water, however, and examined under the microscope, the characteristic fibrous hairs

may be readily recognised. These are coloured yellow by iodine-potassium iodide solution, while the rest of the powder becomes brown. When treated with strong nitric acid, the powder acquires an orange-red colour.

Nux vomica contains the alkaloids *strychnine* and *brucine* in combination with *caffetannic acid*, together with a small amount of a glucoside, *loganin*, about 4% of fatty matter, and a large amount of a horny albuminous substance. There may also be present a few nearly spherical starch grains, from particles of adhering fruit pulp. Nux vomica on ignition yields about 5% of ash.

Caffetannic acid, formerly known as *strychnic* or *igasuric acid*, has been obtained as an amorphous, yellowish-white mass, readily soluble in water and in alcohol. It has a strongly acid and astringent taste and gives a green colour with ferric chloride and a black precipitate with ferrous salts and ammonia. It gives a white precipitate with lead acetate and reduces ammoniacal solution of silver nitrate. On melting with potassium hydroxide, protocatechuic acid is formed.

Loganin exists in nux vomica seeds, but more largely (4 to 5%) in the pulp in which they lie embedded in the fruit. Dunstan and Short (*Pharm. Jour.*, 1884, **14**, 1025) obtained loganin in prismatic crystals by cooling the liquid obtained by exhausting this substance with chloroform-alcohol (4:1). After recrystallisation from alcohol, the colourless prisms contained $C_{25}H_{34}O_{14}$, an empirical formula identical with that of arbutin, from which, however, loganin is distinguished by its much higher m. p. (215°), and by not yielding quinol with dilute sulphuric acid.

Loganin it readily soluble in water and alcohol, but less so in ether, chloroform and benzene. It develops no colour with nitric acid or other oxidising agents, and the aqueous solution is not affected by solutions of lead, iron or silver, and does not reduce Fehling's solution. When gently warmed with strong sulphuric acid, loganin gives a fine red colour, changing to purple on boiling. When boiled with dilute sulphuric acid, it yields a reducing glucose and loganetin, which latter substance behaves with solvents and reagents very similarly to loganin itself.

The Assay of Nux Vomica.

Although nux vomica contains 2 active alkaloids, its value and toxicity depend primarily upon its strychnine content on account of

the much greater physiological activity possessed by this alkaloid. Present-day methods of assay therefore are directed toward the estimation of strychnine alone—although the assay for total alkaloids is of almost equal value—owing to the fact that the alkaloids strychnine and brucine exist in nux vomica in almost equal amounts. Beckurts (*Pharm. Jour.*, 1889, **20**, 341) reported the ratio of strychnine to brucine to vary only between 43:57 and 54:46. In the author's experience in supervising hundreds of assays of nux vomica and its preparations during the past ten years, the yield of total alkaloids has been found to approximate so nearly the double of the yield of strychnine that the former estimation is always made as a check on the accuracy of the latter.

For the estimation of *total alkaloids*, the *method of Keller*[1] is most suitable. The method is carried out as follows:

Take 12 grm. of finely powdered nux vomica, place in a dry 200 c.c. flask, add a mixture of 80 c.c. ether and 40 c.c. chloroform, and stopper tightly. After one-half hour, add 10 c.c. 10% ammonia, and shake repeatedly for 1 hour.[2] 15 to 20 c.c. of water are then added in 2 or 3 portions with vigorous shaking, to aglutinate the drug, and cause the ether-chloroform solution of the extracted alkaloids to separate in a clear layer.

Exactly 100 c.c. of the latter clear liquid (representing 10 grm. drug) are then poured off and transferred to a separator. This is shaken out with 50 c.c. of 0.5% hydrochloric acid, and the extraction repeated with 25 c.c. portions of the latter until a few drops of the acid layer no longer give a cloudiness on addition of Mayer's reagent. The united acid layers are now filtered into a second separator, 30 c.c. of a mixture of 3 parts chloroform and 1 part ether added, the aqueous layer rendered distinctly alkaline with an excess of ammonia, and the mixture agitated thoroughly for 1 to 2 minutes. The separated chloroform-ether (lower) layer is then run off, through a pledget of cotton contained in a small funnel, into a tared Erlenmeyer flask, and the aqueous layer extracted with further portions of chloroform-ether until a few drops of the former, removed with a pipette, no longer give a precipitate with Mayer's reagent. The united chloroform-ether solutions are

[1] *Schweiz. Wochenschr f Chem. u. Pharm*, 1895, 33, 452

[2] In the reviser's laboratory, 8-ounce, narrow-mouthed bottles are preferred to flasks as they may be readily attached to a mechanical shaker and allowed to be shaken while other work is being carried on by the operator. It is most convenient to start an assay late in the afternoon, allow the shaking to continue for an hour or more, and then let the bottle stand over night. In the morning, after one vigorous shaking, the extraction will be complete and the next step of the process can immediately be carried out.

then distilled or evaporated to dryness on the water-bath, the varnish-like residue treated with 5 c.c. of ether and again evaporated (in order to remove traces of chloroform which adhere tenaciously to alkaloidal residues) to constant weight. The weight obtained, multiplied by 10, gives the percentage of total alkaloids in the drug.

As a check on the purity of the alkaloids thus abtained, the residue may be dissolved in 20 c.c. of $N/20$ sulphuric acid, preferably by warming on the water-bath with the addition of a few drops of chloroform to facilitate solution. The solution is then transferred to a 6-oz. bottle, 10 c.c. of ether added to form a layer on the top of the acid solution, a few drops of iodeosin indicator added, and the excess of acid titrated with $N/50$ potassium hydroxide solution. The end point is marked by the appearance of a faint pink colouration in the lower aqueous layer. Each c.c. of $N/20$ acid corresponds to 0.0182 grm. mixed alkaloids, it being assumed that strychnine and brucine are present in equal amounts.

The *method of the French Codex* is essentially the gravimetric method of Keller, differing only in details as to amounts of materials and solvents to be employed. The standard is not less than 2 nor more than 3% of total alkaloids.

The *method of the German Pharmacopœia* differs from the above method in the use of sodium hydroxide instead of ammonia to liberate the alkaloids, and particularly in the method of making the titration. Instead of evaporating the final ether-chloroform extractions to dryness and dissolving them in standard acid, they are placed in a separator and shaken out with 10 c.c. of $N/10$ hydrochloric acid, which is followed by 3 portions of 10 c.c. each of water. The united aqueous-acid extractions are diluted to 100 c.c., an aliquot part taken, and the excess of acid titrated back against $N/100$ potassium hydroxide solution. The standard is not less than 2.5% of total alkaloids.

An accurate method for determining total alkaloids in nux vomica is the *picrolonic acid method of Matthes and Rammstedt* (*Arch. d. Pharm.*, 1907, **245**, 124). 15 grm. of the dried and powdered drug are extracted by maceration with 100 grm. ether, 50 grm. chloroform, and 10 c.c. of 10% sodium hydroxide solution. The drug is aglutinated with water; 50 grm. of the chloroform-ether solution, which has been filtered through a dry filter and which represents 5 grm. drug, are taken and evaporated to about half its volume. 5 c.c. of $N/10$ alcoholic picrolonic acid solution is then added, and after 24 hours, the precipitate

of strychnine-brucine picrolonate is transferred to a Gooch crucible. The precipitate is washed with 2 c.c. of a mixture of alcohol and ether, 1 to 3, dried for 30 minutes at 110° and weighed. The weight obtained, multiplied by 0.5798, gives the amount of total alkaloid in 5 grm. drug, assuming the alkaloids to be present in equal amounts.

Commercial nux vomica as a rule ranges from 2 to 3% in alkaloidal content, although samples of both higher and lower alkaloidal strength have been reported. The international standard for nux vomica adopted by the Brussels Conference is 2.5% total alkaloid. The *United States Pharmacopœia*, however, has adopted a standard of 1.25% strychnine. The official *method of the United States Pharmacopœia, Eighth Revision*, is essentially as follows:

20 grm. of nux vomica in No. 60 powder are placed in a 250 c.c. flask and macerated with frequent shaking for 1 hour with 200 c.c. of a mixture of 137.5 c.c. ether, 44 c.c. chloroform, 13.5 c.c. alcohol, and 5 c.c. ammonia. After 12 hours 100 c.c. of the liquid (representing 10 grm. drug) are transferred to a separator and shaken out with several portions of about 5% sulphuric acid. The combined acid solutions are rendered alkaline with ammonia and shaken out with 3 portions of chloroform. The combined chloroform solutions are then evaporated to dryness in a 100 c.c. flask.

The alkaloidal residue is dissolved in 15 c.c. of 3% sulphuric acid by warming on the water-bath. When cool, 3 c.c. of a cooled mixture of equal volumes of nitric acid (sp. gr. 1.40) and water are added for the purpose of oxidising and destroying the brucine. After shaking the mixture gently 3 times during exactly 10 minutes, the red liquid is transferred to a separator containing 25 c.c. of 10% sodium hydroxide solution. The strychnine is now extracted by shaking out several times with chloroform, and the combined chloroform solutions evaporated to dryness. The alkaloidal residue is finally dissolved in $N/10$ sulphuric acid and the excess of acid titrated back with $N/50$ potassium hydroxide, iodeosin being used as indicator. Each c.c. of $N/10$ acid consumed corresponds to 0.0332 grm. of strychnine.

The principle, involved in this assay, of destroying brucine by means of nitric acid, was first employed in an assay process by J. E. Gerock (*Arch. der Pharm.*, 1889, **227**, 158), who applied the nitric acid, of 1.056 sp. gr., to the mixed picrates of the alkaloids strychnine and brucine, which had been obtained by precipitating an almost neutral solution of the alkaloids with picric acid, collecting the precipi-

tate on a filter, washing, drying, and weighing. On warming with nitric acid, only the brucine picrate was decomposed, leaving behind in unchanged form the strychnine picrate which was collected again and weighed. Later, C. C. Keller (*Proc. A. Ph. A.*, 1894, **42**, 531) applied the nitric acid method directly to a sulphuric acid solution of the mixed alkaloids, the residue from 10 grm. of drug being dissolved in 10 c.c. of 10% sulphuric acid and allowed to stand in the cold for 1 1/2 hours with 1 c.c. of nitric acid of 1.41 to 1.42 sp. gr. Later on, H. M. Gordin (*Proc. A. Ph. A.*, 1902, **50**, 336) worked out better conditions for the destruction of brucine with nitric acid, under which, while the brucine was completely destroyed, the strychnine was scarcely, if at all, affected. He dissolved the alkaloidal residue in 15 c.c. of 3% sulphuric acid, treated the solution with 3 c.c. of a mixture of nitric acid (sp. gr. 1.42) and water for 10 minutes. Unfortunately, when his method was adopted for the *United States Pharmacopœia, Eighth Revision*, the strength of the nitric acid was changed to that corresponding to a sp. gr. of 1.40. Webster and Pursel (*Am. J. Pharm.*, 1907, **79**, 1) and others have pointed out the fact that this official method does not always insure the complete destruction of the brucine, thus leading to apparently too high results. They recommend the addition, after the use of the mixture of nitric acid of sp. gr. 1.40 and water, of 1 c.c. of a 5% solution of sodium nitrite, and increasing the time of standing, from 10 minutes to 38 minutes. The reviser has found, however, that if nitric acid of 1.42 sp. gr. be employed, accurate results will be obtained. If nitric acid of sp. gr. 1.42, which is somewhat stronger than the usual nitric acid of commerce, is not readily obtainable, 1 c.c. of a 5% solution of sodium nitrite will insure the complete destruction of the brucine, and if the latter solution be freshly prepared each time, it is not necessary to increase the time beyond the prescribed 10 minutes.

Preparations of Nux Vomica.

The following table gives the official nux vomica preparations of the *British, United States, German* and *French Pharmacopœias.*

Pharmacopœia	Preparation	Standard
British, 1898	Extract	5% strychnine.
	Liquid extract	1 5 grm strychnine in 100 c c. (About 4/5 grain per fluid dram)
	Tincture	0 25 grm strychnine in 100 c c (About 1/8 grain per fluid dram)
United States, 8th Rev.	Extract	5% Strychnine
	Fluidextract	1 grm strychnine in 100 c c (About 3/5 grain per fluid dram)
	Tincture	0 1 grm strychnine in 100 c c (About 3/50 grain per fluid dram)
German, 5th	Extract	Not less than 16% total alkaloids
	Tincture	Not less than 0 25% total alkaloids
French, 1908	Extract	16% total alkaloids
	Powder	2 5% total alkaloids
	Tincture	0 25% total alkaloids

The lack of international uniformity in the strength of so potent preparations as those of nux vomica is to be deplored, owing to the danger involved of giving an over-dose. Thus the extracts of Great Britain and the United States are about 2/3 as strong as those of Germany and France, whereas the British tincture is twice as strong as those of the latter countries, and 2 1/2 times as strong as that of the United States.

The official *method of assay of the British Pharmacopœia* for the liquid extract is the ferrocyanide method of Dunstan and Short, and is essentially as follows:

10 c.c. are evaporated to a thick syrup on the water-bath, dissolved in 20 c.c. of water, transferred to a separator, mixed with a solution of 5 grm. sodium carbonate in 25 c.c. water, and agitated with 10 c.c. chloroform. The extraction with chloroform is twice repeated, after which the united chloroform extractions are shaken out 3 times with about 10 c.c. of 25% sulphuric acid. The united acid solutions are diluted to 175 c.c., 25 c.c. of 5% solution of potassium ferrocyanide are added, the mixture shaken well for 1/2 hour and set aside for 6 hours. The precipitated strychnine ferrocyanide is now transferred to a filter and washed with much diluted (1/3%) sulphuric acid until the washings are free from bitterness.[1]

[1] The filtrate and washings may be used for the estimation of brucine, by concentrating somewhat, rendering alkaline with ammonia, shaking out with ether-chloroform, and evaporating the ether-chloroform solution.

The precipitate is transferred to a separator, 5 c.c. of 10% ammonia added, and then, after shaking well, the liberated strychnine is extracted with 2 successive portions of chloroform of 15 c.c. each. The united chloroformic solutions are evaporated in a current of warm air, dried for 1 hour on the water-bath, and finally weighed.

The extract of nux vomica, *British Pharmacopœia*, is prepared from the standardised liquid extract, and the tincture from the extract. In the United States, the nitric acid method of destroying the brucine is employed in the assay of the extract, fluidextract, and tincture; the latter is prepared, however, from the extract.

Alkaloids of Curare.

Curare is an exceedingly poisonous extract made from the bark of various species of Strychnos, and used as an arrow poison by tribes of Indians in tropical South America. It is also known variously under the names woorara or woorari, wourali, ourari, and urari. The drug varies greatly[1] owing to the fact that different tribes prepare it from different species, not only of Strychnos, but of other plants. The extract of the bark of *Strychnos toxifera* is probably the most important ingredient of curare, although the barks of *S. Castelnæa*, *S. Gubleri*, and *S. Crevauxii* have also been known to be used.

Curare formerly occurred as a thick syrupy extract, but as now occurring in commerce, it forms a brown or black brittle amorphous mass, resembling catechu in appearance, having no odour but an intensely bitter taste. It is intensely poisonous and should be handled cautiously, care being taken to avoid bringing it in contact with a cut or scratch, as its poisonous properties are manifested particularly when introduced directly into the blood-stream. By mouth it is much less poisonous, owing to its rapid excretion and the destructive action of the gastric juice.

Curare exercises both a paralysing and tetanising action, but it appears to owe its chief poisonous properties to its action on the nerves of motion, which it paralyses, so that an animal under its influence dies of suffocation from paralysis of the muscles of the chest. Hence its physiological effects closely resemble those produced by methyl-strychnine. According to J. Tillie, when the difficulties besetting the

[1] Some tribes also mix with the vegetable extracts certain poisonous animal products, such as different varieties of poisonous ants and the fangs of certain snakes.

examination of the action of curare on the spinal cord are avoided, curare produces tetanus just like strychnine.

Curare appears not to act as a poison when taken into the stomach, but when employed as a hypodermic injection 0.015 grain has been found fatal to a rabbit, and 0.004 grain to a frog. If, after administration of curare, life be maintained by artificial respiration, symptoms of *diabetes mellitus* are observed, and the urine is found to contain sugar.

Curare is used in medicine in the form of a hypodermic injection for the treatment of hydrophobia, tetanus, and strychnine poisoning. Its use should be attended by great caution because of the danger of inducing paralysis of the motor nerve-endings which control respiration.

Commercial curare varies much in physical properties as well as in strength, according to the source. The 3 principal varieties are known by the containers into which they are probably poured, in the form of soft extracts, by the natives, and allowed to harden. These are bamboo or tubo-curare, occurring in bamboo stems; gourd or calabassen-curare, in gourds; and pot or topf-curare, in small, crude, unbaked, earthenware pots. Usually, from 70 to 90% is soluble in warm water, although the water-soluble content is sometimes much reduced by the presence of inorganic constituents such as calcium carbonate and phosphate. A somewhat smaller proportion is dissolved by dilute alcohol, while ether and chloroform exert but slight solvent action on it. The solutions vary in reaction, being sometimes neutral, sometimes slightly alkaline, and sometimes strongly acid.

I. **Bamboo Curare.**—The variety which to-day is the one most commonly found in commerce is the bamboo or tubo-curare which comes from Brazil. Bamboo curare is about 85% soluble in water and contains 2 well defined active principles, the intensely poisonous alkaloid *curarine*, or more properly *tubocurarine*, and the less poisonous alkaloid *curine*.

Curine, $C_{18}H_{19}NO_3$, may be obtained from the aqueous solution or extract of tubocurare by precipitation with slight excess of ammonia, and purifying the impure precipitate by successively treating it with ether, benzene, and methyl alcohol. The solution in ether leaves on evaporation a yellowish residue, which, on boiling with benzene and setting aside in a cool place, deposits crystals of curine containing benzene, in the form of thick rhombic plates. On recrystallising from hot methyl alcohol, pure curine is obtained in the form of colourless crystals, m. p. 212°. Tubocurare yields from 11 to 14% or curine.

Curine is insoluble in water, but is soluble in chloroform and in solutions of the alkali hydroxides. Solutions of curine in weak acids are lævogyrate, and differ from strychnine and from tubocurarine in having a sweetish taste instead of an intensely bitter one. Curine solutions give a voluminous white precipitate with metaphosphoric aicd. With concentrated sulphuric acid, pure curine produces no colour; when the solution is treated with potassium dichromate, a blackish colouration results.

Curine, unlike curarine, does not act on the motor nerves and is much less poisonous, but when injected hypodermically acts as a paralysant on the heart.

Tubocurarine, $C_{19}H_{22}O_4N.OH$, is obtained from the solution from which the curine has been precipitated, by treating the concentrated liquid with alcoholic solution of mercuric chloride, suspending the resulting yellow precipitate in alcohol, decomposing it by means of hydrogen sulphide gas, and precipitating the hydrochloric acid salt from the filtrate by means of ether. Tubocurarine hydrochloride forms an amorphous yellowish-red powder, soluble in water and alcohol to an acid reacting, intensely bitter and poisonous solution. This salt is the commercial so-called "Curarine" on the market to-day. The free base occurs as a reddish-brown syrupy liquid and is easily decomposed. It reacts much like curine, but differs from the latter in producing a yellowish instead of a white precipitate with metaphosphoric acid.

II. Gourd or calabassen-curare was formerly the principal variety on the market but has to-day practically disappeared from commerce. Gourd curare came from Venezuela and was probably prepared chiefly from *Strychnos toxifera*. It was soluble to a slighter extent in water than the bamboo curare, and contained as its principal active constituent the extremely poisonous curarine, differing somewhat from the tubocurarine of bamboo curare.

Curarine, $C_{19}H_{25}ON_2.OH$, is obtained from the aqueous extract of gourd curare in much the same manner as tubocurarine is prepared from tubo-curare—platinic chloride being used, however, instead of mercuric chloride. Curarine hydrochloride forms an amorphous reddish mass, soluble in water and alcohol, but insoluble in ether and chloroform. It is intensely bitter and much more poisonous than tubocurarine.

Curarine differs from curine and tubocurarine as well as from strych-

nine, in dissolving in concentrated sulphuric acid directly with the formation of a bluish-violet colour. Curarine further differs from strychnine in that it forms only an *amorphous* chromate, which remains so even after dissolving in boiling water and re-depositing on cooling. If the precipitate of curarine be kept for some time it decomposes, but if treated without delay with concentrated sulphuric acid it develops a magnificent blue colour, which is often violet in the presence of impurities. The reaction simulates that obtained in a similar manner with strychnine, but curarine can be separated from strychnine by rendering the cold solution alkaline with ammonia, and then filtering. Strychnine will be found in the precipitate, while the curarine will remain in the liquid, owing to its solubility in water. The filtrate may be agitated with chloroform or benzene to remove any trace of strychnine, the aqueous liquid concentrated and the curarine converted into chromate and tested further, as already described.

III. **Pot curare,** which is prepared from *Strychnos Castelnæa* on the upper Amazon, varies grealty in composition and activity. It contains, besides curine, the alkaloids protocurine, protocuridine, and protocurarine, only the last mentioned of which is important, being even more poisonous than curarine. Its formula is $C_{19}H_{24}O_2NOH$, and, although it gives a brown colour with concentrated sulphuric acid which is changed to violet by potassium dichromate, it may be distinguished from strychnine by its failure to form characteristic crystals or crystalline salts.

CINCHONA ALKALOIDS.

By OLIVER CHICK.

Cinchona Barks. French, *Ecorces de Quinquina.* German, *China-rinden.*

The bark of various species of *Cinchona*, which, with about 30 other allied genera, constitute the tribe *Cinchoneæ* (order, *Rubiaceæ*), have been long known for their antifebrile properties. These properties are chiefly due to the extraordinary number of closely analogous alkaloids contained therein, which exist chiefly, though not wholly, in the bark of the trees, and which are absent from all the allied genera, except certain species of *Remijia.*

Nearly 40 species of cinchona have been described, many of which can only be discriminated with great difficulty. The cinchonas form a very intricate genus, one series running into another through a series of intermediate forms, the number of which is limited to some extent in their native country by the fact that particular species are practically confined to certain districts and elevations.

Only some 7 distinct species of cinchona yield bark of any practical importance. These are:

a. **Pale or crown bark,** yielded by *Cinchona officinalis* (Peru) and allied species. It occurs in quills, with a rough, blackish-brown or dark grey surface. It should contain about 5% of alkaloids, of which 3·5% is quinine.

b. **Yellow or calisaya bark,** with its variety *Calisaya Ledgeriana,* is the richest of all the cinchona barks. It now usually occurs in firm, hard quills, which bear well-marked longitudinal and transverse cracks. It was formerly met with in flattened pieces known as "flat yellow bark."

c. **Red bark,** from *C. rubra* and *C. succirubra,* is distinguished by the red colour of the sap and mature bark. It is extensively cultivated in India, and is remarkable for the large proportion of cinchonidine contained in it. (For analyses, see page 485 *et seq.*

d. **Pitayo bark,** from *C. Pitayensis,* is imported in short, brownish, curly pieces, rich in quinine and quinidine.

e. **Columbian and Carthagena barks,** from *C. lucumifolia* and *lancifolia,* are imported in soft quills or broken pieces of very variable quality. The best qualities contain 2% to 3% of quinine, the inferior qualities contain about 2% of alkaloids, of which very little is quinine.

f. **Ledger bark,** from *Cinchona Ledgeriana,* is the richest in quinine of all cinchona barks, and is of the greatest commercial importance.

g. **Cuprea bark,** yielded by *Remijia pedunculata,* is not a true cinchona bark, and is the only known species of any other genus which yields quinine, though the allied alkaloid cinchonamine (page 547) has been found in *R. Purdieana.* Cuprea bark is peculiar in containing the interesting alkaloid cupreine[1] (page 548).

A concise description of the chief kinds of cinchona bark, with their distinguishing characteristics, has been published by W. Elborne (*Pharm. J.,* 1883 [*iii*], **14,** 653).

The *British Pharmacopœia* of 1898 gives the following as the characters of official (red) cinchona bark, from *Cinchona succirubra:*

"In quills, or more or less incurved pieces coated with the periderm, and varying in length from 2 inches to a foot (5 to 30 cm.) or more, the bark itself from about one-tenth to a quarter of an inch (2.5 to 6 mm) thick, or rarely more; outer surface brownish or reddish-brown in colour, more or less rough from longitudinal ridges which are most apparent in a branch bark, with numerous warts often running into lines in the larger pieces; in some varieties marked with numerous transverse cracks which have not thickened edges; inner surface brick-red or deep reddish-brown, irregularly and coarsely striated; fracture shortly fibrous in the smaller, and finely fibrous in the larger, pieces; powder brownish or reddish-brown; no marked odour; taste bitter and somewhat astringent."

The characters which conventionally determine the market-value of "druggists' quills" are often very fallacious, and have no relation to the real quality of the bark. A silvery coating on the epidermis of the bark is one of the points to which a factitious importance is attached,

[1] Formerly, the cinchona trees were invariably cut down and the bark stripped off and dried in the sun or on hurdles over a fire A greatly improved plan is to make longitudinal incisions in the bark of the growing tree, remove about half the bark, leaving the remainder intact, and cover the stem with moss Fresh bark is then formed very rapidly, and this renewed bark not only contains a larger percentage of total alkaloids than the original, but the alkaloids contain a very much larger proportion of quinine.

and renewed bark, though richer in alkaloid than natural, does not sell readily for druggists' purposes owing to the absence of the above characters, though it is readily bought by quinine manufacturers.

A specimen supposed to be one of cinchona bark can be readily identified as such by heating a small quantity in a test-tube, when a carmine-red or purple tar will be produced if the sample contain any of the cinchona alkaloids. Some kinds of cinchona bark are occasionally wholly destitute of alkaloids, and such do not give this indication, as it is produced only when a cinchona base is heated with woody fibre.

Composition of Cinchona Barks.

Cinchona barks contain, in addition to woody fibre, starch, gum, and mineral matters: the characteristic alkaloids; quinovin, and cinchona-red; cinchotannic, quinovic, quinic and oxalic acids; colouring-matters; wax, fat, and traces of volatile oil.[1]

Water extracts only a portion of the alkaloidal constituents of cinchona bark, and a hot infusion becomes turbid on cooling from the separation of sparingly soluble cinchotannates of the alkaloids. The solution obtained by treating cinchona bark with *acidified water* gives a white precipitate with tannin, a whitish precipitate with alkali hydroxides and a yellow crystalline precipitate with platinic chloride. Either of these precipitates yields the characteristic odour of quinoline when subjected to dry distillation.

The ash of cinchona barks from South American sources was found by Carles to contain a sensible amount of *copper*, but this metal was not detected by D. Hooper in the bark from trees cultivated in India (*Pharm. J.*, 1886 [*iii*], **17**, 545), though in other respects the general results are in agreement. The average total ash from upward of 300 specimens of Indian bark was found by Hooper to exceed 3%. Renewed and old natural barks contain less, but the proportion never falls below 2%. Young and branch barks give as much as 4% of ash, and the leaves from 5 to 6%. From 24 to 27% of the ash is soluble in water, and an additional 67 to 70% in acid, leaving 5 to 6% of silica insoluble.

The *British Pharmaceutical Codex* states that red cinchona bark "should not yield more than 5% of ash on incineration."

[1] A full list of the constituents of cinchona barks has been given by E. Léger in "*Les Alcaloïdes des Quinquinas*, 1896, pp. 7–9.

Quinovin, or chinovin, is an indifferent substance analogous to the glucosides which appears to be a constant constituent of the cinchonas, but in a proportion seldom exceeding 2%. It is dissolved on treating the bark with weak sodium hydroxide, and on adding hydrochloric acid to the solution is precipitated in admixture with quinovic acid and cinchona-red. Treatment with milk of lime dissolves the quinovin and quinovic acid, which are reprecipitated by an acid and separated by treatment with chloroform, which dissolves the quinovin only.

By boiling with dilute sulphuric acid for some time quinovin undergoes complete decomposition into quinovic acid and quinovose, a sugar isomeric with rhamnose. Two modifications of quinovin exist, α-quinovin and β-quinovin. They have been studied by Liebermann and Giesel (*Ber.*, 1883, **16**, 926; *Pharm. J.*, 1883 [*iii*], **14**, 987).

Quinovic acid, $C_{32}H_{48}O_6$, is constantly present in cinchona barks in small proportion, and forms a snow-white powder of tasteless needles or scales, quite insoluble in water, ether, or chloroform, and only very sparingly soluble in boiling alcohol or glacial acetic acid. It is best dissolved by adding ammonia to the alcohol, and may be reprecipitated by acetic acid. Quinovic acid decomposes carbonates, and is soluble in ammonia and solutions of the alkali hydroxides and earths, the solutions frothing like soap. The ammonium and calcium salts crystallise from alcohol in needles; the former salt losing its ammonia by exposure to air, or on boiling its solution. On adding an acid to an alkaline solution of quinovic acid, a hydrate of quinovic acid is thrown down as a very voluminous jelly, the whole contents of the vessel solidifying. In this form quinovic acid is very soluble in ether and alcohol. From the solution, the insoluble form of the acid separates in needles on standing. Quinovic acid gives with copper sulphate first a green colour and then a precipitate, and the latter, when washed, has a bitter metallic taste. When heated to about 300°, quinovic acid yields pyroquinovic acid, carbon dioxide, and secondary products.

Cinchotannin or cinchotannic acid, $C_{14}H_{16}O_9$, is a glucoside which is an important constituent of cinchona barks, in which it exists in the proportion of 3 to 4%. It is precipitated as a lead salt by the addition of lead acetate to a decoction of bark previously treated with magnesia to separate colouring-matter. The yellow precipitate when decomposed by hydrogen sulphide gives a solution of cinchotannic acid. It is a yellow, amorphous, very hygroscopic substance, very

soluble in water, alcohol, and ether; gives a green colour with ferric chloride; is precipitated by starch, albumin, gelatin, and tartar-emetic; is hydrolysed by dilute acids into glucose and cinchona-red; gives protocatechuic and acetic acids on fusion with potassium hydroxide; yields pyrocatechol on dry distillation; and is readily decomposed in presence of excess of alkalies, with formation of cinchona-red. The cinchotannates of the alkaloids existing naturally in cinchona bark are difficultly soluble in water, but dissolve readily in acidified water—probably with decomposition.

Cinchona-red or cinchofulvic acid, $C_{12}H_{14}O_7$, is the natural colouring-matter of red cinchona barks in which it is present sometimes to the extent of 10%, and from which it is extracted by treatment with alkalies. It is insoluble in water or ether, but sparingly soluble in alcohol. It is reprecipitated from its alkaline solution by hydrochloric acid. The solution also yields a red precipitate with barium chloride. It yields protocatechuic acid, $C_7H_6O_4$, on fusion with potassium hydroxide. Tschirch (*Woch. f. Chem. u. Pharm.*, 1905, **43**, 125) suggests that cinchona-red is formed by the action of an enzyme on a glucotannoid.

Quinic acid, or kinic acid, $C_7H_{12}O_6$, occurs in cinchona bark to the extent of 5 to 8%. It is prepared from the bark by boiling a quantity of the finely powdered material with dilute sulphuric acid. The liquid is filtered while hot, and to the filtrate freshly precipitated lead oxide is gradually added, until the liquid becomes neutral, and exhibits no longer a red but a pale yellow colour. If too little oxide be added, colouring matter remains in solution; if too much, basic lead quinate is thrown down. The filtrate is freed from lead by hydrogen sulphide, and filtered; calcium hydroxide is then added to precipitate the alkaloids, which are filtered off, the quinic acid in the filtrate being precipitated with basic lead acetate. The washed precipitate is suspended in water, and decomposed by hydrogen sulphide. The filtered liquid is evaporated to a syrup, which yields on cooling a crystalline mass of quinic acid. It crystallises in monoclinic prisms, m. p. 161.6°. It has a strong and purely acid taste, and is soluble in 2.5 parts of water, 35 parts of alcohol, and is almost insoluble in ether. Its solutions are lævorotatory. On dry distillation it yields quinol, phenol, benzene, benzoic acid, and salicyl-aldehyde. When distilled with manganese dioxide and sulphuric acid, or when its salts are heated, quinone, $C_6H_4O_2$, is obtained, which deposits in deep yellow

prisms on the cooler part of the apparatus. This reaction was proposed by Stenhouse as a test for true cinchona bark, but some false barks have been found to give the test.

The alkaloids are the most important constituents of cinchona barks, in which they exist in the form of cinchotannates and quinates.

The proportions of total alkaloids, as also the percentage of quinine, are extremely variable (see *Pharm. J.*, 1883 [*iii*], **14**, 444, 445, 458, 797, 810; 1884 [*iii*], **15**, 411, 453, 480), and chemical analysis is the only means of forming an opinion as to the richness of a specimen of bark. The highest yield of total alkaloid known is about 15%, and even at the present time barks containing less than 2% of total alkaloid come into the market. In eighty specimens of *Calisaya Ledgeriana*, from Java, Moens in 1879 found from 12.50 to 1.09% of total alkaloids, the quinine ranging from 11.6 to 0.8%. In the early days of cinchona cultivation 5% of total alkaloids was considered very high, but at the present time 10% of alkaloids is frequently found (D. Howard, *J. Soc. Chem. Ind.*, 1906, **25**, 97–99). In the same species of cinchona, the natural bark, mossed bark, and renewed bark contain very different percentages of quinine, the last being usually the richest; besides which the external conditions under which the trees are grown largely affect the relative and absolute proportions of the alkaloids in the bark.

Quinine and cinchonine are the cinchona alkaloids of the most frequent occurrence. Cinchonidine is hardly less common, and it occurs very largely in Indian-red bark. Quinidine is not very frequent, and is never present in large amount.

D. Howard has given analyses of barks from cultivated cinchona trees grown near Bagota, United States of Columbia (New Granada), and their characters have been described by E. M. Holmes (*Pharm. J.*, 1891 [*iii*], **22**, 875).

Analyses of a number of cinchona barks from Madras have been published by B. H. Paul (*Pharm. J.*, 1883 [*iii*], **14**, 666). D. Hooper gives the following as the percentage proportions of alkaloids in barks from trees grown on the plantations of the Madras Government:

Source of bark		Quinine sulphate	Quinine	Cinchonidine	Quinidine	Cinchonine	Amorphous alkaloids	Total alkaloids
Species	Description							
C. succirubra.	Natural ..	2 57	1 91	1 14	. .	2 11	0 88	6 04
C. succirubra.	Mossed ...	2 27	1 69	1 68	. ..	2 03	0.98	6.34
C. succirubra	Renewed	2 47	1 84	1.25	.....	1 48	0 71	5.28
C. succirubra	Branch...	1.85	1.38	1.59	. ...	2 28	1 16	6.41
C. succirubra ..	Root..	1 66	1.24	1 43	0 41	0 77	1 27	5.12
C. succirubra....	Renewed (shavings)	3.09	2.30	2.06	.....	1.16	1.45	6.97
C. robusta[1]... .	Natural . .	1 92	1.43	1 58	... ..	2.08	0 31	5.40
C robusta.... .	Mossed ..	2.58	1 92	0 77	... ..	3 16	0.35	6 20
C robusta.. .	Renewed .	5.92	4 40	0 51	.	2 54	1 65	9 10
C robusta.... .	Branch ..	2 20	1 64	1.17	.....	2 71	0 50	6.02
C. micrantha. ..	Natural .	....	. .	1 92	... .	. . .	0 40	2.32
C micrantha. ..	Renewed ..	Trace	Trace	1.12	.....	2 45	1.02	4 54
C. micrantha....	Branch .	. . .	.... ..	1 60	... ...	..... .	0 45	2 05
C. Calisaya.....	Natural..	1.62	1 21	2 13	.....	2 32	0 29	5 95
C. Calisaya... .	Branch...	0.79	0 59	1 93	.... .	0 73	0 48	3 73
C. Anglica[1].... .	Natural...	1 09	0 81	1.49	0.29	0.88	0.44	3 91
C. Anglica.. ..	Branch..	Trace	Trace	2 04	0 25	.....	0.36	2 65
C Ledgeriana .	Natural ..	7 38	5 49	0 82	... .	1 33	0 88	8 52
C. Ledgeriana .	Branch ..	2 97	2 21	1 07	. .	0 49	0 50	4.27
C. Javanica	Natural....	.....	.... .	2.64	1.32	.....	0.48	4.44
C Javanica .	Branch ..	.....	.	1 49	1 43	.. .	0 45	3.37
C. officinalis	Natural...	3.72	2 77	0 39	0 16	1.57	0.50	5.39
C. officinalis ...	Mossed .	4 57	3 40	0 45	0 20	1 50	0 62	6.17
C officinalis .	Renewed	5 66	4 21	0 65	0 22	0 85	0.70	6.63
C paludiana	Natural .	0 05	0.04	0 39	... ...	0 10	0.43	0.96
C. paludiana	Renewed	0 68	0 51	0 28	.....	1.19	0 87	2.85
C Pitayensis . .	Natural	3.14	2 34	1 93	1.10	0 56	0.39	6 32
C. Pitayensis .	Mossed .	5 12	3 81	1 91	0.63	0 95	0.37	7.67
C Pitayensis .	Renewed	3 36	2 50	2 33	0 78	0.52	0.55	6.68
C. Humboltiana	Natural .	3 01	2 24	0 49	Trace	1 55	0.90	5.18
C. Humboltiana	Renewed	1 72	1 28	0 43	. ..	0 64	1.07	3.42

Hooper (*Year-book Pharm.*, 1888, pages 430–438) gives the following as the average centesimal composition of the alkaloids from numerous species of the above barks.

	Red barks[2]	Crown barks	Hybrid barks
Quinine. .	22 2	55 9	41.2
Cinchonidine . . .	36 1	26 7	40.9
Quinidine	.... .	1 5	0.5
Cinchonine .	30 9	8 0	9.7
Amorphous alkaloids .	10 8	7.9	7.7
Total	100 0	100 0	100.0

[1] *Cinchona robusta* is a hybrid or cross between *C. succirubra* and *C. officinalis*, and *C. Anglica* between *C succirubra* and *C Calisaya* (W. T. Thiselton Dyer, *Pharm. J*, 1884 [*iii*], 15, 481).

[2] The mixed total alkaloids of red bark have been introduced into commerce under the name of "Quinetum." This preparation is completely soluble in warm, strong alcohol; 3.1 grm. dissolved in 10 c c. of normal hydrochloric acid should give a clear solution, which, on addition of 2 grm of Rochelle salt, must yield a precipitate equal in weight, after drying, to *at least* 65% of the quinetum taken. (From the Unofficial Formulary of the Dutch Society for the Advancement of Pharmacy, *Pharm J*, 1881 [*iii*], 12, 662.) "Quinetum sulphate" occurs in commerce in a perfectly crystallised form.

Assay of Cinchona Barks.

The complete assay of the various species of cinchona bark, with the view of ascertaining the proportion of the different alkaloids contained in them, is a process at once important and difficult. A great many methods have been proposed which in practised hands yield good results, but it is most difficult to give such a description that a chemist can get accurate results without much practise. Again, a process which is suitable when quinine is the chief alkaloid present becomes difficult of application when the cinchonidine is in excess.

In choosing a process of assaying cinchona bark, due consideration should be given to the kind of information required. Thus, a pharmacist desiring to know the alkaloidal strength of his bark will require a less accurate and elaborate process than a manufacturer buying bark for the extraction of quinine. Again, in some cases it is sufficient to estimate the percentage of total alkaloids, while in others it is very important to ascertain the proportion of crystallised sulphate of quinine which the bark is capable of yielding. On this account, it is desirable to discuss the estimation of the total alkaloids and of the actual quinine separately.

a. The *British Pharmacopœia* of 1898 prescribes the following standard of quality and method of assaying[1] red cinchona bark:

"**Test.**—When used for purposes other than that of obtaining the alkaloids or their salts, it should yield between 5 and 6% of total alkaloids, of which not less than half shall consist of quinine and cinchonidine,[2] as estimated by the following methods:

"Mix 20 grm. of red cinchona bark, in No. 60 powder, with 6 grm. of calcium hydroxide; slightly moisten the powder with 20 c.c of water, mix the whole intimately in a small porcelain dish or mortar, allow the mixture to stand for an hour or two, when it will present the characters of a moist, dark brown powder, in which there should be no lumps or visible white particles. Transfer this powder to a suitable flask fitted with a small reflux condenser, add 130 c.c. of benzolated amyl

[1] Based on a method devised by E R Squibb (*Ephemeris*, 1, 106)

[2] This is not a very exacting requirement Unfortunately no indication is given of the proportion of actual quinine which should be present The editors (*British Pharmacopœia*) omit to recognise red bark in shavings, the most common form in which it is met with in commerce, and notwithstanding that the shavings are often much superior, as regards the amount of quinine, to other forms. This together with the established prejudice as to the appearance of quills tends to favour the use of natural rather than the richer renewed bark, the general effect is to promote the use of the least valuable kinds of bark for pharmaceutical purposes In the present *Pharmacopœia* definition, the quinine standard of cinchona bark is reduced much below that of the 1867 edition, and only corresponds to a content of about 1% of quinine

alcohol,[1] boil them together for about half an hour, decant the liquid on to a filter, leaving the powder in the flask; add more of benzolated amyl alcohol to the powder, and boil and decant as before; repeat this operation a third time, then turn the contents of the flask on to the filter, and wash by percolation with more of the benzolated amyl alcohol until the bark is exhausted. Introduce the collected filtrate while still warm into a stoppered glass separator, add to it 2 c.c. of diluted hydrochloric acid, mixed with 12 c c. of water; shake them well together, and when the acid liquid has separated this may be drawn off, and the process repeated with distilled water slightly acidified with hydrochloric acid, until the whole of the alkaloids have been removed. The liquid should then while warm be carefully and exactly neutralised with solution of ammonia, and concentrated to the bulk of 16 c c. If now about 1.5 grm. of sodium potassium tartrate, dissolved in twice its weight of water, be added to the solution, and the mixture stirred with a glass rod, insoluble tartrates of quinine and cinchonidine will separate completely in about an hour, and these collected on a filter, washed, and dried in a water-oven, will contain 0.8 of their weight of the alkaloids, quinine and cinchonidine, which, multiplied by 5, gives the weight of those alkaloids present in 100 grm. of the bark. To the mother-liquor from the preceding process add solution of ammonia in slight excess. Collect, wash, and dry the precipitate,[2] which will contain the other alkaloids. The weight of this precipitate, multiplied by 5, and added to the percentage weight of the quinine and cinchonidine, gives the percentage of total alkaloids."

b. The following method of estimating the total alkaloids, and ether-soluble alkaloids, of cinchona bark is that of the *United States Pharmacopœia* of 1900, and with slight modifications is that of the *German Pharmacopœia* of 1900, and the *Japanese Pharmacopœia* of 1907. The estimation is carried out on 15 grm. of bark in No. 80 powder dried at 100°.

"Introduce the cinchona into an Erlenmeyer flask or bottle of about 200 c.c. capacity, and add a mixture of 125 c c. of ether and 25 c.c. of chloroform; then insert the stopper securely, shake the flask vigorously, and allow it to stand for 10 minutes. Then add 10 c.c. of ammonia water, and allow it to stand for 5 hours, shaking at frequent intervals.

[1] Prepared by mixing 3 volumes of benzene with 1 of amyl alcohol.
[2] It would be better to extract the alkaloids with chloroform.

Next add 15 c.c. of distilled water, shake the flask vigorously, and allow it to stand for a few minutes, so as to cause the powder to settle readily. When the supernatant liquid is quite clear, decant into a measuring flask or cylinder exactly 100 c.c. of the supernatant liquid (representing 10 grm. of cinchona), transfer this to a separator and add 15 c.c. of normal sulphuric acid, or sufficient to make the liquid distinctly acid. Shake the separator vigorously for 1 minute, and allow the 2 layers of liquid to separate completely. Draw off the lower aqueous layer into a flask Then add 5 c.c. of normal sulphuric acid and 5 c.c. of distilled water to the separator and shake it vigorously for about 1 minute, allow the liquids to separate as before, and again draw off the lower aqueous layer into the flask. Repeat the operation, using 5 c.c. of distilled water in the separator (without acid), drawing off the aqueous liquid into the flask. Filter the combined acid liquids into a measuring cylinder, and wash the filter and flask with enough distilled water to make the contents of the cylinder measure exactly 50 c.c. Pour half (25 c.c.) of the acid liquid into a separator marked No. 1, and the remaining half (25 c.c.) into another separator marked No. 2, which set aside.

"I. **For Anhydrous Cinchona Alkaloids.**—To separator No. 1 add 25 c.c. of a mixture of chloroform 3 vols. and ether 1 vol., also 5 c.c. of ammonia water, or sufficient to render the liquid alkaline. Insert the stopper and shake the separator carefully for 1 minute, and then draw off the lower layer into a tared flask or beaker. Add 20 c.c. more of the chloroform-ether mixture to the separator, insert the stopper, and shake the liquid carefully for 1 minute, again drawing off the lower layer into the tared flask. Repeat the operation with 10 c.c. of chloroform, and draw this off into the tared flask. Evaporate the chloroform-ether solutions in the tared flask or beaker slowly and carefully to dryness on a water-bath. Add 3 c.c. of ether to the dry residue, and again evaporate to dryness. Then place the flask or beaker in an air-bath and heat at 110° until the weight after cooling remains constant. This weight in grm. multiplied by 20 will give the percentage of anhydrous cinchona alkaloids (total alkaloids) in the cinchona.

"II. **For Ether-soluble Alkaloids.**—To separator No. 2, containing the other 25 c.c. of acid liquid, add 25 c.c. of ether and 5 c.c. of ammonia water, or sufficient to render the liquid alkaline. The temperature of the liquid should be kept below 20°, by cooling it, if necessary.

Shake the separator moderately for 2 minutes, and allow the liquid to stand for 10 minutes at 15°; after the liquids have separated, draw off and reject the lower aqueous layer and transfer the ethereal liquid to a tared beaker. Add 5 c.c. more of ether to the separator, rinse carefully and add the rinsings to the tared beaker. Evaporate the ether carefully by the aid of a water-bath, dry the beaker and contents in an air-bath at 110° for 2 hours, cool, and weigh. This weight in grm. multiplied by 20 gives the percentage of the anhydrous ether-soluble alkaloids contained in the cinchona. *Note.*—Ether-soluble alkaloids include quinine, quinidine, and cinchonidine."

Other methods for the estimation of the total alkaloids still in use by a few quinologists are the following:

c. That proposed by J. E. De Vrij, and adopted as the official method of the *United States Pharmacopœia* of 1887. It depends upon treatment of the powdered bark with quick-lime and water, the dried mixture being extracted with alcohol. The alcohol extract is acidified with sulphuric acid and the alcohol evaporated off. The acid liquid is made alkaline with sodium hydroxide and extracted with chloroform.

d. The method of Prollius (*Arch. Pharm.*, 1881, **219**, 85; 1882, **220**, 350), adopted by the *German Pharmacopœia* of 1882. The dry and powdered bark is extracted with a mixture of ether, alcohol and ammonia, from which extract the alkaloids are washed out with dilute hydrochloric acid, precipitated with sodium hydroxide, and extracted with chloroform.

e. The method of Hager, the accuracy of which was confirmed by O. Medin (*Zeitsch. anal. Chem.*, 1870, **8**, 477; 1872, **11**, 447). The dry powdered bark is boiled with potassium hydroxide solution (sp. gr. 1.35), then dilute sulphuric acid (sp. gr. 1.115) is added, and the whole boiled again. The residue is then allowed to settle, an aliquot portion of the liquid filtered, and the alkaloids contained therein precipitated as picrates with a cold, saturated, aqueous solution of picric acid.

A review of the various methods suggested for the estimation of quinine in barks, and in mixtures of cinchona alkaloids, has been given by Waldemar Hill, *Arch. Pharm.*, 1903, **241**, 54–110.

The following method is described by C. Zebel (*Chem. Zeit.*, 1891, **15**, 735) for the commercial extraction of the bark:

"The finely-powdered bark is ground to a thin paste with lime, sodium hydroxide, or sodium carbonate, and extracted with warm paraffin oil. On standing the oil separates, when it is run off and shaken with

sulphuric acid; this solution is boiled, and while boiling is neutralised with sodium carbonate and allowed to cool. Quinine sulphate crystallises out on cooling, while cinchonidine, cinchonine, and quinidine remain in solution as sulphates. The quinine sulphate is purified by recrystallisation after treatment with animal charcoal. The mother-liquor containing the other alkaloids is treated with sodium hydroxide and extracted with weak alcohol Of the 3 bases precipitated by the alkali, quinidine and cinchonidine are dissolved by the spirit, while cinchonine is left behind; the 2 former can then be separated by means of their neutral tartrates, that of quinidine being considerably the more soluble."

Chemically pure quinine is manufactured by preparing the acid sulphate, which after undergoing sufficient purification is reconverted into the neutral salt.

Separation of Cinchona Bases.

The separation of the alkaloids of cinchona and allied barks is an extremely complex operation, and as respects the rarer alkaloids outside the scope of this work. But the accurate separation even of the commoner alkaloids, such as is frequently required for commercial purposes, is very difficult, and its accurate performance presents special obstacles to an inexperienced analyst. In some cases it is sufficient to estimate the proportion of crystallisable quinine, but in other cases it is necessary to estimate also the cinchonine, cinchonidine, and occasionally the quinidine, quinamine, and amorphous alkaloids. For the separation of quinine from the admixed alkaloids, ether is usually employed, but the separation effected by this solvent is not an absolute one, all the free cinchona bases being more or less soluble in ether, especially in the presence of quinine. The anhydrous sulphates of quinine and cinchonidine are almost insoluble in chloroform free from alcohol (see page 487), but in presence of sulphate of cinchonine or quinidine sensible quantities pass into solution. Crystallisation of the quinine sulphate from water affords a simple and fairly accurate mode of separation, which has the advantage of being similar to the process employed by the manufacturer, and hence is regarded by many as furnishing the best proof of the yield likely to be obtained in practice. The following method of separating the quinine in the form of sulphate is described by J. Muter (*Analyst*, 1880, **5**, 223): Treat the total alkaloids, or the ether-residue from 20 grm. of bark, with

warm distilled water slightly acidified with dilute sulphuric acid, till the mixture is perceptibly acid. Add water to make 70 c.c. for each 1 grm. of alkaloids taken, and then heat and add very dilute sodium hydroxide with constant stirring till the liquid is exactly neutral, with a faint tendency to acidity. Digest the liquid at 85° for 5 minutes; then cool, and leave at 15° for 1 hour. Filter the liquid through a small double filter (2 3/4 in diam), the 2 filters being previously trimmed to equal weight, and receive the filtrate in a graduated cylinder. Wash carefully with water at 15° till the filtrate and washings measure 90 c c. for each 1 grm. of the mixed alkaloids. The filter and contents are now completely dried at 100° and weighed, the second filter being used as a counterpoise. To the weight in grm. add .000817 grm. for each c.c. of filtrate and washings. The sum divided by 0 855 gives the corresponding amount of crystallised sulphate, and this number multiplied by 5 gives the crystallised quinine sulphate obtainable from 100 grm. of dried bark

The quinine sulphate so obtained is apt to contain cinchonidine sulphate, and should be tested for this admixture as directed on page 522. The remaining alkaloids may be recovered from the mother-liquors by concentrating the liquid somewhat, adding sodium hydroxide in excess, and agitating with chloroform. On evaporating the chloroform, the bases will be obtained in a solid state, and may be separated as described on page 493.

The following scheme for the separation of the principal cinchona bases is founded on a method described by De Vrij (*Pharm. J*, 1871 [*iii*], **2**, 642). The process requires a considerable weight of alkaloids, and does not yield strictly accurate results. Traces of quinidine and cinchonidine are dissolved by the ether, and are only recovered on treatment of the amorphous alkaloids with a limited quantity of ether as directed.[1]

In presence of much quinine the solubility of cinchonidine in ether is notably increased.

The precipitation of the quinine as herepathite cannot be recommended, as the presence of cinchonidine, which is always obtained in notable quantity in the ethereal solution B, invalidates the result (*Pharm. J.*, 1881 [*iii*], **12**, 441, 1016; 1885, **16**, 205; 1886, **17**, 654). Moreover, in practice it is found difficult to free the crystallised here-

[1] The solubility of the cinchona bases in ether at 15° is given by A B Prescott as being: for quinine, 1 25, quinidine, 1 30, cinchonidine, 1 188, and for cinchonine, 1.371. The amorphous cinchona alkaloids are readily soluble in ether

SEPARATION OF CINCHONA ALKALOIDS. (De Vrij.)

A weight of not less than 2, and preferably 5, grm. of the mixed alkaloids in a free state is finely powdered,[1] and treated in a closed tube with 10 times its weight of ether (free from alcohol). The mixture is well shaken and left at rest for 12 hours, when it is filtered, and the residue washed with a small quantity of ether.

A The residue is dried and weighed It may contain *cinchonine*, *cinchonidine*, and *quinidine* It is dissolved in a slight excess of dilute acetic acid, and the solution rendered neutral to litmus by cautious addition of sodium hydroxide. The cinchonidine is then precipitated as tartrate, the quinidine as hydriodide, and the cinchonine as the free alkaloid, the operations being conducted exactly as described on page 494, with the exception that quinine and amorphous alkaloids having been previously removed, the processes and calculations necessitated by their presence may be omitted.

B The ethereal solution is evaporated to dryness, and the residue weighed. It consists of *quinine*, *amorphous alkaloids*, and *quinamine*, with heavy traces of *quinidine* and *cinchonidine*. It is dissolved in 10 parts of proof spirit acidified with 1/20 of sulphuric acid To this solution an alcoholic solution of iodine is gradually added as long as a precipitate is produced. Excess of iodine must be carefully avoided In presence of much quinine, a black precipitate of herepathite is immediately produced, but if the quantity is small some time is required for its appearance In such a case only a small quantity of iodine solution must be added, and the liquid well stirred, and left 12 hours. The precipitate is filtered off, and washed with strong alcohol

C The precipitate consists of herepathite It is dried at 100°, weighed. The weight, multiplied by 5505, gives the quantity of *quinine* in the mixed alkaloids operated upon The precipitate may also be treated with sodium thiosulphate or sulphurous acid and the alkaloid liberated by ammonia, extracted with ether, and titrated or weighed

D The solution is treated with sulphurous acid till colourless, and then carefully neutralised with sodium hydroxide. The alcohol is evaporated off, and the liquid treated with excess of sodium hydroxide or ammonia, and agitated with chloroform. The residue left on evaporating the chloroform consists of *amorphous alkaloids*, with considerable traces of *quinidine* and *cinchonidine*. The two latter will remain undissolved on treatment with a limited quantity of ether, and the amorphous alkaloids may be examined by De Vrij's test, described on page 545

[1] It is not always an easy matter to obtain the mixed alkaloids in a condition of fine powder, especially if their total amount has been determined by evaporating a chloroform or other solution in a flask. For the treatment of such residues, E. R. Squibb (*Ephemeris*, I, 111) recommends that 5 grm. of glass, which has been reduced in a mortar to a mixture of coarse and fine powder, should be placed in a flask containing the alkaloid, from 5 to 10 grm. of bark, this being followed by 5 c c of ether, of sp gr. not higher than 0 725. The flask is corked and shaken vigorously so as to grind up the alkaloidal residue and mix it thoroughly with the glass. Then pass the liquid through a very small filter (2.8 inches) previously wetted with ether, and wash with drops of ether delivered from a pipette till the filtrate measures 10 c.c. Change the receiver and continue the washing till another 10 c c. has been collected Then evaporate each portion to dryness, and correct the weight left by the first (quinine, etc) by that of the residue from the second, which represents the traces of quinidine, cinchonidine, etc , soluble in 10 c.c. of ether.

pathite from adhering iodine. To obviate this error, De Vrij proposed washing the crystals with a saturated solution of herepathite in 92% alcohol; but this introduces a new error of adhering herepathite solution. In precipitating quinine with an alcoholic solution of iodine, periodised iodosulphate of quinine is formed in part, and to avoid the error from this source De Vrij recommends the use as a precipitant the iodosulphate of "quinoïdine," in place of the alcoholic solution of iodine (*Pharm. J.*, 1875 [*iii*], **6**, 461; 1881 [*iii*], **12**, 601). The use of the iodosulphate of quinoïdine prevents any subsequent isolation of the amorphous alkaloids of the bark under examination.

Instead of converting the quinine in the ethereal solution B into herepathite, David Howard (*Watts' Dict. Chem.*, 2d ed., **2**, 177)agitates the ethereal liquid with excess of dilute sulphuric acid, and, after heating to boiling, adds dilute ammonia till neutral to litmus, using no more water than is necessary. On cooling, the *quinine* crystallises out almost entirely as sulphate, which salt is almost insoluble in a cold solution containing ammonium sulphate. The crystals are filtered off, washed with a little cold water, pressed between blotting-paper, and dried at 100°. 84.8 parts of the anhydrous salt represent 100 parts of the crystallised sulphate. The product should be tested for cinchonidine (page 519), which may be present in small quantity. The alkaloids existing in the mother-liquid from the quinine sulphate are then recovered by concentrating the liquid somewhat, adding sodium hydroxide in excess, and shaking with chloroform. The bases are extracted from the separated chloroform by dilute acetic acid, and the solution treated as in A.

The mixed alkaloids of yellow cinchona bark consists chiefly of quinine, and hence the portion soluble in ether represents the most useful constituents of the bark. Pale and red barks, on the other hand, contain a considerable proportion of alkaloids insoluble or sparingly soluble in ether. Hence the use of chloroform in the general process for assaying cinchona barks (see page 488).

In some cases, the alkaloids soluble in ether are contaminated to a considerable extent with colouring matter. In this event, the following is a good method of obtaining colourless quinine sulphate: The ether-residue is dried thoroughly and weighed. It is then dissolved in 30 c.c. of absolute alcohol, and $N/10$ sulphuric acid cautiously added from a burette, till the liquid is neutral or *very* faintly acid to litmus-paper. Each c.c. is equivalent to 0.0324 grm. of anhydrous

alkaloids. The liquid is next evaporated nearly to dryness, and a volume of $N/10$ sulphuric acid added equal to that previously required for neutralisation. 30 c.c. of hot water are added, and the liquid boiled till complete solution results. Purified animal charcoal is next added, in quantity equal to the weight of the ether-residue, the liquid heated on the water-bath for 20 minutes, filtered, and the residue washed twice with boiling water acidified with sulphuric acid. The filtrate is brought to a concentration of 70 c.c. for each 1 grm. of ether-residue taken, and then cautiously neutralised with sodium hydroxide while boiling.

Instead of commencing the separation of the alkaloids by ether, Moens recommends that the neutral solution of the mixed alkaloids should be treated with excess of solution of potassium sodium tartrate (Rochelle salt), which throws down the quinine and cinchonidine as tartrates. The same procedure is adopted in the *British Pharmacopœia* (see page 486). The precipitated tartrates are washed with a little cold water, decomposed by excess of alkali, and the quinine and cinchonidine separated by ether; the quinine dissolved being either directly weighed, or, preferably, converted into sulphate and tested for cinchonidine (page 519). The tartrate mother-liquor is then tested for cinchonine and quinidine as described on page 539.

The following method for the separation of the cinchona bases insoluble, or nearly insoluble, in ether may be applied to the residue left on treatment of the mixed alkaloids with ether, as in De Vrij's process (page 492). It may also be applied directly to the mixed alkaloids extracted from a sample of bark, in which case it may be carried on simultaneously with Muter's process for the production of crystallised quinine sulphate as described on page 493.

This process, with experience, gives very good results, the sum of the separated alkaloids frequently amounting to 99% of the mixed bases operated on. It is well suited for the assay of Indian barks. The least satisfactory part of the process is the separation of the cinchonine from the amorphous bases by dilute spirit. The presence of much quinidine prevents the complete precipitation of the cinchonidine and quinine as tartrates; while the precipitate with potassium iodide, if tenacious or resinous instead of crystalline, contains cinchonine, with or without quinidine. By the moderate addition of alcohol the cinchonine may be kept in solution, and the quinidine obtained in a crystalline state.

ANALYSIS OF CINCHONA BASES. SEPARATION OF CINCHONINE, CINCHONIDINE, AND QUINIDINE.

The mixed alkaloids extracted from the bark, or from the filtrate from the crystallised quinine sulphate by treatment with sodium hydroxide and chloroform, or the residual alkaloids left on treating the total alkaloids with ether (see page 492), are dissolved in dilute sulphuric acid, and the solution exactly neutralised by sodium hydroxide A saturated solution of Rochelle salt is next added in excess, the liquid cooled to 15°, and repeatedly stirred during 1 hour Crystalline streaks in the track of the glass rod consist of cinchonidine (or quinine) tartrate The precipitate is collected on a double tared filter, and washed first with a 5% solution of Rochelle salt and then with a little cold water, the filtrate and washings being collected in a graduated cylinder

The precipitate is dried at 100 to 105° and weighed, the outer filter being used as a counterpoise The amount found is corrected by adding 00083 grm for each 1 c c measured by the filtrate and wash-water The sum multiplied by 0 797 gives the weight of *cinchonidine* If quinine has not previously been separated, the amount of crystallised sulphate found must be multiplied by 915, and the product subtracted from the weight of the tartrate before calculating it to cinchonidine A preferable plan is to dissolve the precipitate off the filter with dilute sulphuric acid, add ammonia, and extract with ether, weighing or titrating the alkaloid

A The filtrate is concentrated to its original bulk, cooled, a drop of dilute acetic acid added, and then excess of a saturated solution of potassium iodide (free from any alkaline reaction) The liquid is left for 2 hours at 15°, being frequently stirred Any streaks in the track of the glass rod are produced by quinidine hydriodide The liquid is filtered on a double counterpoised filter, and the precipitate cautiously washed with cold water

The precipitate is dried at 100° and weighed Its weight is corrected by the addition of 00077 grm for each 1 c c of filtrate and washings (B) The sum, multiplied by .7168, gives the *quinidine*, or the precipitate may be decomposed by ammonia, the alkaloid extracted by ether, and weighed

B Filtrate is measured and made distinctly alkaline with sodium hydroxide, and the precipitated *cinchonine* is extracted by agitation with chloroform, which is separated, evaporated, and the residue weighed The weight found is corrected by deducting 00052 for each 1 c c measured by filtrate A, and 00066 for each c c of filtrate B Any *amorphous alkaloid* may be dissolved out by alcohol of 0 94 sp gr

Titration of Cinchona Alkaloids.

Many chemists have investigated the estimation of cinchona alkaloids by titration with various standard solutions, using different indicators. The general conclusion arrived at is that, although a great saving of time may be effected by titration methods, they do not compare favourably for accuracy with methods depending on the weighing of the alkaloids. The molecular weights of the alkaloids being very high a small error in titration makes a large error in the amount of alkaloid found. Moreover, a pure neutral salt of an alkaloid gives an alkaline indication to some indicators, the alkalinity increasing with the temperature of the solutions. Tutin (*Pharm. J.*, 1909 [*iv*], **29**, 600) prepared a solution of quinine sulphate giving an alkaline indication when hot, and an acid indication when cold. For these reasons, gravimetric methods are preferable to volumetric methods where strict accuracy is required.

Quinine sulphate of commerce, having, when anhydrous, the formula $(C_{20}H_{24}O_2N_2)_2.H_2SO_4$, is practically neutral to brazil-wood, lacmoid, cochineal, or hæmatoxylin, but is strongly alkaline to methyl-orange, the end-point with the last indicator corresponding to the formation of the readily-soluble *acid* sulphate of quinine, $C_{20}H_{24}O_2N_2.H_2SO_4$. Hence twice the volume of standard acid will be required by a given weight of quinine when Methyl-Orange is employed, as when the other indicators are used. Phenolphthaleïn is useless as an indicator for the cinchona alkaloids, and cinchonine and cinchonidine cannot be accurately estimated by titration (Allen, *Analyst*, 1896, **21**, 85–87).

The directions in the table on page 492 can be modified with considerable saving of time by titrating the alkaloids. Thus the tartrate of cinchonidine is dissolved in boiling water, and the liquid titrated with $N/20$ potassium hydroxide, each 1 c.c. of which represents 0.0147 grm. of cinchonidine (or other alkaloid) precipitated as tartrate. The quinidine iodide, which should be washed with a little of the precipitant instead of with water, is immersed with the filter in boiling water, the solution then being titrated with $N/20$ potassium hydroxide. Each 1. c.c. of the standard solution represents 0.0162 grm. of quinidine precipitated as hydriodide.[1] Phenolphthaleïn is used as indicator in these titrations as the free alkaloids have no action on phe-

[1] This procedure does not dispense with the necessity of making a correction for the amount of quinidine lost in the mother-liquor and washings.

nolphthaleïn, but, as stated by Allen (see above), the results obtained are not accurate.

The following is the official method of the *German Pharmacopœia*, 1900, for titrating the total alkaloids of red cinchona bark:

To 12 grm. of the bark in fine powder, dried at 100°, add 90 grm. of ether, and 30 grm. of chloroform. Shake with 10 c.c. of sodium hydroxide solution, allow to stand for 3 hours, and then let the powder collect by adding 10 c.c. of water. Filter 100 grm. of the clear chloroform-ether solution into a small flask, and distil off half of it. Put the remaining half into a separator, and wash the small flask 3 times with 5 c.c. of a mixture of 3 parts of ether and 1 part of chloroform, and add the washings to the separator. Shake the mixture with 25 c.c of $N/10$ hydrochloric acid, if necessary, with the addition of a little ether. When complete separation of the chloroform-ether takes place, run off the lower acid layer and filter it into a 100 c.c. flask. Add 10 c c. of water, successively 3 times, to the chloroform-ether solution and shake, the aqueous layer being separated and filtered each time through the original filter paper into the 100 c.c. flask. Finally wash the filter paper with water, mix the whole filtrates and washings, and make the mixture up to 100 c.c. with water. To 50 c.c. of this solution add 1 c.c. of alcohol in which a small piece of hæmatoxylin has been freshly dissolved, and titrate the resulting yellowish solution with $N/10$ potassium hydroxide solution. Not more than 4.3 c.c. of the latter solution should be ruquired in order to colour it immediately bluish-violet.

The above test requires the bark to contain at least 5.3% of total alkaloids. In the *German Pharmacopœia* of 1910 this method is modified in a few details, and the bark is required to contain 6.5% of total alkaloids. J. Messner (*Zeitsch. angew. Chem.*, 1903, **16**, 444–450, 468–477) recommends pure lacmoid as the best indicator to use.

J. Katz (*Ber. deutsch. pharm. Ges.*, 1910, **20**, 316–330) obtained good results by titrating the cinchona alkaloids in 90% alcoholic solution with $N/10$ alcoholic potassium hydroxide solution using Parrier's blue as indicated.

C. Kippenberger (*Zeitsch. anal. Chem*, 1896, **35**, 422) has suggested the use of $N/20$ iodine solution in 6–8% potassium iodide solution, which has been standardised against quinine, for the titration of the cinchona alkaloids. Quinine being a diacid base, its periodide is $C_{20}H_{24}O_2N_2(HI)_2,I_4$.

A valuable series of experiments on the titration of alkaloids as existing in pharmacopœial tinctures, in which several indicators were employed and compared, has been published by Farr and Wright (*Pharm. J.*, 1894 [*iii*], **25**, 124).

Engelhardt and Jones (*Pharm. J.*, 1910 [*iv*], **30**, 236) recommend that the anhydrous cinchona alkaloids, obtained on extracting a sample of the bark, be dissolved in a mixture of equal parts of alcohol and ether, and, after the addition of water, titrated with $N/10$ acid using hæmatoxylin as indicator. They obtained good results with the four anhydrous alkaloids, quinine, quinidine, cinchonidine and cinchonine.

Cinchona Alkaloids.

Any satisfactory classification of the cinchona bases in the present imperfect state of our knowledge of their constitution, and in some cases even of their empirical formulæ, is manifestly impossible. Isomerism is common, and on slight provocation quinine and some others suffer polymerisation, with or without losing the elements of water, forming amorphous "apo-" or anhydro-bases.

Perhaps the most suggestive method of classifying the cinchona bases and their allies is to arrange them according to the number of atoms of oxygen in the molecule, and subdivide these classes according to other analogies.

The following (pages 499, 500) is a tabular list of the alkaloids hitherto isolated from the various species of cinchona and allied barks. It contains the names of all the natural cinchona bases, the existence of which as chemical individuals has been fairly well established up to the present time; but it must not be supposed to include all that actually exist.

As is evident from the table, isomerism is very common among the cinchona bases. Thus the two best-known bases are qui*nine* and cincho*nine*. Isomerides of these bases coexist with them in the bark, and are called respectively quin*idine* and cinchon*idine*.

These 4 bases are the chief crystallisable alkaloids of cinchona barks, but there exist with them, or are formed in the process of manufacture, certain amorphous isomerides called respectively quin*icine* and cincho*nicine*. It is doubtful how far these bases pre-exist in the bark, the natural amorphous alkaloids being probably the anhydro-derivatives *diquinicine* and *dicinchonicine*, and distinct from the amorphous

TABLE OF CINCHONA BASES.

Alkaloid	Formula	Chief source	m p	Optical rotation[1] $[\alpha]_D$	Other characters
I. Cinchonine Class: Paricine	$C_{16}H_{18}ON_2$	*C lutea* and *C succirubra* from Darjeeling	130°	0	Pale yellow, amorphous, bitter powder. Sparingly soluble nitrate.
Cinchotine, or Hydrocinchonine, Cinchonamine	$C_{19}H_{24}ON_2$	Crude cinchonine sulphate	286°	+	Slender prisms and scales.
		Remijia Purdieana	194°	+121 1	Very poisonous Hexagonal prisms No thalleioquin reaction. Page 547
Hydrocinchonidine, or Cinchamidine		Cinchonidine sulphate mother-liquors	230°	−98 4	Plates or flat needles. Not fluorescent.
Cinchonine	$C_{19}H_{22}ON_2$	Various species of *Cinchona*	264°	+226 5 (c=0 5)	Rhombic prisms. Page 540
Cinchonidine		Almost always present *C rubra*, especially	207°	−70° (chloroform, c=4, 15°)	Prisms Page 557.
Homocinchonidine		With cinchonidine	207°	−107	Prisms or plates Not fluorescent No thalleioquin reaction.
Cinchonicine		By heating cinchonine	50°	+47 2	Amorphous Crystallisable salts Page 544
Paytine	$C_{21}H_{24}ON_2$	From white bark of Payta	156°	−49 5 (c=0 45, 96 % alcohol 15°)	Crystallises with 1 H_2O in fine prisms
Paytamine					Amorphous Amorphous salts.
II. Quinamine Class: Quinamine	$C_{19}H_{24}O_2N_2$	*C succirubra* from Brit India and Java	172°	+104 5	Long prisms Page 536
Conguinamine		*C officinalis*, *C calisaya*	121°	+204 6	Long, shining, triclinic prisms. Page 537
Javanine		*C calisaya* from Java			Rhombic plates Solution in H_2SO_4 intensely yellow
Cupreine	$C_{19}H_{22}O_2N_2$	*C cuprea* or *Remijia pedunculata*	198°	−175 3 (c=1 5, 16°)	Grouped prisms. Page 548

[1] Except where otherwise stated the solvent is 97% alcohol, c=2, temp =15°.

TABLE OF CINCHONA BASES.—CONTINUED.

Alkaloid	Formula	Chief source	m. p.	Optical rotation[1] $[\alpha]_D$	Other characters
III. Quinine Class:					
Hydroquinine	$C_{20}H_{26}O_2N_2$	In mother-liquors from quinine sulphate	172°	−142 2 (c=2 4, 95% alcohol at 20°).	Bitter needles Fluorescent. Thalleioquin reaction. Page 537.
Hydroquinidine . .			166°	+	Needles or tables Fluorescent. Thalleioquin reaction.
Quinine . .	$C_{20}H_{24}O_2N_2$	*Calisaya officinalis*, etc	172°	−145 2	Page 507. $[\alpha]_D^{15}$ = −145 2+ 0 657c, c=1 to 10 in 97% alcohol
Quinidine . . .			168°	+236 8	Page 535
Quinicine . . .		By heating quinine sulphate.	60°	+44 1 (chloroform)	Amorphous or oil Non-fluorescent Page 543.
IV. Cusconine Class:					
Chairamine ...	$C_{22}H_{26}O_4N_2$	*Remijia Purdieana* (Cusco or False Cuprea Bark)	233°	about + 100	Needles or prisms containing $1H_2O$
Conchairamine .			120°	+68 4	Prisms.
Chairamidine			127°	+7 3 (c=3)	Amorphous powder.
Conchairamidine .			114°	60 (c=3)	Crystallisable
Concusconine .			206° (anhydrous)	+	Monoclinic prisms with $1H_2O$.
Aricine . .			188°	−58 2	Non-bitter, shining prisms Acetate crystalline, nearly insoluble in cold water
Cusconine . . .			110°	−54 3	Monoclinic plates with $2H_2O$ Not fluorescent
Cusconidine					Yellow, amorphous
Cuscamine ..	.	—*C. Pelleterana* . .	218		Flat prisms.
Cuscamidine. .					... .
V. Anhydro-bases: Dicinchonicine	$C_{38}H_{44}O_2N_4$?	*C rosulenta* and *C succirubra*	40	+66	Yellowish, amorphous No thalleioquin reaction. Page 545
Diquinicine	$C_{40}H_{46}O_2N_4$?	*C rosulenta* and "quinoidine"		+	Amorphous, and amorphous salts. Fluorescent. Thalleioquin reaction. Page 545

[1] Except where otherwise stated the solvent is 97% alcohol, c=2, temp.=15°.

products formed from the crystallisable bases by the action of heat or acids.

In addition to these isomers and anhydro-derivatives of the cinchona bases, there exist various homologues and isologues of them. Quinine itself is probably a methyl-cupreine and a methoxy-cinchonine.

Certain of the cinchona bases (*e. g.*, cupreine) exhibit a remarkable tendency to form stable crystalline compounds with other of the bases. It is probable that the existence of these remarkable compounds, having different physical properties in the form of salts as well as in the free state, has led to the isolation and description of various bases which will hereafter be found to be compounds.

The less important cinchona bases have no recognised position in commerce or medicine, but they are liable to be present to a greater or less extent in specimens of commercial alkaloids called by the better-known names. Commercial quinine is liable to retain traces of cinchonine, quinidine and hydroquinine, and generally contains notable proportions of cinchonidine. Hydrocinchonidine is sometimes present in commercial cinchonidine, hydrocinchonine is usually present in commercial cinchonine, while quinidine contains hydroquinidine and hydroquinine. Quinamine and conquinamine are probably not unfrequently present in commercial cinchona alkaloids.

Constitution.

The constitution of the cinchona bases is at present very imperfectly understood. Cinchonine, cinchonidine and cinchonicine have the same empirical formula $C_{19}H_{22}ON_2$, while quinine, quinidine and quinicine have the formula $C_{20}H_{24}O_2N_2$. Cinchonine and cinchonidine, quinine and quinidine are physical isomerides, their differences probably depending upon an asymmetric carbon atom in the molecule. Cinchonicine differs from cinchonine, and quinicine from quinine by a molecular change of the group $=N-COH$ into $=NH-CO$. Quinine probably differs from cinchonine by the replacement of a hydrogen atom by the methoxyl group, $-OCH_3$. The cinchona bases known to contain hydroxyl or methoxyl groups are:

Cinchonine and cinchonidine, $C_{19}H_{21}N_2(OH)$;
Quinine and quinidine, $C_{19}H_{20}N_2(OH)(OCH_3)$;
Quinicine, $C_{19}H_{21}ON_2(OCH_3)$;
Hydrocinchonine and hydrocinchonidine, $C_{19}H_{23}N_2(OH)$;
Hydroquinine and hydroquinidine, $C_{19}H_{22}N_2(OH)(OCH_3)$;

Cupreine, $C_{19}H_{20}N_2(OH)_2$;
Quinamine, $C_{19}H_{22}N_2(OH)_2$
Concusconine, $C_{21}H_{22}O_2N_2(OCH_3)_2$.

Cinchonicine is a ketone. Cupreine has been converted into quinine by heating it at 100°, under pressure, with metallic sodium and a solution of methyl chloride in methyl alcohol (Grimaux and Arnold, *Compt. Rend.*, 1891, **112**, 774; **114**, 672).

The oxidation products of the cinchona bases, especially those of cinchonine, have been very fully studied with the view of throwing light on the constitution of the bases. On careful oxidation with permanganate, cinchonine yields formic acid, CH_2O_2, and *cinchotenine*, $C_{18}H_{20}O_3N_2$. On oxidation with chromic acid it yields *cinchoninic acid*, C_9H_6NCOOH, and a syrupy mass containing *cincholoiponic acid*, $C_8H_{13}O_4N$ (a pipecolinedicarboxylic acid), *loiponic acid*, $C_7H_{11}O_4N$ (a pyridinedicarboxylic acid), and *meroquinene*, $C_9H_{15}O_2N$ (which is *cincholoiponic acid* in which the group $-CH=CH_2$ takes the place of a $-COOH$ group). The constitutional formulæ of all these acids have been determined. Cinchoninic acid has been shown to be the decomposition product of one-half of the cinchonine molecule ($C_9H_6N.CH_2$), while the other 3 acids are the products of the second half of the molecule ($C_9H_{13}OHN$). The final products of oxidation are *cinchonic acid* (a pyridinecarboxylic acid), and *cinchomeronic acid*, $C_5H_3N(COOH)_2$, (a pyridinedicarboxylic acid). Cinchonidine and cinchonicine yield products of oxidation similar to, or isomeric with, those of cinchonine. Cinchonine has been proved to contain a hydroxyl group (probably linked to the carbon atom next to the nitrogen), and an ethylene group, both in the "second half" of the molecule.

Quinine on oxidation with chromic acid yields *quininic acid*, $C_{11}H_9O_3N$ (which is *p-methoxycinchoninic acid*), and *cincholoiponic acid*. It is assumed therefore that quinine only differs from cinchonine by a methoxyl group in the para position in the first half of the molecule. Quinidine and quinicine yield products of oxidation similar to, or isomeric with, those obtained from quinine.

Cinchonine on fusion with potassium hydroxide yields, among other products, quinoline; while quinine on similar treatment yields quinolidine (paramethoxyquinoline).

The conclusion derivable from the researches on the constitution

of the cinchona bases is that they are derivatives of a hydroquinoline of which probably only one side is hydrogenated. The following formulæ illustrate these deductions:

Quinoline, C_9H_7N.
Hydroxyquinoline, $C_9H_8(OH)N$.
Tetrahydroquinoline, $C_9H_{10}NH$.
Diquinoline, $C_9H_7N.C_9H_7N$.
Diquinolyline, $C_9H_{10}N.C_9H_{10}N$.
Cinchonine, $C_9H_6N.CH_2.C_7H_{10}(OH)(CH=CH_2)N$.
Quinine, $C_9H_5(OCH_3)N.CH_2.C_7H_{10}(OH)(CH=CH_2)N$.

Although the knowledge of the constitution of the cinchona bases is not yet sufficiently perfect to allow of their formation from pyridine or quinoline, it is interesting to note that two distinct basic substances isomeric with quinine have been prepared synthetically. One of these discovered by C. A. Kohn (*J. Soc. Chem. Ind.*, 1889, **8**, 959) has the constitution of an *α-hydroxy-hydroethylene-quinoline*, $C_9H_{10}(OH)N.C_2H_4.N.C_9H_{10}(OH)$. It was obtained by the action of 1 molecule of ethylene dibromide on 2 molecules of α-1′-hydroxy hydroquinoline, obtained by reducing hydroxyquinoline by tin and hydrochloric acid. It is a weak base, forming small glittering prisms which melt at 223°, and are readily soluble in chloroform and benzene, with difficulty in hot alcohol and insoluble in water. It has weak antipyretic characters.

The other synthetical isomer of quinine was prepared by Wallach and Otto (*Annalen*, 1891, **253**, 251) by the action of β-naphthylamine on pinol nitrosochloride: $C_{10}H_7NH_2+C_{10}H_{16}ONOCl=HCl+C_{20}H_{24}O_2N_2$. The product is a basic crystalline substance, m. p. 194–195°, insoluble in water, slightly soluble in hot spirit, and readily soluble in ether. The solutions, both of the base and its salts, are strongly fluorescent.

An excellent summary of all the research work that has been carried out in order to investigate the constitution of the cinchona alkaloids, and their decomposition and addition products, has been written by Ezio Comanducci (*Sammlung chem. u. chem. techn. Vorträge*, 1911, **16**, Bände 4–7).

General Properties of Cinchona Bases.

The cinchona alkaloids all have well-defined basic characters, some of them being sufficiently powerful to displace ammonia from its compounds. Their salts are usually crystallisable.

In the free state, the cinchona alkaloids are colourless or faintly yellow solids, often readily fusible, but not volatile without decomposition. Cinchonine, however, may be distilled in an atmosphere of hydrogen, or *in vacuo*, without decomposition. They are generally but slightly soluble in water, but dissolve more readily in alcohol, and generally with great facility in ether and chloroform. Such as are soluble in the last two liquids are removed from their ammoniacal solutions by agitation with ether or chloroform, but in no case will ether or chloroform remove them from an aqueous *solution* acidified with sulphuric or hydrochloric acid. On the other hand, the anhydrous sulphates of many of the cinchona alkaloids are soluble in chloroform, and still more readily in a mixture of chloroform and absolute alcohol. This fact is sometimes utilised for detecting adulterations (page 525).

The solutions of some of the cinchona alkaloids in excess of dilute sulphuric acid exhibit a strong blue fluorescence, which is visible even in very dilute liquids. This fluorescence is destroyed by adding an excess of sodium chloride or other haloid salt.

The solutions of the cinchona alkaloids exert a well-marked rotatory action on polarised light, the rotation being in some cases right- and in others left-handed. The specific rotation is affected in a remarkable manner by the solvent employed and by the proportion of free acid present, which circumstances greatly reduce the practical value of the optical activity for the identification and quantitative estimation of the unmixed alkaloids.

On adding a fixed alkali, alkaline carbonate or ammonia to the solution of a salt of one of the cinchona bases, the sparingly soluble alkaloid is usually separated in a free state, and is in some cases soluble in an excess of the precipitant. On agitating the alkaline liquid with chloroform, the precipitated alkaloid is usually dissolved,[1] and may be recovered in a free state by separating the chloroform, and evaporating it to dryness at a steam-heat. By adding more chloroform to the aqueous liquid, and repeating the agitation, the complete extraction of the alkaloid may be ensured, and the process made quantitative. Ether may be substituted for chloroform in the case of quinine and other alkaloids readily dissolved by it.

The cinchona bases are tertiary amines; for when treated with an alkyl iodide they form additive-compounds which are converted by

[1] This is not the case with cupreine and some other alkaloids, which form definite compounds with the fixed alkalies in the same manner as morphine.

treatment with oxide of silver into powerful soluble bases analogous to tetrethyl-ammonium hydroxide.

Many of the cinchona alkaloids form two series of salts; neutral (improperly called "basic"), and acid salts. The neutral sulphates of the cinchona alkaloids have, when anhydrous, the general formula $B_2H_2SO_4$. They have a neutral reaction to most indicators, but are strongly alkaline to Methyl-Orange, and are generally very sparingly soluble in water; but the corresponding acid or bisulphates (BH_2SO_4) are generally readily soluble. From quinine, cinchonidine and cupreine still more acid sulphates have been prepared of the formula $B.2H_2SO_4$.

The sulphates of many of the cinchona bases possess the property of combining with iodine, the compounds produced being in some cases of a very complex character. Certain of these "iodosulphates," of which the quinine compound or herepathite is the type, possess the remarkable optical properties of the tourmaline (see page 513).

When a salt of one of the natural cinchona bases is heated for a prolonged period to a high temperature, the alkaloid undergoes a curious change. It becomes incapable of crystallising, a property sometimes extending to its salts. The change occurs most readily by exposing the acid sulphate of the alkaloid to a temperature of 100° till anhydrous, and then increasing the heat for some time to about 130°; or by similarly heating a mixture of neutral sulphate and glycerin. *Dilute acid* solutions of the alkaloids heated under pressure do not undergo this change, which fact indicates that the change is due to physical, and not to chemical, conditions. No means are at present known by which the modified alkaloid can be restored to its original crystallisable condition

When the cinchona bases are heated with strong hydrochloric acid (sp. gr. 1.125) to 150° for 6 to 10 hours, they are converted into apo- or anhydro-derivatives of basic character, the change in the case of quinine and quinidine being attended with evolution of methyl chloride (Hesse, *Annalen*, 1880, **205**, 314).

When the sulphates of quinine, cinchonine, and cinchonidine are dissolved in concentrated sulphuric acid at the ordinary temperature, they are converted into "iso-bases" (Hesse, *Annalen*, 1888, **243**, 131), which differ in several respects from the parent alkaloids. Hydroquinine, hydroquinidine, and hydrocinchonidine are converted by the same treatment into the corresponding sulphonic acids, which are compounds of distinct basic character.

With platinic chloride, the hydrochlorides of the cinchona bases form platinichlorides of the general formula BH_2PtCl_6, but many of them also form salts containing $B_2H_2PtCl_6$. Salts of this constitution are produced on adding sodio-platinic chloride to neutral solutions of quinine, quinidine, cinchonidine, and homocinchonidine (Hesse, *Annalen*, 1881, **207**, 309). The auri-chlorides of the cinchona bases are mostly unstable, and liable to speedy decomposition with separation of finely-divided metallic gold.

Certain of the cinchona bases give a deep green colouration or precipitate when their solutions are treated with chlorine or bromine water, and ammonia is subsequently added. This reaction is known as the "thalleioquin test" (see also page 511).

Most of the cinchona bases are very completely precipitated by tannic and picric acids, potassio-mercuric iodide, and certain other reagents. These reactions are sometimes used for their detection and separation.

On treatment in solution with bromine-water in slight excess, the cinchona bases are converted into bromo-derivatives. The number of atoms of bromine taken up varies with the constitution of the alkaloid. According to T. Fawssett (*Pharm. J.*, 1888 [*iii*], **19**, 915), quinine, quinidine, and cupreine react with approximately Br_6, hydroquinine with Br_4, and cinchonine, cinchonidine, and "amorphous quinine, with Br_2. On heating the cinchona bases, or their hydrochlorides or sulphates, with acetic anhydride to about 80° for a few hours, they are converted into acetyl-derivatives (Wright and Beckett, *J. Chem. Soc.*, 1876, **29**, 655; O. Hesse, *Annalen*, 1880, **205**, 314). With the exception of the acetyl-derivative of quinine, all these compounds are amorphous. They can be dried at 100° without change, are readily soluble in dilute acids, and are thrown down as resinous precipitates by alkalies. On treatment with alcoholic potassium hydroxide they are hydrolysed into acetic acid and the original bases. The acetyl-derivatives of quinine and quinidine give the thalleioquin reaction.

The more important properties of the leading cinchona alkaloids may be summarised as follows:

A {
- *Hydrated crystals* are formed by Quinine, Quinidine, Paytine, Cupreine, Cusconine, Chairamine.
- *Anhydrous crystals* are formed by Cinchonine, Cinchonidine, Quinamine.
- *No crystals* are formed by Paricine, Quinicine, Diquinicine, Dicinchonicine.

B { *Readily soluble in Ether.*—Quinine, Quinamine, Paytine, Quinicine, Javanine.
Sparingly soluble in Ether.—Cinchonidine, Quinidine, Cupreine.
Almost insoluble in Ether.—Cinchonine.

C { *Dextrorotatory* solutions in alcohol are formed by Cinchonine, Cinchonamine, Quinamine, Quinidine, Chairamine, Quinicine, Diquinicine.
Lævorotatory solutions in alcohol are formed by Cinchonidine, Hydro-cinchonidine, Homocinchonidine, Paytine, Cupreine, Quinine, Hydroquinine, Cusconine, Aricine.

D { *Fluorescent* solutions in dilute sulphuric acid are formed by Quinine, Quinidine, Hydroquinine, Hydroquinidine, Diquinicine.
No fluorescence is exhibited by solutions of Cinchonine, Cinchonidine, Hydrocinchonidine, Homocinchonidine, Quinamine, Quinicine, Dicinchonicine, Cusconine, Cupreine.

E { *Thalleioquin* is formed by Quinine, Quinidine, Quinicine, Diquinicine, Hydroquinine, Hydroquinidine, Cupreine.
Thalleioquin is *not* formed by Apoquinidine, Cinchonine, Cinchonidine, Homocinchonidine, Hydrocinchonidine, Cinchonicine, Dicinchonicine, Quinamine, Cinchonamine.

Quinine. Quinia.

$C_{20}H_{24}O_2N_2$; or $C_9H_5(OCH_3)N.CH_2.C_7H_{10}(OH)(CH=CH_2)N$.

Quinine is the most important of the cinchona bases, and appears to possess the most powerfully febrifuge properties. Its mode of preparation from the bark is based on the same principle as its estimation in the same (see page 490).

The chemical constitution of quinine is not thoroughly understood, but such knowledge as exists is epitomised on page 502. The complete synthesis of the alkaloid has not hitherto been effected, but cupreine has been apparently converted into quinine by the introduction of a methyl group.

From alcohol, benzene, and some other solvents quinine may be obtained in crystals, but on the evaporation of its ethereal solution it separates as a gelatinous or resinoid mass, which is never crystalline. This behaviour is important, as most other cinchona bases give crystalline-ether residues.

Free quinine usually appears in commerce as a soft, granular, white powder, which has a crystalline structure.

As obtained by the precipitation of one of its salts by an alkali, quinine forms a bulky, white precipitate, which coagulates into a resinoid mass by very slight elevation in temperature. According to O. Hesse the precipitate at first formed at the ordinary temperature is amorphous and anhydrous, but it soon takes up water and becomes crystalline. It then contains $3H_2O$. If the ammonia be added in large excess, and the solution is not too concentrated, the trihydrate is obtained in small needles, and the same compound can be obtained from an ethereal solution below 10°. But the resinoid mass left on the spontaneous evaporation of a solution of quinine in ether usually contains water in proportion corresponding to a monohydrate, and when the crystallised trihydrate is exposed in an desiccator over sulphuric acid, it effloresces and loses its water more or less perfectly. At 20°, over concentrated sulphuric acid, the trihydrate soon loses the whole of its water, but over equal volumes of conc. sulphuric acid and water a monohydrate results. At 15°, in the open air, the trihydrate is unaltered, but at 20° it effloresces and loses $1H_2O$, the residue having the composition of a dihydrate. Commercial quinine contains from 8 to 11% of water, and hence is approximately a dihydrate. The *French* and *German Pharmacopœias*, and the *British Pharmaceutical Codex*, however, require the trihydrate for the official alkaloid. The precipitate produced by ammonia at a low temperature in concentrated solutions of quinine sulphate is usually a dihydrate. Hydrates of quinine containing 8 and 9 H_2O have also been described. When the trihydrate is exposed at a temperature of 40° for a short time, and then at 60°, the whole of the water is driven off, and this change occurs rapidly at 100°. Resinoid quinine loses its water with some difficulty at 100° unless previously powdered, but at 120° becomes anhydrous very rapidly (see *Pharm J.*, 1885 [*iii*], **16**, 385, 897, 937).

Anhydrous quinine, obtained by drying the trihydrate over sulphuric acid and heating to 125°, melts at 174.9°, and that prepared by heating the benzene compound to 120° at 171.6–172°.[1]

Quinine is odourless. When in solution or finely divided it has an intense and purely bitter taste. It has valuable febrifuge properties,

[1] According to Hesse (*Annal.*, 1890, **258**, 133) on prolonged heating of a solution of quinine in alcohol to 30° the alkaloid is converted into an isomeride for which he proposes the unsuitable name of homoquinine. This melts at 174 4°–175°, and is reconverted into quinine by prolonged heating with dilute sulphuric acid.

and is poisonous to frogs and other of the lower animals. It has decided antiseptic properties, retarding or arresting the alcoholic, lactic, butyric, amygdalous, and salicylous fermentations, but not the digestive action of pepsin.

Quinine is very sparingly soluble in water. According to Sestini the solubility of the anhydrous alkaloid in water is 1 in 1,667 at 20° and 1 in 902 at 100°, the trihydrate requiring 1,428 and 773 parts of water at the same temperatures.

In dilute solutions of the fixed alkalies quinine is less soluble than in water, 1 part of the alkaloid being soluble in 3,450 parts of potassium hydroxide (1 in 20) at 25°. Ammonia has when first applied considerably greater solvent action than water, but most of the alkaloid is deposited on long standing, as 1 part of alkaloid dissolves in 2,286 parts of 10%, or 2,505 parts of 32.5%, ammonia at 15°. Certain ammonium and calcium salts notably increase the solubility of quinine in aqueous liquids. Anhydrous quinine dissolves in 0.6 parts of alcohol, 4.5 parts of ether, 1.9 parts of chloroform, 120 parts of benzene, at 25°. The trihydrate dissolves in 0.6 parts of alcohol, 1.3 parts of ether, 1.6 parts of chloroform, 166 parts of benzene, at 25°.[1] It dissolves readily in carbon disulphide, and in about 200 parts of glycerin. It is only sparingly soluble in petroleum ether, even when hot.

Quinine exercises a powerful lævorotatory action on polarised light, the value of $[\alpha]_D$ being, according to Hesse, $-145.2°+0.657\ c$ at 15°, for the solution of the hydrated alkaloid in 97% alcohol. In ether, quinine gives $[\alpha]_D^{15} = -158.7+1.911c$ where $c = 1.5$ to 6; while in a mixture of 2 parts of chloroform and 1 part of alcohol $[\alpha]_D^{15} = -141.0$, when $c = 2$.

Quinine affords no visible colour or other reactions with strong acids. By cautiously dissolving quinine hydrate or sulphate in a mixture of equal volumes of concentrated nitric and sulphuric acids, amorphous dinitroquinine, $C_{20}H_{22}(O_2N)_2O_2N_2$, is produced, nearly insoluble in ether and forming uncrystallisable salts (E. H. Rennie, *J. Chem. Soc.*, 1881, **39**, 469). The action of permanganate and chromic acid mixture on quinine is described on page 502.

Quinine is a powerful base, its solutions having a marked alkaline reaction to litmus and methyl-orange, and neutralising the strongest acids. It does not redden phenolphthaleïn.

[1] Quinine is deposited from its solution in warm benzene in crystals containing $(C_{20}H_{24}O_2N_2)_2,C_6H_6,2H_2O$ (*Chem. News*, 1883, 48, 4).

Detection and Estimation of Quinine.

The detection and estimation of quinine, when it occurs unmixed with other alkaloids or organic matter, is very readily effected, but the problem becomes more complex in the presence of other cinchona bases.

The following reactions are yielded by a solution of quinine in a moderate excess of dilute sulphuric acid:

1. Solutions of quinine in dilute sulphuric acid exhibit a strong blue fluorescence. The effect is best observed in very dilute liquids, and is intensified by addition of excess of sulphuric acid. The hydrochloride and other haloid compounds of quinine (including the thiosulphate and cyanogen compounds) exhibit no fluorescence till excess of sulphuric acid is added, and the fluorescence of solutions of the sulphate is destroyed by very small quantities of hydrochloric acid or other chlorides, but can be again produced by adding excess of dilute sulphuric acid. Alcoholic solutions of quinine exhibit but little fluorescence, and solutions of the alkaloid in solvents immiscible with water, none at all. Under favorable conditions, the fluorescence of quinine becomes an extremely delicate test for the presence of the alkaloid.[1] Fluorescence is also produced by quinidine, hydroquinine and hydroquinidine, and diquinicine, but not by quinamine, cinchonine or its isomers, cusconine, cupreine, or quinicine.

2. According to A. Weller (*Arch. Pharm.*, 1886, **224**, 161), on adding chlorine-water to a strong solution of quinine the solution acquires a more or less intense red colour. Bromine-water is a preferable reagent, and on adding a few drops to a saturated solution of quinine hydrochloride a yellow precipitate is formed, which gradually disappears with formation of a rose-red colouration, changing to cherry-red. The colour disappears after a time, but can be reproduced by adding more bromine-water, and the reaction is more delicate and prompt if the quinine solution be previously gently warmed. Acids

[1] The fluorescence of quinine is best observed by holding a test-tube filled with the solution in a vertical position before a window, when a bluish "bloom" will be perceived on observing the liquid from above against a dark background Another plan is to make a thick streak of the solution on a piece of polished jet or black marble, or on a plate of glass smoked at the back, and to place the streaked surface in front of, and at right angles to, a well-lighted window. (See also Vol. I.)

The fluorescence of quinine solutions is not perceptible by gas-light, but may be brought out by burning a piece of magnesium ribbon in the proper position The use of blue glass, which transmits the ultra-violet rays which produce the fluorescence of quinine, while excluding the less refrangible rays, is sometimes recommended. In this case the light transmitted by the glass should be concentrated by means of a lens

and excess of bromine-water prevent the reaction, which is also produced by quinidine, but not by cinchonine or cinchonidine.

3. If a solution of quinine, rendered as nearly neutral as possible, be treated first with chlorine or bromine, and then with excess of ammonia, a green substance called thalleioquin is produced, which in concentrated solutions forms a precipitate, and in more dilute a deep green liquid. When carefully applied, the test, which is due to Brande, is extremely delicate. Bromine is a more sensitive reagent than chlorine. The following is the best mode of applying the test: To 10 c.c. of the solution of quinine add 3 c.c. of chlorine-water, or 0.5 c.c. of saturated bromine-water. Agitate well, and then add 1 drop of strong ammonia solution, or sufficient to render the liquid distinctly alkaline. If the proportion of quinine exceed about 1 per 1,000 of solution, a green substance is precipitated, soluble in absolute alcohol, but insoluble in ether or chloroform. In more dilute liquids, even if the proportion of quinine does not exceed 1 in 20,000, a deep green colouration is produced. If the green ammoniacal solution be just neutralised with acid, a blue colouration is obtained, and on adding more acid a colour ranging from violet to red, but changing to green again on adding excess of ammonia.

H. Trimble has proposed to use this reaction for the approximate colorimetric determination of quinine, but the test is very unreliable and is useless from a quantitative point of view (E. Léger, *J. Pharm. Chim.*, 1904, **19**, 281–284, 434–435; E. Comanducci, *Chem. Zentr.*, 1910, **1**, 1885).

The thalleioquin reaction is also given by quinidine, cupreine, hydroquinine, hydroquinidine and diquinicine, but not by quinamine, or cinchonine and its isomers. It is prevented by morphine.

4. If, after the addition of chlorine- or bromine-water, the quinine solution be treated with a few drops of solution of potassium ferro- or ferricyanide, ammonia being *subsequently* added, a red colouration is produced instead of a green. The reaction is not so delicate as the thalleioquin test, but affords useful confirmatory evidence of the presence of quinine. A. Vogel modifies the test by adding bromine-water and potassium ferrocyanide to the solution to be tested, and then shaking with a fragment of marble, which, in presence of quinine, is at once covered with a red film. Strychnine, cinchonine, and caffeine do not give similar reactions.

5. On adding a fixed alkali, alkaline carbonate, or ammonia to a

solution of a salt of quinine, a bulky white precipitate of the free alkaloid (more or less hydrated) is produced. The precipitate is very sparingly soluble in cold water or excess of these precipitants, with the exception of ammonia. The precipitate cannot be conveniently filtered off, washed, and weighed, as it is not wholly insoluble, and melts with very slight increase of temperature. Its state of hydration is also very uncertain. But, if the liquid containing the precipitated alkaloid be agitated with ether or chloroform, or a mixture of the two, the quinine passes readily and completely into solution, and may be obtained in the solid state by evaporating the solvent. The process is readily made quantitative by operating with care and repeating the agitation of the liquid with the solvent, and the quinine may be weighed in the anhydrous state as $C_{20}H_{24}O_2N_2$, after being dried at 100° till constant in weight; or after exposure for 15 or 20 minutes to a temperature of 120°. The estimation of quinine in this manner is capable of yielding very accurate results, and is of very extensive and rapid application.

6. When quinine exists in a free state, as it is obtained in process 5 by the evaporation of its solution in ether or chloroform it may be estimated roughly by dissolving it in slight excess of $N/10$ acid and titrating the excess of acid with $N/10$ alkali (see page 497). Each 1 c.c. of $N/10$ sulphuric acid (=4.9 grm. of H_2SO_4 per litre) corresponds to .0324 grm. of anhydrous quinine. This titration by standard acid, of course, merely indicates the total alkaloid present, in terms of quinine. The process furnishes a very useful check on the estimation from the weight of the chloroform or ether-residue, and brings the alkaloid into a convenient form for further examination by one of the following processes:

7. On adding tincture of iodine to a solution of acid sulphate of quinine in dilute alcohol, a curious compound is produced, called, after its discoverer, Herepathite, and having the formula $4C_{20}H_{24}O_2N_2,3H_2SO_4,2HI,I_4+3H_2O$.[1] This compound, called also the iodosulphate of quinine or sulphate of iodo-quinine, is the type of a series of similar substances formed by the action of iodine on the sulphates of the cinchona bases. Herepathite is but little soluble in cold water or dilute alcohol, and requires 1,000 parts of hot water for solution;

[1] Herepathite may be readily prepared by dissolving the quinine sulphate in 10 parts of proof spirit containing 5% of sulphuric acid, and adding an alcoholic solution of iodine so long as a black precipitate is produced. The precipitate is filtered off, washed, and recrystallised from hot alcohol.

but it dissolves in boiling rectified spirit, and is deposited on cooling in tabular crystals, remarkable for their dichroism and their action on light, a thin film of herepathite polarising the transmitted light as completely as tourmaline. Herepathite is re-converted into quinine sulphate by treatment with sulphurous acid, thiosulphates, hydrogen sulphide, and other readucing agents.

Iodosulphate of quinine possesses far less solubility than the corresponding compounds of the other cinchona bases.[1] This fact has been utilised by J. E. de Vrij for the estimation of quinine (*Pharm. J.*, 1875 [*iii*], **6**, 461), but the method has a limited practical value.

E. B. Stuart (*Pharm. J.*, 1881 [*iii*], **12**, 1016) finds the herepathite reaction equally delicate with the thalleioquin test, and quite as easy of application. The salt of quinine should be dissolved in dilute alcohol, and dilute sulphuric acid, the presence of which is essential, added. Very dilute tincture of iodine is then added, drop by drop, with constant agitation, when the precipitate suddenly appears and quickly subsides. Precipitation as herepathite may be used with advantage for separating quinine from morphine even when the relative proportions are as 1:1,000.

8. André (*J. Pharm. Chim.*, 1862, **41**, 341) proposed a method of estimating quinine and separating it from other cinchona bases by precipitation as the chromate from neutral solutions of the alkaloids.

[1] B. Y. Shimoyama (*Pharm. J.*, 1885 [iii], 16, 205) gives the following figures for the solubility of quinine herepathite in 90% alcohol at different temperatures:

Temperature	Alcohol without acid	Acidified alcohol
15°	1 in 869 pts	1 in 255 pts
16°	1 in 841 pts	
17°		1 in 117 pts
18°		1 in 101 pts
20°	1 in 733 pts	
25°	1 in 660 pts	
90°	1 in 638 pts	

The solubilities of the iodosulphates of the principal cinchona alkaloids in acidified alcohol at 15° were found to be as follows

Alkaloid	Solubility	Iodine, %
Quinine herepathite	1 in 255 pts	32.37
Cinchonidine herepathite	1 in 92 pts.	53 68
Quinidine herepathite	1 in 61 pts.	42.70
Cinchonine herepathite	1 in 42 pts.	24.90

De Vrij (*Arch. Pharm.*, 1887 [*iii*], **24**, 1073) states that neutral quinine chromate is soluble in 2,733 parts of water at 12°, or 2,000 parts at 16°. Although the chromate is a good confirmatory test for quinine, Hesse (*Pharm. J.*, 1887 [*iii*], **17**, 585, 665; 1888, **18**, 582) finds that cinchonidine and hydroqinine are in part thrown down with the quinine, which renders the method inapplicable for separating quinine from its most constant associates.

Quinine is distinguished:

1. From cinchonine: *a*, by its fluorescence; *b*, its lævo-rotation; *c*, the thalleioquin test; *d*, the crystallisation of the sulphate; *e*, its solubility in ether; *f*, its solubility in ammonia; *g*, the sparing solubility of the iodosulphate; *h*, by the insolubility of its neutral tartrate.

2. From cinchonidine by most of the above reactions, except *b* and *h*, and less sharply than cinchonine by those tests depending on relative solubility (*d*, *e*, *f*, *g*).

3. From quinidine by *b*, *d*, *f*, *g*; also by (*i*) yielding no crystalline precipitate with potassium iodide, and (*j*) the insolubility of the sulphate in chloroform.

4. From quinamine by *b*, *e*, *h*; and (*k*), the sparing solubility of the sulphate.

5. From cupreine by *a*, and (*l*) the insolubility of the precipitated alkaloid in excess of soda.

Methods for the separation of quinine from the associated *cinchona bases* are given on pages 492, 517, *et seq.*

The separation of quinine from *morphine* may be effected, as already stated (page 511), by precipitation as herepathite; also by treating the free alkaloids with chloroform or ether, which leaves the morphine undissolved.

From *strychnine*, quinine may be separated as indicated under "Easton's syrup."

Salts of Quinine.

Quinine is a strong base, completely neutralising acids, and forming crystallisable salts having a slight alkaline indication to litmus. These salts react with phenolphthalein as if the acid were in an uncombined state. Quinine also forms a series of acid salts, of which the acid sulphate of quinine is the type, which are neutral to methyl-orange.

Several of the salts of quinine are official in the *Pharmacopœia*, and others are extensively used in medicine.

Quinine Sulphate. Diquinic sulphate. $(C_{20}H_{24}O_2N_2)_2H_2SO_4$. This important salt, sometimes called "disulphate" or "basic sulphate" of quinine, forms, in the hydrated state, the ordinary medicinal sulphate of quinine of commerce.

Sulphate of quinine is usually met with in exceedingly light scales, or long, flexible filiform needles,[1] having a nacreous aspect and a pure and intensely bitter taste.

The crystallised sulphate of quinine of commerce usually contains about 15.2% of water, a proportion which corresponds closely to $7.5H_2O$, which is the official salt of the *British Pharmacopœia.*

Hesse states that pure crystallised quinine sulphate, which has not effloresced, contains $8H_2O$, or 16 17% of water, and this has been confirmed by Robiquet, by Schorlemmer, and by Koppeschaar. This salt is the official one of the *French Pharmacopœia.* Samples of the commercial salt are sometimes met with containing about 14.5% of water, a proportion which corresponds to $7H_2O$, and this has been taken as the official salt of the *United States Pharmacopœia.* The evidence is in favour of crystallised quinine sulphate containing $8H_2O$, and the salt efflorescing very rapidly, even when packed in corked bottles, accounts for the lower percentage usually found in the commercial article. Another reason suggested by Kopppeschaar is that cinchonidine sulphate crystallises with $6H_2O$; moreover, any cinchonidine sulphate in the quinine salt does not exist in the state of a mixture, but in combination with a portion of the quinine sulphate, the compound salt enclosing $6H_2O$.

Crystallised quinine sulphate is rendered perfectly anhydrous by exposure to a temperature of 100°. If a higher temperature be employed for its dehydration, there is a danger of some of the alkaloid undergoing conversion into quinicine (see page 544). If the anhydrous sulphate of quinine be exposed to moist air, it rapidly obsorbs from 4.8 to 5% of water, a proportion which corresponds to the formula

[1] Chemically pure quinine sulphate, free from hydroquinine, crystallises in heavy needles. The light character of the commercial salt is chiefly due to the presence of small admixtures of the sulphates of hydroquinine and cinchonidine, and possibly of hydrocinchonidine and homocinchonidine 1% of cinchonidine is sufficient to produce the light silky appearance, and this persists with a larger proportion "A few years ago, when the bark of Remijia, which contains no cinchonidine, was first treated, the latter alkaloid was added, as the pure solutions yielded large brilliant needles unfamiliar in commerce, for the same reason the bark of cuprea was never treated, except by being mixed with other barks" The sulphates of the bases of the cinchonidine group can be separated from quinine sulphate without interfering with its light form when there is a sufficient amount of hydroquinine present. According to Carles, an addition of 4 grm of ammonium sulphate to 1 litre of a hot saturated solution of quinine sulphate causes the latter salt to crystallise on cooling in a very voluminous form.

$B_2H_2SO_4 + 2H_2O$.[1] On the other hand, the crystallised salt rapidly loses water on exposure to air, until it acquires the composition of the $2H_2O$ substance. The same quantity of water is retained when the crystallised salt is dried over sulphuric acid, or crystallised from strong alcohol.

Quinine sulphate requires 750 parts of cold water for solution, but dissolves in about 30 parts of water at 100°. It is far less soluble in water containing magnesium, sodium, or ammonium sulphate than in pure water. A solution of quinine sulphate is so completely precipitated by a strong solution of Rochelle salt that the alkaloid can scarcely be detected in the solution by the fluorescence or thalleioquin test. On the other hand, the solubility of sulphate of quinine in water is increased by the presence of ammonium chloride, or of potassium nitrate or chlorate.

In alcohol, quinine sulphate dissolves more readily than in water, requiring 6 parts at a boiling temperature, or 65 parts at 15°. Quinine sulphate dissolves in about 24 parts of cold glycerin, the solution being precipitated by addition of water. Crystallised quinine sulphate is not soluble in fixed oils, ether, chloroform, or petroleum spirit. (It is said to dissolve in benzene.) In the anhydrous state, 1 part of quinine sulphate is soluble in about 1,000 parts of chloroform (see page 525). It is easily soluble in a mixture of 2 parts of chloroform and 1 part of absolute alcohol.

In dilute sulphuric acid, quinine sulphate is readily soluble, owing to the formation of *acid quinine sulphate*, $C_{20}H_{24}O_2N_2,H_2SO_4$. This salt is readily obtainable in crystals containing $7H_2O$. The crystallised salt loses $6H_2O$ in the desiccator, and becomes anhydrous at 100°. This salt is the official quinine acid sulphate of the *United States Pharmacopœia*. (The normal sulphate is also official.) It is described as being colourless, transparent or whitish, orthorhombic crystals or small needles; odourless and having a very bitter taste. It effloresces on exposure to the air, and turns yellow on exposure to light. Soluble in 8.5 parts of water, 18 parts of alcohol, 1,770 parts of ether, 920 parts of chloroform, and in 18 parts of glycerin at 25°; soluble in 0.68 part of water at 80° and in 0.5 part of alcohol at 60°. When heated the salt softens at 60°, becomes semi-fluid at 70°, and melts at about 160°, with decomposition.

[1] H. P. Parsons recommends the official adoption of this hydrate as a definite and stable form of quinine sulphate.

It loses all of its water of crystallisation at 100°. The salt on melting is converted into acid quinicine sulphate. Acid quinine sulphate dissolves in 10 parts of water at 15°, and more readily in hot water. It is soluble in 45 parts of alcohol at 15°. These solutions are strongly fluorescent.

Normal quinine sulphate has a specific rotation in absolute alcoholic solution of $[\alpha]_D^{17} = -157.4$ ($c = 22$, of the 7 1/2H_2O salt). Excess of acid increases the rotatory power. A solution of quinine sulphate in excess of dilute sulphuric acid has a specific rotation of $[\alpha]_D = -235.0$ at 15°, calculated for the anhydrous salt (Tutin, *Phar. J.*, 1909 [*iv*], **29**, 600).

Sulphate of quinine is largely employed as a febrifuge and tonic, the official dose ranging from 1 to 10 grains. It has marked antiseptic properties.

The fluorescence of sulphate of quinine is considered on page 510; its reaction with iodine on page 512; and with the thalleioquin test on page 511.

Examination of Commercial Quinine Sulphate.

The salts of quinine, except the tannate (page 529), can all be examined by the following methods applicable to the quinine sulphate, provided they are first treated with 10 parts of boiling water and their own weight of sodium sulphate. The sulphate of quinine which deposits on cooling and the mother-liquor obtained can then be examined in the usual way.

Commercial quinine sulphate was formerly subject to adulterations of a very gross character. Among the substances employed to sophisticate it are said to have been starch, gum, stearin, salicin, phloridzin, sugars, magnesium sulphate, sodium sulphate, chalk, asbestos, boric acid, etc. Some of these additions are apocryphal and the majority are certainly obsolete.

Ammonium salts should be tested for qualitatively by boiling some of the sample with sodium hydroxide, or quantitatively by washing a weighed portion of the quinine salt with cold water and testing the liquid with Nessler's reagent. Mineral additions would be readily recognised on igniting the sample, which, when pure, will leave no sensible ash. Starch, chalk, stearin, and boric acid would remain insoluble on treating the substance with cold dilute sulphuric acid,

and gum would be precipitated on adding excess of alcohol to the solution thus obtained. Soluble impurities generally may be detected and estimated by dissolving the sample in hot water and adding excess of barium hydroxide water. The alkaloid is then removed by agitation with ether. After removing the ethereal layer, a stream of carbon dioxide is passed through the aqueous liquid to precipitate the excess of barium hydroxide, and the whole well boiled and filtered. Sulphate and carbonate of barium will be left insoluble, and the filtrate will contain any sugar or other soluble impurity present in the original sample, and the observation of the weight of the residue left on evaporation will allow of a determination of the amount. In presence of *sugar* the liquid will exert a dextrorotatory action, and in presence of *salicin* a lævorotatory action on polarised light.

Treatment of the original solid sample with concentrated sulphuric acid, attended by gentle warming, will suffice for the qualitative detection of some impurities. Sugar and manitol will become charred, while salicin develops a striking red colour. Good commercial quinine sulphate dissolves with faint yellow colour in strong sulphuric acid, and the tint is not deepened on warming gently.

Similar general impurities may be rapidly tested for by a test devised by Hesse, and described on page 526. *Salicin*, if present in greater proportion than 1%, may be detected by this test. The residue insoluble in the chloroform-mixture will be coloured deep red by concentrated sulphuric acid, and will reduce Fehling's solution after boiling with dilute sulphuric acid. The reaction with strong sulphuric acid will be produced by the original sample if the proportion of salicin be considerable. Smaller proportions of salicin may be detected in the filtrate from the precipitate produced by adding barium hydroxide to the aqueous solution of the sample. Another test for salicin is to dissolve 0.25 grm. of the sample in 4 c.c. of water and 4 drops of concentrated hydrochloric acid. If salicin be present, on boiling the liquid for some minutes a white turbidity will be produced, due to the formation of saliretin.

Quinine sulphate has occasionally been largely adulterated with or entirely substituted by the cinchonine hydrochloride. This fraud is recognisable by testing for chlorides with nitric acid and nitrate of silver, and for cinchonine as described on page 522.

The most common impurity of commercial quinine sulphate is an admixture of one or more of the sulphates of *other cinchona alkaloids*,

especially *cinchonidine* and *hydroquinine.* This admixture is often purely accidental, owing to imperfect separation of the other alkaloids during manufacture, but is no doubt sometimes provided for and secured by suitable arrangements of the manufacturing operations, while occasionally an intentional admixture of other alkaloids has occurred.

Various qualities of quinine sulphate are produced by manufacturers. The chief are: 1. The pure salt or "heavy sulphate," of which the use is extremely limited, chiefly on account of the heavy cost entailed in freeing it from the last 2 or 3% of impurities. This is the official salt of the *German Pharmacopœia* of 1890 and 1900. 2. Products satisfying the requirements of the *French Pharmacopœia*, and containing up to 3% of other alkaloids. 3 Products satisfying the requirements of the *German Pharmacopœia* of 1882, and the *British Pharmacopœia*, which may contain up to 10% of other alkaloids. Other products may have a certain commercial importance, but have no "legal status" in civilised countries.

The best samples of commercial quinine sulphate are seldom free from cinchonidine, but contain not more than 2 or 3%; while other kinds contain from 5 to 10, and even 20% of cinchonidine sulphate, and on one accasion B. H. Paul found 60%. In addition, *hydroquinine* is a very constant impurity in quinine sulphate, usually occurring to the extent of 2 to 4% (D. Howard, *Pharm. J.*, 1896 [*iv*], **3**, 505); and, according to Hesse, *hydrocinchonidine* and *homocinchonidine* may also be met with in quinine from certain sources. The presence of even 1% of *cinchonine* or *quinidine* in quinine sulphate is far more likely to be intentional than due merely to accident or careless manufacture, but these alkaloids are apt to be met as accidental impurities in quinine hydrochloride.

The detection and estimation of foreign alkaloids in commercial quinine sulphate has received much attention, and considerable ingenuity has been exercised in the solution of this somewhat difficult problem.

Owing to the obstinacy with which quinine and cinchonidine form more or less definite compounds when crystallising together, as sulphates from water and as alkaloids from ether, greater difficulty is experienced in the recognition and estimation of cinchonidine sulphate in quinine sulphate than in the detection and estimation of the other alkaloids when present in notable proportion.

To detect cinchonidine in quinine sulphate Liebig proposed shaking the salt with ether and ammonia, but this will pass a sample containing upward of 10% of cinchonidine. Kerner proposed a test depending upon the difference in solubility of the sulphates, quinine sulphate being soluble in about 750 parts of cold water, while cinchonidine sulphate is soluble in about 100 parts. This method modified and improved by Paul, and by Hesse, is the one generally adopted by the various pharmacopœias. The test is usually carried out by shaking 2 grm. of the quinine sulphate with 20 c.c. of water, filtering off the undissolved crystals (in the later modifications after heating and cooling), and gently mixing 5 c.c. of the clear filtrate with ammonia (sp. gr. 0.96). The amount of ammonia required to just redissolve the precipitate first formed is an indication of the purity of the salt tested, and the ammonia solution should not deposit any crystals on standing for 24 hours. The following is a table showing the methods recommended by the various pharmacopœias for carrying out the test, with the amount of ammonia allowed by each:

MODIFICATIONS OF THE KERNER TEST OF THE VARIOUS PHARMACOPŒIAS FOR QUININE SULPHATE.

	Pharmacopœia	Wt taken in grm.	Containing % H_2O	Dried for	at	Water used	Heated for at		Cooled for at		c c of NH_4OH allowed
1.	British Pharmaceutical Codex	2	15 4	2 hours	50°	20 c c	65°	1/2 hour	15°	1/2 hour	6 c c
2	French, 1884	2	15 4	.. ..		20 c c		1/2 hour	15°	1/2 hour	7 c c
3	French, 1909	1	16 2			30 c c	100°	To dissolve	15°	1/2 hour	5 c c
4.	German, 1882	2	15 4	.		20 c c			15°	1/2 hour	7 c c
5.	German, 1900.	2	15 4	2 hours	40 to 50°	20 c c.	65°	1/2 hour	15°	2 hours	4 c c
6.	Italian ..	2	4 6	[2 hours][1]	[50°][1]	20 c c	100°	1 hour	15°	1/2 hour	7 c c
7.	Netherlands.	1	4 6	[1 hour][1]	[40 to 50°][1]	20 c c.	60 to 65°	1/2 hour	15°	2 hours	4 5 c c
8.	United States, 1905.	1 8	14 5	[2 hours][1]	[50°][1]	20 c c.	65°	1/2 hour	15°	2 hours	7 c c.

[1] The figures in brackets indicate that the drying at the temperature named is carried out before the salt is weighed out.

The results obtained by this method are only empirical owing to the difficulty of dissolving out cinchonidine sulphate from crystals of quinine sulphate, and also on account of cinchonidine sulphate forming a double salt with quinine sulphate in crystallising out below 50° from its solutions. As pointed out by D. Howard (*Pharm. J.*, 1896 [*iv*], **3**, 505) and by Tutin (*Pharm. J.*, 1909 [*iv*], **29**, 600) quinine sulphate is almost insoluble in even a very dilute solution of ammonium sulphate, and 0.1% of this salt in an impure quinine sulphate will make it appear purer than pure quinine sulphate. Moreover, should pure quinine sulphate contain a little free alkaloid the ammonia required in testing it will be far in excess of the amount required by the neutral salt. For these reasons reliance can be placed on Kerner's test only when the absence of alkaline sulphates has been proved, and when the neutrality of the salt has been assured. The former point is readily ascertained by evaporating 5 c.c. of the filtrate (which should be neutral to litmus) to dryness, and constant weight, on the steam-bath. If the residue weighs not more than 0.008 grm., the absence of soluble inorganic salts is proved. Biginelli (*Monit. Scient.*, 1908, **22**, 175–185) recommends that the limit of ash for quinine sulphate should not exceed 0.1%. The great recommendation for Kerner's test is that it is the only test that readily indicates the presence of hydroquinine and other little-known alkaloids, about the therapeutic effects of which little is known, and which certainly should not be present in excessive quantities.

On the point whether it is better to treat the sample with water at 60° or to 100°, authorities are at variance. Hesse considers that at a boiling heat more of the quinine sulphate will pass into solution, and hence there will be a greater tendency to the re-formation of the double salt when crystallisation takes place. Kerner and Weller also recommend the use of water at 60°. E. Jungfleisch (*J. Pharm. Chim.*, 1886 [*v*], **15**, 5; *Pharm. J.*, 1886 [*iii*], **17**, 585) gives the preference to a boiling temperature, and points out the tendency to erratic results if less heat be employed. Paul (*Pharm. J.*, 1886 [*iii*], **17**, 595) considers that the best results can only be obtained by using nearly sufficient water to effect the complete solution of the quinine sulphate at the b. p.

The *British Pharmacopœia* of 1898 gives the following methods of testing commercial sulphate of quinine for accompanying alkaloids. The salt "should not contain much more than 5% of other cinchona alkaloids."

Test for Cinchonidine and Cinchonine.—Dissolve 4 grm. of the quinine sulphate in 120 c c. of boiling water. Cool the solution slowly to 50°, with frequent stirring. Separate, by filtration, the purified quinine sulphate which has crystallised out. Concentrate the filtrate by evaporation until it is reduced to 10 c.c. or less; transfer to a small stoppered flask, and, when cold, shake with 10 c.c. of ether and half that amount of solution of ammonia. Set aside in a cool place for not less than 24 hours. Collect the crystals, which consist of cinchonidine and cinchonine combined with quinine, on a tared filter, wash with a little ether, dry at 100° and weigh. These should not amount to more than 0.12 grm.

Test for Quinidine.—Dissolve 1 grm. of the quinine sulphate in 30 c c. of boiling water; cool, and filter. To the solution add solution of potassium iodide and a little alcohol (90%) to prevent the precipitation of amorphous hydriodides. Collect any separated quinidine hydriodide, wash with a little water, dry and weigh. The weight represents about an equal weight of crystallised quinidine sulphate.

Test for Cupreine.—Shake the recrystallised quinine sulphate, obtained in testing the original quinine sulphate for cinchonidine and cinchonine, with 25 c c. of ether and 6 c.c. of solution of ammonia, and to this ethereal solution, separated, add the ethereal liquid and washings also obtained in testing the original sulphate for the 2 alkaloids just mentioned. Shake this ethereal liquid with 6 c.c. of a 10% solution of sodium hydroxide, adding water if any solid matter should separate. Remove the ethereal solution, wash the aqueous solution with more ether, and remove the ethereal washings. Add diluted sulphuric acid to the aqueous liquid heated to boiling, until exactly neutral. When cold, collect any crystallised sulphate of cupreine on a tared filter, dry, and weigh.

Test for Cinchonine and Amorphous Alkaloids.—Dissove 1 grm. of the quinine sulphate in 30 c.c. of boiling water. Add 1 grm. of sodium potassium tartrate. Allow to cool, with frequent shaking; filter. The solution when evaporated to small bulk should give little or no precipitate with solution of ammonia.

The foregoing tests are, of course, not intended for the detection and estimation of minute traces of accompanying alkaloids in quinine sulphate, as the therapeutic value of the salt is not so greatly affected by small quantities of other alkaloids. De Vrij (*Chem. Zentr.*, 1885, 968) has suggested the addition of sufficient sulphuric acid to convert

the bases into acid salts before separating them by fractional solution and crystallisation. Hesse (*Pharm. J.*, 1886 [*iii*], **17**, 486), who expresses a high opinion of this method if carefully performed, recommends the following mode of operating: 5 grm. weight of the sample is dissolved by the aid of heat in 12 c.c. of *N* sulphuric acid contained in a small porcelain basin, and the solution poured into a funnel closed at the bottom,[1] in which it is allowed to cool. At the end of 2 hours crystallisation is complete, the stopper is removed, and the mother-liquor allowed to drain away as completely as possible, its removal being assisted by suction. The upper portion of the crystals is then pressed down with a glass rod and washed with 3 c.c. of cold water, added drop by drop while the suction is kept up. The whole solution is then mixed with 16 c.c. of ether (sp. gr. 0 721 to 0 728) and shaken up.[2] 3 c c. of ammonia (sp. gr. 0.960) is next added, and the whole well shaken again. After standing 1 day the ether is removed with a pipette, and the crystals which have separated are collected on a filter and washed with water saturated with ether. The filter is then placed on an absorbent surface, the crystals again washed with some ether, and dried at 100° and weighed.

D. Howard (*Pharm. J.*, 1896 [*iv*], **3**, 505) points out that although the cinchonidine almost entirely remains in the acid liquor, owing to the great solubility of the acid sulphate of quinine, the solution contains so much quinine that the cinchonidine largely remains dissolved in the ethereal solution. In order to obtain accurate results by this method, a sufficient quantity of the quinine sulphate must be taken to allow the solution separated from the first crop of crystals to be evaporated to a small bulk, again crystallised, and the mother-liquor carefully separated from the crystals by a filter pump and shaken with ether and ammonia. If a complete analysis is required, the crystals of impure cinchonidine should be dissolved in absolute alcohol and 2.1 c.c. of 50% sulphuric acid for each grm. of crystal added. The cinchonidine will then crystallise out almost entirely as tetrasulphate, and, by neutralising the mother-liquor and evaporating off the spirit, the quinine may be obtained as sulphate. The ethereal solution still containing cinchonidine should be evaporated, the alkaloid crystallised as acid sulphate, and the mother-liquor again treated with ether

[1] This may be conveniently effected by a glass rod introduced from above, and having the lower end covered with a short length of india-rubber tubing The same rod can be afterward used for pressing down the crystals

[2] If the sample contain more than 10% of cinchonidine the volume of ether must be increased.

and ammonia. These last operations are, however, hardly necessary in practice, for when the test is carefully carried out, the quinine crystallising with the cinchonidine very nearly balances the cinchonidine dissolved by the ethereal solution of quinine.

A method of assaying quinine sulphate for cinchonidine, based upon the optical rotation of the solution, has been recommended by several authorities and is equally distrusted by others. Oudemans was among the first to experiment in this direction, and Hesse proposed a definite process of assay, based on the rotation of the sulphate. Koppeschaar proposed to employ the tartrates by preference, while R. H. Davies operated on the sulphates. De Vrij has strongly recommended the optical method of examination, giving preference to the tartrates. Jungfleisch and Paul and Cownley have expressed strong distrust of the optical method, considering it manifestly impracticable to determine proportions of 1 and 1.5% of cinchonidine in quinine sulphate containing even minute proportions of the cinchonine and quinidine salts; and D. Howard states that no published method gives the mixed tartrates of quinine and cinchonidine sufficiently pure to render the polarimetric assay absolutely reliable. Hesse has modified his former high opinion of the method, and points out that it is invalidated by the presence of hydroquinine, which is invariably present in commercial quinine sulphate, and is not separated by converting the bases into tartrates. A. E. Léger (*J. Pharm. Chim.*, 1904, **19**, 427–434) condemns Oudeman's method, but states that the mixture of quinine, cinchonidine, and cinchonine sulphates may be accurately estimated optically if the mixture be recrystallised, a saturated solution of quinine sulphate being employed as solvent. The cinchonine sulphate remains in solution and is filtered off. The purified quinine and cinchonidine sulphates are then converted into tartrates, the optical rotation of which is estimated by Oudeman's method.

The presence of 1% of hydroquinine sulphate reduces the rotation to the same extent as 0.42% of the cinchonidine salt, and its presence accounts for the excessive and discordant figures for cinchonidine often obtained by those who rely on the optical method of assay. Hydroquinine cannot be perfectly separated from quinine by several repeated recrystallisations of the neutral sulphate, but it can be completely got rid of by converting the alkaloid into the acid sulphate and recrystallising this from water or alcohol, when the hydroquinine remains in the mother-liquor.

For the optical assay, Koppeschaar (*Zeitsch. anal. Chem.*, 1885, **24**, 362) recommends that the quinine and cinchonidine should be converted into tartrates by precipitating the neutral solution with Rochelle salt, and the precipitate washed with a little cold water and dried at 125–130°; 0.400 grm. of the dry product is then dissolved in 3 c.c. of normal hydrochloric acid, and the solution diluted with water at 15° to a volume of 20 c.c. The solution is placed in a jacketed tube kept at 15°, and the rotatory power observed by a polarimeter employing monochromatic (sodium) light. From the angular rotation the specific rotatory power of the tartrate is then calculated by the formula $S=\frac{100a}{2l}$; where S is the specific and a the angular rotation, and l the length of the tube in decimetres. From the figure thus obtained, the percentage of quinine tartrate, x, in the mixed tartrate may be ascertained by the following (Koppeschaar's) formula:

$$x=\frac{100(S-137.67)}{82.4}$$

Each 1° of diminution in the specific rotation below 220.07° corresponds to about 1.2% of cinchonidine tartrate in the mixed tartrates. The angular rotation is diminished by 0.077° only by the presence of 1% of cinchonidine tartrate. Notwithstanding the extreme accuracy of observation necessary, Hooper (*Pharm. J.*, 1886 [*iii*], **17**, 61) has found the optical estimation of quinine in the mixed tartrates to give very satisfactory results. Hesse found the specific rotation of quinine, hydroquinine, and cinchonidine tartrates, for Oudemans' concentration B, to be respectively, −212.5°, −176.9°, and −132.0°.

For the detection of *cinchonine*[1] or *quinidine* in quinine sulphate, Hesse proposes to dry the salt at 100°, and agitate 1 grm. with 15 c.c. of chloroform free from alcohol. The liquid is passed through a small filter. If 10 c.c., on evaporation at a gentle heat, leave an amorphous residue weighing more than .035 grm., cinchonine or quinidine sulphate is certainly present. If the residue be crystalline and less than the above weight, it may be tested for the foreign alkaloids by heating it with 5 c.c. of water, adding 0.5 grm. of potassium sodium tartrate, cooling, filtering from the precipitated quinine and cinchonidine tartrates, and

[1] According to Laborde (*Pharm. J.*, 1882 [*iii*], 13, 684) the presence of cinchonine materially alters the physiological effects of quinine salts

mixing the filtrate with an equal volume of ammonia. If quinidine or cinchonine be present, a precipitate will be formed, and may be further examined by agitation with ether (see page 541), or by treatment with potassium iodide (see page 522). Cinchonidine sulphate, if present, will remain undissolved by the chloroform, but will swell up into very bulky needles, which suck up the chloroform like a sponge and do not yield it again without pressure.

L. Schafer (*Arch. Pharm.*, 1887, **225**, 64, 1033) has described a method of testing commercial quinine sulphate, based upon the precipitation of the boiling aqueous solution by neutral potassium oxalate. After cooling and filtering, the filtrate is tested by addition of sodium hydroxide.

O. Schlickum (*Arch. Pharm.*, 1887, **225**, 128) has investigated De Vrij's chromate method (page 514), and finds it applicable, under certain conditions, to the examination of quinine sulphate. On precipitating a solution of this or other neutral quinine salt with neutral potassium chromate, and filtering after four or more hours, the filtrate remains clear on addition of sodium hydroxide, if the quinine salt was pure. In presence of 1.5% of cinchonine sulphate, or 1% of the quinidine or cinchonidine salt, a turbidity is produced at once or after a time.

A criticism of the accuracy of the various methods proposed for estimating quinine has been given by W. Hille (*Arch. Pharm.*, 1903, **241**, 54–110).

A test for the purity of quinine sulphate, devised by Hesse and adopted by most pharmacopœias, consists in heating 1 grm. of the sample for a short time to 40–50°, in 7 c.c. of a mixture of 2 volumes of chloroform and 1 of absolute alcohol. If the sample be pure it is completely dissolved, and the solution remains quite clear on cooling. Sulphates of other cinchona bases and various organic and inorganic impurities remain insoluble (compare page 525).

A somewhat similar test has been described by E. Hirschsohn, according to which 0.2 grm. of the quinine sulphate should be briskly agitated with 5 c c. of a mixture of 30 parts of petroleum ether of 0.680 sp. gr. with 70 parts of chloroform. The liquid is filtered, and diluted with 3 or 4 times its volume of petroleum ether, when an admixture of 0.1% of sulphates of other cinchona bases will give rise to a turbidity or precipitate.

For the detection of *amorphous alkaloid* in commercial quinine

sulphate, De Vrij recommends the following method: The sample is dissolved in dilute acid, and shaken with ammonia and ether for estimation of total alkaloid. Sufficient $N/10$ oxalic acid is added to the ether-residue to convert the alkaloid into neutral oxalate, and the liquid is evaporated at a steam heat and the residue thoroughly dried in the water-bath. It is then dissolved in chloroform, and the liquid filtered if necessary. The clear solution is next treated in a test-tube with a few drops of water, when crystals of quinine oxalate will appear in the chloroform. If the sample were pure the aqueous layer will remain clear and uncoloured, but if amorphous alkaloid be present it will be dissolved by the water and colour it yellow.

Quinine Hydrochloride.—Hydrochlorate of quinine. B,HCl. This salt forms long asbestos-like prisms containing $2H_2O$ which become anhydrous at 100° without previously melting. The dehydrated salt fuses at 158–160° without change, and is not converted into quinicine, as stated by Pasteur (Hesse). If an aqueous solution of quinine hydrochloride saturated at 15° be allowed to stand for some time at about 0°, large octahedral crystals containing $3H_2O$ separate out.

All the pharmacopœias state that quinine hydrochloride contains $2H_2O$, but the salt met with in commerce usually contains about 8% of water, and consequently contains about 82% of quinine against 73·5% in the crystallised sulphate. Quinine hydrochloride is soluble in about 40 parts of cold water, in 3 parts of cold alcohol (90%), in 1 part of boiling water or 90% alcohol, and in 9 parts of cold chloroform. The anhydrous salt is very soluble in chloroform. Quinine hydrochloride is very frequently substituted for the sulphate, on account of its greater solubility and purity. Thus it is used in making the Tincture of Quinine, *British Pharmacopœia*. The hydrochloride is the more expensive salt, owing to the increased difficulty of crystallising and the high percentage of quinine contained in it.

Quinine hydrochlorides may be prepared by acting on the sulphate with barium chloride. Hence it is apt to contain either undecomposed quinine sulphate, or else barium chloride. The latter impurity is, of course, very objectionable.

The official method of the pharmacopœias for the assay of quinine hydrochloride is a modification of Kerner's test. 2 grm. of the hydrochloride are dissolved in 20 c.c. of hot water together with excess of powdered sodium sulphate. The mixture is cooled to 15°, and the

test carried out as for quinine sulphate (see page 520). The method is obviously a faulty one on account of quinine sulphate being almost insoluble in a solution of sodium sulphate, and more soluble in a solution of sodium chloride than in water, both of which conditions are present in the test. Quinine hydrochloride is more likely to be contaminated with the similar salts of cinchonine and quinidine than with the hydrochlorides of cinchonidine and homocinchonidine.

Quinine hydrochloride has on several occasions been accidentally mixed with or replaced by the corresponding salt of *morphine*. The impurity may be detected by warming the salt with dilute nitric acid, which acquires a yellow or red colour if morphine be present; or the salt may be placed in a porcelain crucible and moistened with very neutral ferric chloride, which will produce a green or blue colour if morphine be present. The production of a blue colour with mixed solutions of ferric chloride and potassium ferricyanide (page 384) is also well adapted for the detection and approximate estimation of morphine in presence of cinchona bases. Lastly, the aqueous solution of the salt may be treated with ammonia and agitated with a small quantity of ether, when any morphine (or cinchonine) will remain undissolved.

Quinine Dihydrochloride. *Acid Hydrochlorate of Quinine, B.2HCl.* This salt forms a white crystalline powder, soluble in 0.75 parts of cold water, 5 parts of alcohol, or 7 parts of chloroform, and is insoluble in ether. According to the official formula the salt should contain 12% of water, corresponding to $3H_2O$, but as met with in commerce it contains only 3 to 5% of water. From absolute alcohol it is obtained in fine needly crystals containing $1H_2O$ which is rapidly lost on exposure to warm air. From dilute alcohol prismatic crystals are obtained containing 1 molecule of alcohol and 1 molecule of water, the alcohol of crystallisation being lost on drying at 37°.

There is an increasing demand for this salt for subcutaneous use.

The remarks on the assay of the hydrochloride apply also to the assay of the dihydrochloride.

Quinine Hydrobromide, $BHBr+H_2O$. The salt forms white silky crystals which are efflorescent. It is soluble in about 50 parts of cold water, in 0.7 parts of alcohol, 10 parts of chloroform, 1 part of boiling water, and in glycerin. Its solutions, are not, as has been stated, fluorescent. It begins to fuse at 152° forming a syrupy liquid at 200°.

Quinine Dihydrobromide. *Acid Hydrobromate of Quinine,*

B.$2HBr+3H_2O$. This salt forms yellowish prismatic crystals or powder, soluble in 7 parts of cold water, very soluble in boiling water and in alcohol, but insoluble in ether. The hydrated salt melts between 81 and 82°.

Quinine Carbonate, $B_2H_2CO_3+H_2O$, is obtained by passing carbon dioxide into water containing freshly precipitated quinine hydrate, and exposing the resultant solution to the air. If forms translucent needles, effloresces rapidly in the air, decomposing at 110°, and soluble in water or alcohol but insoluble in ether.

Quinine Chromate, $B_2H_2CrO_4+2H_2O$. The anhydrous salt rapidly re-absorbs $2H_2O$ on exposure to air. It is soluble in 2,400 parts of water at 15° and in 160 parts of boiling water, and has been recommended by De Vrij for the estimation of quinine (page 514). It becomes anhydrous at 80°, and decomposes at a higher temperature, at 100° turning a bronze-green colour.

Quinine Oxalate, $B_2H_2C_2O_4+6H_2O$, forms delicate needles soluble in 1,030 parts of water at 10°. The oxalates of the other frequently occurring cinchona bases are comparatively easily soluble, and L. Schafer has based on this fact a method of separating small proportions of these bases from quinine (page 526).

Quinine Valerate forms colourless rhomboidal plates, having a pearly lustre and a faint odour of valeric acid. It is not deliquescent, and fuses at a low temperature. Quinine valerate requires 110 parts of cold or 40 of boiling water for solution, and is easily soluble in alcohol. It is liable to contain much the same impurities as the sulphate (see page 520). Sulphate and hydrochloride of quinine, and valerate and acetate of zinc are also liable to be present.

Quinine Tannate has come into use in medicine on account of its comparatively tasteless character. The commercial product varies greatly in its composition, the bitter taste decreasing with the amount of alkaloid contained in the specimen.

For the preparation of quinine tannate, Peltz recommends the precipitation of a saturated solution of 1 part of quinine hydrochloride by 3 of tannin (in 10% solution previously neutralised by ammonia). After standing 24 hours, the washed precipitate is dried at a low temperature. So prepared, quinine tannate is a yellowish-white amorphous powder, soluble in about 50 parts of cold water or alcohol. Its solution gives the reactions of tannic acid.

On precipitating quinine sulphate with tannic acid, a mixed com-

pound of variable constitution is precipitated (P. Biginelli, *Gazzetta*, 1907, **37**, 205; F. Mararo, *Gazzetta*, 1908, **38**, 427).

The *Dutch Pharmacopœia* of 1905 states that quinine tannate should be prepared by dissolving 6 parts of quinine alkaloid in 12 parts of strong alcohol. The solution is heated on the water-bath and 13 parts of dry tannic acid gradually added with constant agitation. The quinine tannate is then precipitated by the addition of 100 parts of water. This process yields a tannate containing 29 to 31% of quinine alkaloid.

In some cases, the quinine in the commercial tannate is largely replaced by other cinchona bases. The following analyses by Jobst (*Arch. Pharm.*, 1878, **212**, 331; *J. Chem. Soc.*, 1878, **34**, 678) illustrate the composition of commercial "tannate of quinine."

	1	2	3	4	5	6	7
Water lost at 120°	7 2	9 7	9 1	9 8	10 2	10 7	11 4
Quinine . .	31 37	22 72	4 46	4 93	6 23	10 00	7 40
Quinidine			11 97	2 43	Trace		
Cinchonidine			7 33	13 10	23 80		
Cinchonine				3 35	Trace		
Total alkaloid	31 37	22 72	23 76	23 82	27 03	10 00	7 40

To ascertain the proportion of total alkaloid in quinine tannate, Jobst powders 1 grm. of the sample and mixes it with milk of lime. The mixture is dried on the water-bath, and the resulting powder exhausted with chloroform. The chloroform is filtered, evaporated, and the residue weighed after drying at 120°. The alkaloid thus separated can be further examined as described on page 523. There seems no reason why the mixture of the sample with milk of lime should not be agitated directly with chloroform, thus avoiding the evaporation to dryness of the aqueous liquid. A similar process is adopted by S. Neumann, who agitates the finely divided tannate with strong solution of sodium hydroxide and excess of ether. The presence of solid particles in suspension, either in the ethereal or alkaline solution, shows that the sample is impure or that it has not been completely decomposed.

Quinine tartrate, $B_2H_2C_4H_4O_6+H_2O$, forms a crystalline precipitate, soluble in 910 parts of cold and more readily in hot water.

It becomes anhydrous at 100°, and is the best form for observing the optical activity of quinine (page 525).

Other salts of quinine met with in commerce are the arsenate, benzoate, cacodylate, citrate, ethylcarbonate, formate, glycerophosphate, hydriodide, acid hydriodide, hydrofluoride, hypophosphite, lactate, phosphate, and salicylate.

Quinine Citrate in combination with ferric citrate constitutes the *Ferri et Quininæ Citras*, *British Pharmacopœia* and *United States Pharmacopœia*.

Iron and Quinine Citrate occurs in commerce in the form of thin transparent deliquescent scales, varying in colour from a delicate greenish golden-yellow to yellowish-brown, according to the proportion of ammonium citrate present. The preparation should be somewhat slowly, but freely and completely, soluble in cold water. It is insoluble in alcohol or ether. The aqueous solution has a very bitter and chalybeate taste, and should be only very slightly acid. On adding ammonia to the cold solution, white quinine hydrate is thrown down, and the liquid assumes a darker colour. No ferric hydroxide is precipitated unless the liquid be heated, or a fixed alkali substituted for the ammonia.

Citrate of iron and quinine is liable to several sophistications.

The proportion of *water* in the sample may be ascertained by drying a weighed quantity in the water-oven. It averages 8%, and should not exceed 10 to 12%.

Adulteration with *potassio-citrate* or *potassio-tartrate* of iron would be detected by the strongly alkaline reaction of the residue left on igniting the substance, a genuine preparation yielding an ash neutral or only very faintly alkaline to litmus paper. The substitution of tartaric acid for the citric acid of the sample is now improbable, but may be detected as described in Vol. I.

The proportion of *oxide of iron* can be estimated in the pure preparation with sufficient accuracy by igniting a known weight of the sample. After testing the ash for fixed alkali, a few drops of nitric acid should be added and the residue again ignited. This treatment ensures the complete combustion of the carbon. Citrate of iron and quinine ought to yield from 18 to 20% of ferric oxide on ignition. A more accurate estimation of the iron can be made in the ash, if desired.

Excess of *citric acid* is indicated by the extra acidity of the sample,

but the commercial substance frequently contains a much larger proportion of acid than is prescribed in the *British Pharmacopœia*.

Sulphates are almost invariably present in citrate of iron and quinine, owing to imperfect washing of the ferric hydroxide employed, or to the introduction of the quinine as sulphate instead of precipitated hydrate. The employment of sulphate of quinine is said to render the preparation liable to yield a turbid solution, but it has the advantage of preventing the inevitable loss of alkaloid attending the preparation of quinine hydrate.[1]

The *British Pharmacopœia* requires that the citrate of iron and quinine should contain 15% of alkaloid. The following is the official method of testing the compound: When incinerated, with free access of air, it leaves a residue which when moistened with water is not alkaline to test-paper (absence of fixed alkali).[2] 5 grm. dissolved in 45 c.c. of water and treated with a slight excess of solution of ammonia should yield a white precipitate, which, when dissolved out by repeated treatment of the liquid with ether, and the latter evaporated, and the residue completely dried at 120°, weighs 0.75 grm. This precipitate is almost entirely soluble in a little purified ether; when burned it leaves but a minute residue; neutralised by sulphuric acid, it should answer to the characters of and the tests for quinine sulphate.

The proportion of alkaloid in the citrate of iron and quinine of commerce is often notably less than the 15% required by the *British Pharmacopœia* (see *Pharm. J.*, 1886 [*iii*], **17**, 234; 1888 [*iii*], **19**, 259; 1889 [*iii*], **20**, 1052). Very commonly only 13% is present, and occasionally (in Allen's experience) from 9 to 11%, even in the case of preparations manufactured by English firms of fairly good repute. Foreign specimens sometimes contain only 4 or 5% of alkaloid, and substitution of the quinine by other cinchona bases is common. Amorphous alkaloids are not unfrequently present in considerable proportion.

The *United States Pharmacopœia* states that the double citrate should contain at least 11.5% of dried quinine and 13.5% of metallic iron. The *German Pharmacopœia* states that the citrate should

[1] F. W. Fletcher states that a preparation made with sulphate of quinine contains less calcium salts than when quinine hydrate is used, since the calcium salts introduced in the water employed for washing the alkaline ferric hydroxide are retained by the latter, and are subsequently precipitated as calcium sulphate, instead of remaining in the finished product

[2] The commercial product nearly always leaves a slightly alkaline residue, owing to the ferric hydroxide being precipitated with sodium hydroxide some of which is held tenaciously by the precipitate.

contain 9 to 10% of pure quinine alkaloid, and at least 30% of ferric oxide.

Tincture of quinine, *British Pharmacopœia,* was formerly directed to be made by dissolving 160 grains of crystallised quinine sulphate in 20 fluid ounces of tincture of orange-peel, by the aid of a gentle heat, the solution being filtered after 3 days. This was an unsatisfactory preparation, as in cold weather, or when too weak a spirit was used, it was apt to deposit crystals of quinine sulphate, and so alter in strength. In some cases, at least, the deposit consisted largely of calcium sulphate. In the *Pharmacopœia* of 1898, 175 grains of quinine hydrochloride is substituted for the sulphate, so that the tincture is somewhat stronger than the old preparation. To ascertain the proportion of quinine in the tincture, 1 fluid ounce should be concentrated, and shaken with ether to remove the essential oil of orange-peel. After removing the ether, the aqueous liquid should be cooled, an excess of ammonia added, and then the whole shaken with ether in the usual way (see page 199).

Wine of quinine, *British Pharmacopœia,* contains 1 grain of quinine hydrochloride in each fluid ounce of orange wine. It is apt to be deficient in alkaloid or the quinine may be replaced by other cinchona alkaloids. For its assay, 2 fluid ounces may be concentrated to 1/2 ounce, and then treated like the tincture of quinine (see above). If the alkaloid prove insoluble in ether, a mixture of chloroform and amyl alcohol must be substituted for the ether.

Ammoniated tincture of quinine, *British Pharmacopœia,* is obtained by mixing 2 fluid ounces of ammonia (sp. gr. 0.959) with 18 fluid ounces of 90% alcohol, adding 175 grains of quinine sulphate, and shaking until a clear solution is produced. It is assayed in a similar manner to wine of quinine (see above).

Hydroquinine, $C_{20}H_{26}O_2N_2$, was discovered by Hesse (*Ber.*, 1882, **15**, 856) in the mother-liquors from which quinine sulphate had been crystallised, and subsequently in the commercial salt itself, in which it is sometimes present to the extent of 4%.[1] Quinine can only partially be freed from hydroquinine by repeated crystallisation of the neutral sulphates, but the hydroquinine can be completely separated

[1] The proportion of hydroquinine in the bark is very small, and bears no constant relation to that of the quinine To obtain the hydroquinine pure the alkaloids should be repeatedly crystallised as acid sulphates, the residual quinine destroyed at 0° by potassium permanganate, the hydroquinine liberated from the filtered liquid by sodium hydroxide, extracted with ether or chloroform, and the neutral sulphate repeatedly recrystallised from boiling water.

by converting the alkaloid into the acid sulphate and recrystallising this from water or alcohol, when the hydroquinine remains in the mother-liquor.

As precipitated from a cold solution of a salt by sodium hydroxide, hydroquinine is amorphous, but gradually becomes crystalline. In the latter condition it contains $2H_2O$, which is driven off at 115°. From chloroform and ether the alkaloid crystallises in delicate concentric groups of needles.

The anhydrous alkaloid melts at 172°, with decomposition.

Hydroquinine dissolves readily in alcohol, ether, chloroform, benzene and ammonia, but not in alkali hydroxide solutions, and is only very sparingly soluble in water.

Hydroquinine resembles quinine in its lævo-rotation, fluorescence of its acid solutions, behaviour with the thalleioquin test, and in its physiological action. It differs from quinine by only very slowly decolourising a solution of potassium permanganate.

Crystalline compounds of hydroquinine with cupreine, quinidine, cinchonidine, and some other cinchona bases have been obtained, but not with cinchonine or hydrocinchonine.

Hydroquinine has the usual well-marked basic characters of the cinchona alkaloids, and forms neutral and acid salts.

The sulphate, $B_2H_2SO_4+6H_2O$, forms short prisms, soluble in 348 parts of water at 15°. The salt BH_2SO_4 forms long needles, very soluble in water. Heated to 140° the acid sulphate is converted into the corresponding salt of its isomer hydroquinicine, but with dilute sulphuric acid this change does not occur.

The *tartrate* crystallises with $2H_2O$ in prisms which become anhydrous at 120° and are soluble in 545 parts of water at 17°. The *chromate* is more soluble than the quinine salt, but crystallises with it, and can only be partially separated by boiling with water. $BHCl+2H_2O$ is readily soluble. On mixing its solution with potassium iodide, the *hydriodide* separates as an oily mass which gradually solidifies but does not become crystalline. The *acid salt*, $B(HI)_2+4H_2O$, crystallises in brilliant yellow needles, readily soluble in hot water to a colourless solution, from which the yellow salt separates again on cooling.

When heated to 140° with strong hydrochloric acid, hydroquinine loses a methyl group, and is converted into hydrocupreine, $C_{19}H_{24}O_2N_2$.

Hydroquinicine neutralises acids completely and forms some crystallisable salts. When an ethereal solution of the base is gradually

mixed with a solution of oxalic acid in ether, neutral hydroquinicine oxalate is formed as an amorphous brown mass, readily soluble in chloroform; whereas the oxalate of quinicine, obtained similarly, forms a voluminous precipitate, consisting of very minute needles.

Quinidine. Conquinine. $C_{20}H_{24}O_2N_2$.

This base is isomeric with quinine, and occurs frequently in cinchona barks (especially *Cinchona Pitayensis*) in association with quinine and other alkaloids. It also occurs in cuprea bark.

Quinidine (see also page 194) crystallises from alcohol with $2.5H_2O$ in large monoclinic efflorescent prisms or needles. From absolute alcohol it crystallises in large prisms enclosing 1 molecule of alcohol. From ether permanent rhombohedra containing $2H_2O$ are obtained, and from boiling water permanent plates with $1.5H_2O$. The whole of the water is driven off at 120°. It separates from benzene in anhydrous needles which melt at 171.5°, with decomposition. It is soluble in 2,000 parts of water at 15°, in 22 parts of ether at 20°, in 26 parts of alcohol at 80°, and is not very soluble in chloroform, benzene, or carbon disulphide.

Quinidine is strongly dextrorotatory, its solutions in 97% alcohol giving $[\alpha]_D^{15} = +236.77 - 3.01\,c$, where $c = 1$ to 3.

Quinidine resembles quinine in its taste and physiological effects in being deposited in hydrated crystals from alcohol, in its tolerabl ready solubility in ether, in giving the thalleioquin reaction, and in the fluorescence of its solution in dilute sulphuric acid. It is distinguished from quinine by the permanent bulky precipitate its solutions yield on successive treatment with chlorine water, potassium ferricyanide, and ammonia; by the very sparing solubility of its *hydriodide*, and by its solutions being dextrorotatory.

Quinidine Sulphate, $B_2H_2SO_4 + 2H_2O$, crystallises in white needles or long hard prisms which require 108 parts of cold or 7 of boiling water for solution. It dissolves in 7 parts of cold alcohol, and in 20 of chloroform, but is almost insoluble in ether. The salt differs from the sulphates of the other cinchona alkaloids in requiring a temperature of 120° to render it anhydrous, and in readily taking up the water again in moist air.

Quinidine sulphate is an official remedy in France. It is examined for other alkaloids by a test slightly modified from one devised by De Vrij (*Pharm. J.*, 1877 [*iii*], **8**, 745), who utilises the fact that

quinidine hydriodide requires 1,200 parts of water for solution. To test the purity of the commercial sulphate of quinidine, 0.5 grm. is dissolved in 10 c.c. of water at 60°, and an equal weight of iodide of potassium free from any alkaline reaction added. If the sample be pure, quinidine hydriodide is precipitated on stirring and cooling as a heavy sandy powder, and if the liquid be allowed to stand for half an hour with frequent agitation and is then filtered, addition of 1 or 2 drops of ammonia will cause no turbidity in the clear filtrate. A slight turbidity indicates a trifling admixture of other alkaloids, but if a decided precipitate occur the alkaline liquid should be shaken with a mixture of amyl alcohol and chloroform, or chloroform only, and the solvent evaporated to ascertain the proportion and nature of the admixture, which may be cinchonidine or quinine, but is usually cinchonine. The appearance of the precipitated hydriodide is some indication of the presence of impurity, as in the presence of cinchonine or cinchonidine it is resinous instead of being sandy.

For the detection of *inorganic impurities* (*e. g.*, calcium or sodium compounds) in commercial quinidine sulphate, Hesse treats 1 grm. of the sample with 7 c.c. of a mixture of 2 volumes of chloroform with 1 of alcohol of 95%. Complete solution will take place in the absence of impurities.

The presence of *cinchonidine* sulphate in the quinidine salt may be detected by treating the sample with pure chloroform Unless only a very small proportion of the impurity be present, part of it will remain undissolved. Smaller quantities may be detected by shaking the chloroform solution with cold water, in which the whole of the cinchonidine and part only of the quinine salt will dissolve, and the former will be precipitated on addition of Rochelle salt.

A solution of quinidine sulphate in chloroform is at first colourless, but on keeping becomes yellow with a slight green reflection. On shaking this solution with water, the colouring-matter is extracted and the aqueous liquid shows a magnificent green fluorescence.

Quinamine. *Chinamine.* $C_{10}H_{24}O_2N_2$.

This alkaloid was first discovered by Hesse in the bark of *Cinchona succirubra*, and has since been detected in *C. officinalis*, *rosulenta*, and several varieties of *Cinchona Calisaya*, particularly *Ledgeriana*.[1]

[1] The mother-liquors from the crystals of quinine sulphate are precipitated with Rochelle salt, the filtrate treated with ammonia, and the precipitate washed with ether. The ethereal washings are treated with acetic acid, the liquid neutralised, and while warm treated

Quinamine crystallises in delicate hair-like anhydrous needles, which melt at 172°. $[\alpha]_D^{15}$ in 2% alcoholic solution (97% alcohol) is +104.5°.

Quinamine is nearly insoluble in cold water, more readily in boiling. Hot alcohol dissolves it freely. It also dissolves in boiling ether, petroleum spirit, and benzene.

Quinamine itself is almost tasteless, but its solutions in acids are very bitter. The solution in excess of dilute sulphuric acid exhibits no fluorescence. Acid solutions of quinamine are very prone to decomposition with formation of an amorphous alkaloid called quinamidine, isomeric with quinamine. Quinamicine is also formed and under certain conditions apoquinamine, $C_{19}H_{22}ON_2$, results. When tested with chlorine or bromine water and ammonia, solutions of quinamine yield a yellowish amorphous precipitate, but no green colour. The solid alkaloid, when moistened with strong nitric acid, gives a yellow colouration.

Conquinamine, $C_{19}H_{24}O_2N_2$, occurs with quinamine, but in smaller proportion. It may be separated from the latter base by fractional crystallisation of the nitrates, oxalates, or hydrobromides, the conquinamine salts being in each case the less soluble (*Annalen*, 1881, **209**, 38, 62). Conquinamine forms colourless or golden-yellow tetragonal crystals, m. p. 121–123°, easily soluble in ether, chloroform, and benzene. $[\alpha]_D^{15} = +204.1°$ for a 4% solution in absolute alcohol (Oudemans, *Annalen*, 1881, **209**, 46). $B_2H_2SO_4 + x\,H_2O$ is *very soluble*. The *aurichloride* is a yellow precipitate, becoming purple. Conquinamine closely resembles quinamine. When heated with concentrated hydrochloric acid, it yields apoquinamine, $C_{19}H_{22}ON_2$.

Cinchonidine. $C_{19}H_{22}ON_2$.[1] (See also page 499.)

This base is contained in several species of cinchona, but is especially characteristic of the red bark of *C. succirubra*. It was formerly confused with quinidine, and the name conchinine was used to distinguish them.

with potassium thiocyanate, till on cooling cinchonine can no longer be detected Quinidine is then precipitated, together with colouring matter The filtered liquid is treated with sodium hydroxide, and the resinous precipitate dissolved in a minimum of hot 80% alcohol, from which quinamine crystallises on cooling

[1] Cinchonidine was formerly believed to contain $C_{20}H_{24}ON_2$; but its conversion by heating with concentrated hydrochloric acid into apocinchonidine, $C_{19}H_{22}ON_2$, without formation of methyl chloride, and analyses of hydrochloride, sulphate, and platinichloride establish the formula given in the text.

Cinchonidine crystallises in short anhydrous prisms or thin plates, soluble in 16 parts of alcohol and 188 of ether. It is readily soluble in amyl alcohol and chloroform. It is lævorotatory, $[\alpha]_D^{15}$ (where $c=4$), in chloroform solution being $-70.0°$ (Hesse); while in dilute hydrochloric acid solution ($c=5$) $[\alpha]_D^{15}= -174.6°$. In 97% alcohol $[\alpha]_D^{15} = -107.48 + 0.297\ c$, where $c=1$ to 5.

Cinchonidine resembles quinine in the sign of its optical activity, in the insolubility of the anhydrous neutral sulphate in chloroform, and in the sparing solubility of the tartrate in water. According to Hesse, it forms a crystalline compound with quinine containing $C_{20}H_{24}O_2N_2+2C_{19}H_{22}ON_2$. It is distinguished from quinine by its lower specific rotation, its more sparing solubility in ether, its non-fluorescence, by not giving the thalleioquin reaction, and by the greater solubility of its neutral and acid sulphate and iodosulphate. The accurate separation of cinchonidine from quinine presents great difficulties, and is discussed at length on page 523 *et seq.* Cinchonidine has only about two-thirds of the therapeutic activity of quinine.

Cinchonidine is isomeric with cinchonine, from which it differs by its lævo-rotation; its greater solubility in ether; the insolubility of its tartrate in water; the insolubility of the anhydrous sulphate in chloroform; and the formula of the crystallised sulphate.

By boiling cinchonine with potassium hydroxide and amyl alcohol part of the alkaloid is converted into cinchonidine. (Koenigs and Husmann, *Ber.*, 1896, **29**, 2185.)

The following table shows the formulæ and solubilities of the principal salts of cinchonidine:

Salt	Formula	Appearance	Solubility in water	
			Cold	Hot
Hydrochloride..	$BHCl+1H_2O$	Double pyramids or octahedra	30	Readily soluble
Hydrobromide ..	$BHBr+1H_2O$.	Long colourless needles.	40	Freely soluble.
Sulphate ..	$B_2H_2SO_4+xH_2O$	Silky lustrous needles, or thin quadratic prisms	100	4
Oxalate.....	$B_2H_2C_2O_4+6H_2O$.	Prismatic crystalline powder	252 at 12°	
Tartrate.....	$B_2C_4H_6O_6+2H_2O$.	Crystalline precipitate.	1265 at 10°	

Cinchonidine sulphate, $B_2H_2SO_4$, is remarkable for the number of hydrates it is capable of forming. From a moderately concentrated aqueous solution it crystallises with $6H_2O$ in brilliant needles; from a hot and concentrated aqueous solution in hard prisms or acicular silky crystals containing $3H_2O$ (official in the *British* and *United States Pharmacopœias*); and from alcohol in fine prisms with $2H_2O$. A hydrate containing $5H_2O$ has been described by Hesse.[1] The hexahydrate is somewhat efflorescent. All water is lost at 100°, and $2H_2O$ re-absorbed in moist air.

Cinchonidine sulphate is sometimes contaminated with an admixture of the corresponding salts of cinchonine and quinidine. To detect these, Hesse (*Zeitsch. anal. Chem.*, 1876, **15**, 464) dissolves 0.5 grm. of the salt in 20 c.c. of water at 60°, and adds 1.5 grm. of Rochelle salt. A crystalline precipitate of the sparingly soluble cinchonidine tartrate is produced. After standing 1 hour the liquid is filtered, and the filtrate tested with a drop of ammonia. Any turbidity or precipitate is due to the presence of more than 0.5% of cinchonine or 1.5% of quinidine. These may be distinguished by treating the filtrate with potassium iodide as described on page 526.

Hager recommends the use of 0 1 grm. of cinchonidine sulphate, 0 3 of Rochelle salt, and 20 c.c. of cold water. The liquid is frequently agitated, filtered after 1 hour, and tested with a few drops of ammonia. As thus performed, the test is less strict than that of Hesse, but perhaps, on that account, is better suited for medicinal purposes.

The precipitate of *cinchonidine tartrate* obtained in the above tests is soluble in about 1,200 parts of cold water, but almost wholly insoluble in a strong solution of Rochelle salt. After drying at 100°, it contains 80.84% of cinchonidine. It will contain quinine if any of that base were present in the sample. In such case the solution of the precipitate in excess of dilute sulphuric acid will be notably fluorescent.

Hesse has also proposed to distinguish the sulphates of the cinchona bases by their behaviour with chloroform. The *anhydrous* neutral sulphates of quinine and cinchonidine are almost insoluble in alcohol-free chloroform, while the corresponding salts of cinchonine and quinidine dissolve readily (see pages 525, 536). Cinchonidine sulphate requires, when anhydrous, 300 of boiling or 1,000 parts of cold chloroform, the

[1] Five commercial samples of cinchonidine sulphate examined by A. B Prescott, lost, at 100°, proportions of water ranging from 6 36 to 7.04%. $B_2H_2SO_4+3H_2O$ requires 7 30%.

undissolved portion becoming gelatinous. In the presence of cinchonine or quinidine sulphate its solubility in chloroform is increased. It is soluble in 100 parts of cold water, 60 parts of alcohol, and is almost insoluble in ether or benzene. The anhydrous salt melts at 205°, with decomposition.

The presence of quinidine and quinine in cinchonidine sulphate can be recognised by the thalleioquin reaction and the fluorescence of the solution in dilute sulphuric acid.

Homocinchonidine, $C_{19}H_{22}ON_2$ (see also page 499), accompanies cinchonidine in many cinchona barks, especially that of *C. rosulenta*, and passes into the dark sulphate mother-liquors in the quinine manufacture. It crystallises from alcohol in anhydrous prisms, or from a dilute solution in leaflets, almost insoluble in water, but soluble in chloroform. $B_2H_2SO_4+6H_2O$ crystallises from hot water in white needles, but from strong solutions the salt separates as a white mass, which after drying resembles magnesia.

Hesse states that homocinchonidine is an essentially different substance from cinchonidine, and that it is not possible to convert one into the other. The two bases may be separated by fractional crystallisation of their sulphates from aqueous solution. Homocinchonidine sulphate to which has been added 1% of quinine sulphate crystallises in the form of cinchonidine sulphate, and, by many chemists, it is regarded as a pure form of the latter salt, the only point of difference being the crystalline form.

Hydrocinchonidine, $C_{19}H_{24}ON_2$, occurs in the mother-liquors from cinchonidine sulphate. It crystallises from dilute alcohol in leaflets, and from absolute alcohol in short anhydrous prisms, m. p. 230°. It is less soluble in alcohol than cinchonidine, and is almost insoluble in water, ether or chloroform. It is lævorotatory, and is identical with cinchamidine.

Cinchonine. $C_{19}H_{22}ON_2$; or $C_9H_6N.CH_2.C_7H_{10}(OH)(CH=CH_2)N$.[1]

This important alkaloid is almost invariably present in cinchona barks. When the free bases are crystallised from alcohol the cinchonine is deposited largely before the quinine; unless the latter base is present in relatively large amount, in which case the greater part should be previously removed by crystallising the sulphates.

Cinchonine crystallises from alcohol in anhydrous shining prisms or needles. It melts at 264° to a colourless liquid, and partially

[1] The constitution of cinchonine is discussed on page 501.

sublimes at a higher temperature. According to Hlasiwetz, it may be readily sublimed in a current of hydrogen or ammonia.

Cinchonine is soluble in 3,670 parts of water at 20°, and in 2,500 at 100°. 1 part of cinchonine dissolves in 120 parts by weight of cold rectified spirit or 28 of boiling alcohol, in 350 parts of chloroform, in 371 of ether, and in 109 parts of amyl alcohol. It requires only about 13 parts of a mixture of 6 grm. of chloroform with 1 of rectified spirit, and is soluble in 23 parts of a mixture of 4 of chloroform and 1 of amyl alcohol.

A. B. Prescott found the following to be the solubility of cinchonine in different physical conditions, and at the boiling-point of the solvent:

Condition of alkaloid	Parts by weight of washed solvent required			
	Ether	Chloroform	Amyl alcohol	Benzene
Crystallised	719	828		
Amorphous	563			
"Nascent,"[1]	526	178	22	37 6

It will be seen from these results that amyl alcohol is by far the best solvent for cinchonine, except a mixture of amyl alcohol and chloroform. On the other hand, ether is the best solvent for effecting an approximate separation of cinchonine from quinine.

When heated to a high temperature with an alkali, cinchonine yields quinoline, C_9H_7N, together with other products. With iodine trichloride, cinchonine yields a yellow precipitate.

Cinchonine is not precipitated in the cold from a solution containing tartaric acid by adding sodium hydrogen carbonate. On heating the liquid, however, carbonic acid escapes and cinchonine is separated.

Cinchonine is not precipitated from a dilute neutral solution by Rochelle salt, its tartrate being soluble in 33 parts of cold water.

The precipitate formed by ammonia in solutions of cinchonine is not soluble in excess of the reagent. The precipitate is amorphous when first produced, but speedily becomes crystalline.

Cinchonine is sharply distinguished from quinine by the very limited solubility of the free base in ether, by the solubility of the anhydrous neutral sulphate in chloroform, by the solubility of its neutral

[1] To obtain the alkaloid in the "nascent" state, the solvent was added to its sulphuric acid solution, which was then warmed to the b p of the former The liquid was next made slightly alkaline with ammonia, shaken, kept warm for 5 minutes, and filtered.

tartrate in water, by its failure to give the thalleioquin reaction, by its dextrorotatory power, and by the non-fluorescence of its solution in excess of dilute sulphuric acid. Methods of detection and separation based on these facts are given on pages 520 and 521.

Cinchonine sulphate, $(C_{19}H_{22}ON_2)_2H_2SO_4 + 2H_2O$, forms short, hard, shining, clino-rhombic prisms, with dihedral summits. The salt becomes anhydrous at 100°, and melts with partial decomposition at about 200°. Cinchonine sulphate has a very bitter taste, dissolves in 58 parts of cold water, 32 parts of water at 80°, 10 parts of cold alcohol, and in 5.2 parts of alcohol at 60°. It is insoluble in ether or benzene. The *anhydrous* salt is soluble in 60 parts of cold or 22 of boiling chloroform, a fact which distinguishes it from the sulphates of cinchonidine and quinine.

A solution of cinchonine sulphate does not give the thalleioquin reaction, and is not rendered fluorescent by dilution with very weak sulphuric acid.

The mode of assaying of cinchonine sulphate is sufficiently indicated under the head of "Quinine Sulphate" (page 517 *et seq*).

Cinchonine hydrochloride, $C_{19}H_{22}ON_2,HCl + 2H_2O$, is readily soluble in water and alcohol, and somewhat so in ether and chloroform. It has been not unfrequently employed to adulterate sulphate of quinine. In such case the solution of the sample in very dilute sulphuric or nitric acid will give a while, curdy precipitate of silver chloride on adding silver nitrate. Cinchonine will be detected by the tests for that alkaloid.

When heated in a dry test-tube, cinchonine hydrochloride gives purple fumes much resembling the vapour of iodine. The *sulphates* of the cinchona bases do not give this reaction.

Hydrocinchonine, or cinchotine, $C_{19}H_{24}ON_2$, generally occurs with cinchonine, and is sometimes found to the extent of 10% in commercial cinchonine, from which it is obtained by recrystallising the alkaloid from alcohol. The alcoholic filtrate is acidified with sulphuric acid and treated at 0° with potassium permanganate solution till a red colour persists. The hydrocinchonine is then precipitated from the filtrate with ammonia.

Hydrocinchonine crystallises in anhydrous prisms, m. p. 286°. It is soluble in 136 parts of 90% alcohol at 15°, and in 534 parts of ether at 20°. It is almost insoluble in cold water, but dissolves notably in hot water. It is dextrorotatory. Rabe gives $[\alpha]_D^{12} = +192.1$,

when $c = 0.406$ in 99% alcohol (*Annalen*, 1910, **373**, 100). Hydrocinchonine sulphate, $(C_{19}H_{24}ON_2)_2H_2SO_4 + 12H_2O$, crystallises in efflorescent prisms, soluble in about 30 parts of cold water. The hydrochloride, $BHCl + 2H_2O$, crystallises in fine needles, soluble in 47 parts of water.

Amorphous Cinchona Bases.

Quinoïdine is obtained in quinine factories by precipitating the brown mother-liquors with ammonia. When first used quinoïdine contained a great proportion of the alkaloids other than quinine contained in the bark, but as the processes of manufacture of the crystalline alkaloids have been improved it consists of the uncrystallisable bases in the bark in most uncertain proportions, with any quinicine and cinchonicine that may have been formed in the process of manufacture. The uncrystallisable bases of the bark consist largely of anhydrobases.

Commercial quinoïdine is a dark brown, brittle, "extractiform" mass, softening below 100°, and having usually a slight alkaline reaction. It is a product which has never been very favorably regarded in this country, though is has received official recognition in the *German Pharmacopœia* of 1882 and the *United States Pharmacopœia* of 1883, but has been omitted from the latest editions of both. Both works limit the ash to 0.7%. By the latter it is described as almost insoluble in water, freely soluble in alcohol, chloroform, and dilute acids, and partly soluble in ether and benzene. When triturated with boiling water, the liquid, after filtration, should be clear and colourless, and should remain so after addition of an alkali. The *German Pharmacopœia* requires that quinoïdine should dissolve clear in an equal weight of 1 part of dilute acetic acid with 9 parts of water, so as to leave scarcely any residue; and it must also form a clear solution with 9 times its weight of cold dilute spirit. Quinoïdine is said to be liable to adulteration with mineral matters, resins, liquorice, glucose, etc., all of which sophistications would be detected by one or other of the above tests.

Quinicine, $C_{20}H_{24}O_2N_2$, is a yellowish, amorphous, anhydrous substance, which melts at about 60°, assuming a reddish-brown colour which becomes darker at 100°. It is nearly insoluble in water, but has a bitter taste. The alcoholic solution has a strong alkaline reaction,

and absorbs carbon dioxide from the air. The alkaloid is readily soluble in chloroform or ether. Quinicine gives a green colouration when treated in solution with chlorine- or bromine-water and ammonia, but is distinguished from quinine and quinidine by producing a white amorphous precipitate with sodium hypochlorite or solution of bleaching powder. In applying this test the liquid should be slightly, but not strongly, acidified with hydrochloric acid. Quinicine may be separated from the accompanying alkaloids by adding ammonia, when the ammonium salt formed dissolves the liberated alkaloid, which may then be recovered by agitation with ether. If sodium hydroxide be employed instead of ammonia the alkaloid is thrown down as an oily mass.

A solution of quinicine in excess of dilute sulphuric acid has a yellow colour but exhibits no fluorescence.

Quinicine forms a crystalline oxalate, bitartrate, sulphocyanide, and hydriodide, and double salts with the chlorides of platinum and gold. Hesse claims to have prepared a crystalline sulphate and bisulphate, but later workers have been unable to obtain either of these salts in a crystalline state. Quinicine is dextrorotatory, a 2% solution in chloroform giving $[\alpha]_D^{15} = +44.1°$.

Quinicine solutions are not precipitated by Rochelle salt. They are completely precipitated by adding excess of potassium thiocyanate, which throws down quinicine thiocyanate as an oil which subsequently solidifies. It is soluble in pure water, but insoluble in solutions of alkaline thiocyanates.

Cinchonicine, $C_{19}H_{22}ON_2$, when precipitated by sodium hydroxide from the solution of one of its salts, forms a yellow viscous mass readily drawn out into colourless strings. It liquefies at about 50°, and at 80° turns brown. At higher temperatures (*e.g.*, 100°) it becomes dark brown, and is converted into a substance resembling "quinoïdine." Upon cooling it remains soft.

In most reactions, including its behaviour with ammoniacal salts and with hypochlorites, cinchonicine closely resembles quinicine, and hence is distinguished from cinchonine and cinchonidine. It is distinguished from quinicine by not giving a green colour with chlorine- or bromine-water and ammonia.

Cinchonicine is bitter, and in the free state has a strongly alkaline reaction. The free alkaloid liberates ammonia from its salts, and combines with carbon dioxide. It forms a crystalline oxalate, hydro-

gen tartrate, hydriodide, and platinichloride. It is dextrorotatory, a 1% solution of the base in chloroform or absolute alcohol giving $[\alpha]_D^{15} + 47.2°$.

Anhydro-bases. Certain amorphous bases, distinct from quinicine and cinchonicine, exist ready-formed in cinchona barks. They are not convertible into quinicine or cinchonicine, and appear to be formed by the coalescence of 2 molecules of the crystallisable alkaloids, accompanied in the case of quinine and quinidine with the elimination of a molecule of water. Thus:

$$\underset{\text{Quinine or Quinidine.}}{2C_{20}H_{24}O_2N_2} - H_2O = \underset{\text{Diquinicine.}}{C_{40}H_{46}O_3N_4}.$$

$$\underset{\substack{\text{Cinchonidine or} \\ \text{Cinchonine.}}}{2C_{19}H_{22}ON_2} = \underset{\text{Dicinchonicine.}}{C_{38}H_{44}O_2N_4}.$$

The molecular formula of these alkaloids is probably not as represented above, since Hesse on determining the molecular weight by the cryoscopic method obtained a similar result to that given by cinchonine.

These bases constitute the greater part of the amorphous alkaloid contained in commercial quinoïdine. They are wholly amorphous, as also are all their salts. The solution of diquinicine in excess of dilute sulphuric acid is fluorescent, gives the thalleioquin reaction, and is dextrorotatory. Dicinchonicine does not possess these characters.

De Vrij has pointed out a distinction between quinicine, cinchonicine, and the natural amorphous alkaloids. If the neutral oxalates of the bases be rendered anhydrous by heating at 100°, and the dry salts treated with chloroform, they behave in a characteristic manner. Oxalate of *quinicine* dissolves sparingly in chloroform at the ordinary temperature, but freely in the boiling liquid. On cooling, the solution deposits the greater part of the oxalate in crystals. Anhydrous *cinchonicine* oxalate dissolves freely in cold chloroform. By adding a few drops of water on the surface, the solution is transformed in a few minutes into a solid mass. The oxalates of the *natural amorphous alkaloids* are very soluble in chloroform. The solution remains clear on adding a few drops of water, but the water dissolves out some of the oxalate from its chloroform solution. The amorphous oxalate is highly deliquescent, but the oxalates of quinicine and cinchonicine remain unchanged in the air.

Alkaloids of Remijia Barks.

The barks of the various species of *Remijia* vary greatly in the alkaloids which they contain. Thus, while the bark of *R. pedunculata* contains quinine and the allied alkaloid cupreine, that of *R. Purdieana*, which anatomically closely resembles the former, and has been confounded with it, contains no alkaloid closely related to quinine except comparatively small proportions (0.1 to 0.2%) of cinchonine and cinchonamine. Cusconidine, which occurs in the bark of *R. Purdieana*, is also found in that of *Cinchona Pelletierana*, together with cusconine and aricine, which two bases do not appear to be present in Remijia bark. The bases isolated from this bark by Hesse were (in addition to cinchonine and cinchonamine) concusconine, chairamine, conchairamine, chairamidine and conchairamidine, the formulæ and certain characters of which are given on page 500.[1] Concuscamidine does not appear to be a definite substance. All these alkaloids, like aricine and cusconine, contain 4 atoms of oxygen, and form a group only distantly related to cinchonine and cinchonamine. Concusconine has the same empirical formula as cusconine, aricine, and brucine, and resembles the strychnos alkaloids in some of its reactions. It crystallises with $1H_2O$, and is dextrorotatory, while cusconine has a lower m. p., crystallises with $4H_2O$, and rotates to the left. Concusconine resembles chairamine and its isomers in giving a deep green colouration when the solution in hydrochloric or sulphuric acid is mixed with concentrated nitric acid, a property which is not common to cusconine or aricine. *Echitamine* or *ditaine*, an alkaloid contained in the bark of *Alstonia scholaris*,[2] only differs by H_2 from chairamine

[1] To extract the whole of the alkaloids, amounting to 2 to 3%, Hesse treated the finely ground bark with hot alcohol, distilled off the solvent, treated the residue with excess of sodium hydroxide, and agitated with ether On shaking the separated ethereal layer with dilute sulphuric acid, a pale yellow, curdy mass (A) separated, a portion of which remained suspended in the ether and part in the yellow acid liquid (B) On separating the latter (B) and adding very dilute nitric acid, *cinchonamine* nitrate was precipitated (mixed with the nitrates of some of the bases of group A), while *cinchonine* remained in solution. The curdy precipitate (A) was digested with dilute sodium hydroxide, the liberated alkaloids washed and air-dried, dissolved in hot alcohol, and treated with one-eighth of their weight of sulphuric acid (H_2SO_4) Almost all the *concusconine* immediately precipitated as sulphate, a small additional quantity separating on cooling *Chairamine* hydrochloride was precipitated on adding strong hydrochloric acid to the cold alcoholic mother-liquor The filtrate from this was warmed and treated with a little potassium thiocyanate, and the precipitate of *conchairamine* thiocyanate filtered off On adding more of the reagent, till the dark coloured solution became light brown, a pitchy mass separated, after the removal of which the solution was treated with excess of ammonia and shaken with benzene. The benzene was extracted with acetic acid, and the acetic solution treated with a saturated solution of ammonium sulphate, which precipitated a mixture of the sulphates of *chairamidine* and *conchairamidine*, separable by fractional crystallisation from hot water, in which the latter salt is the less soluble

[2] Dita Bark, from *Alstonia* or *Echites scholaris* (Philippine Islands), has febrifuge properties, and contains the following alkaloids, together with several peculiar indifferent compounds. For the extraction and separation of the alkaloids the bark is extracted with hot alcohol, the solvent distilled off, the residue treated with ammonia and shaken with ether.

and its isomers, to which it presents a considerable resemblance. *Alstonine*, $C_{21}H_{20}O_4N_2$, an amorphous alkaloid, which occurs together with *alstonidine* and *porphyrine* in the bark of *Alstonia constricta*, is strongly fluorescent in acid solutions, and is not improbably related to the cusconidine group. Hesse suggests that *gelsemine*, $C_{24}H_{28}O_4N_2$, the poisonous alkaloid from the root of *Gelsemium sempervirens* (yellow jesamine), is related to these alkaloids, and points out that the colouration it gives with nitric acid somewhat resembles the reaction of concusconine.

A full description of the alkaloids of *Remijia Purdieana* and *Cinchona Pelletierana* barks has been published by O. Hesse (*Annalen*, 1877, **185**, 296, 323; 1884, **225**, 211; *J. Chem. Soc*, 1880, **38**, 328; 1885, **48**, 64; *Pharm. J.*, 1884 [*iii*], **15**, 772). Aricine has been re-investigated by Moissan and Langrin (*Compt. Rend.*, 1890, **110**, 469). Cinchonamine and cupreine are described below.

Cinchonamine, $C_{19}H_{24}ON_2$ (see page 500), occurs in the bark of *Remijia Purdieana* (false cuprea bark), a tree growing in the Columbian provinces of Antioquia. Its isolation is described on page 546. It is soluble in alcohol, ether, chloroform, benzene, and carbon disulphide but only sparingly in water or petroleum spirit. It is very bitter, poisonous,[1] does not yield methyl chloride when heated with strong hydrochloric acid, nor give a colouration with ferric chloride, or with the thalleioquin test. It is said to be insoluble in strong hydrochloric acid, but dissolves in strong nitric acid with bright yellow, and in strong sulphuric acid with reddish-yellow colour.

The hydrochloride, B.HCl, crystallises in soft laminæ, of a white glistening appearance. The *sulphate*, $B_2H_2SO_4$, forms colourless prisms, very soluble in water, less soluble in dilute alcohol. A full description of the salts of cinchonamine has been given by B. F. Howard and F. Perry (*J. Soc. Chem. Ind.*, 1905, **24**, 1281). The

which dissolves the *ditamine* The residue is treated with solid potassium hydroxide and extracted with chloroform, which is evaporated, and the residue treated with concentrated hydrochloric acid, when *ditaine* hydrochloride separates while echitenine remains dissolved

Echitamine or Ditaine, $C_{22}H_{28}O_4N_2+4H_2O$, forms glassy prisms, m. p. 206°. $[\alpha]_D^{15}$ −28·8° ($c=2$, 97% alcohol) Very bitter Moderately soluble in water, alcohol, and ether A strong base not precipitated by ammonia Decomposes sodium chloride, setting free sodium hydroxide Reduces Fehling's solution after boiling with hydrochloric acid. Concentrated sulphuric acid dissolves ditaine with purple-red colour, nitric acid gives a purple-red, changing to green

Ditamine, $C_{19}H_{19}O_2N$, an amorphous powder melting at 75°, soluble in alcohol, ether, and chloroform

Echitenine, $C_{20}H_{27}O_4N$, brownish, amorphous, melting above 120° Forms amorphous salts

[1] Séc and Bockefontaine (*Compt Rend*, 1885, 100, 366) found cinchonamine (sulphate) 6 times as toxic as quinine, cinchonidine, or cinchonine An injection of 0.25 grm killed a guinea-pig in a few minutes

nitrate, $C_{19}H_{24}N_2O.HNO_3$, forms short prisms, m. p. 195°, almost insoluble in cold water. The use of this alkaloid for the estimation of nitrates in vegetable tissues, and for the detection of small quantities of nitric acid in effluents and mineral waters was proposed by Arnaud. It has been successfully employed in the form of the hydrochloride for the estimation of nitrates in various inorganic and organic compounds by B. F. Howard and O. Chick (*J. Soc. Chem. Ind.*, 1909, **28**, 53).

Cupreine, $C_{19}H_{22}O_2N_2$, or $C_{19}H_{20}(OH)N_2OH$. This interesting alkaloid was discovered by Paul and Cownley in the bark of *Cinchona cuprea* or *Remijia pedunculata*, a tree growing in the districts surrounding the Magdalena River and the Upper Orinoca. Since 1881, cuprea bark has been largely used for the manufacture of quinine[1] but now very little of this bark is obtainable.

Cupreine crystallises form alcohol in the anhydrous form, but from ether in concentric prisms containing $2H_2O$. When the alcoholic solution is diluted with water, the precipitate contains B_3+H_2O. The hydrates lose their water at 125°, and melt at 198°. Cupreine is only sparingly soluble in ether or chloroform, but readily in alcohol. The alcoholic solution is lævorotatory ($[\alpha]_D^{16}=-175.3°$; $c=1.5$, 97% alcohol), alkaline, gives a dark reddish-brown colouration with ferric chloride, and responds to the thalleioquin test. The solution of cupreine in dilute sulphuric acid is not fluorescent. The free base precipitated by ammonia is only slightly soluble in excess, and may be extracted by ether. When cupreine is liberated from a salt by a fixed alkaline hydroxide, it dissolves on adding an excess of the reagent, forming (with soda) a definite crystallisable compound containing $C_{19}H_{21}ON_2.ONa$, from the solution of which the alkaloid cannot be extracted by ether.[2] This behaviour is due to the presence of a hydroxyl-group having a phenolic character (compare Morphine, page 356). The cupreinates of potassium and sodium are very soluble in water, and the corresponding compounds of calcium, lead, and silver have a strong alkaline reaction, and are more or less soluble in water. From the fact that alkalies form only mono-derivatives, while

[1] For the preparation of cupreine, the crude quinine sulphate from the cuprea bark is dissolved in dilute sulphuric acid, excess of sodium hydroxide added, and the quinine extracted by agitation with ether. The separated alkaline liquid is neutralised with sulphuric acid, when cupreine sulphate crystallises out The sulphate is treated with ammonia and boiling ether, from which the cupreine crystallises on cooling.

[2] When cupreine and potassium or sodium hydroxide are mixed in molecular proportions, a portion of the alkaloid (10 to 20%) is extracted on agitation with ether, but this may be prevented by using some excess of alkali.

2 atoms of the hydroxyl of cupreine can be replaced by acetyl,[1] it is probable that the hydroxyl-atoms have different functions, as is the case with those of the morphine-molecule.

When heated with hydrochloric acid (sp. gr. 1.125) to 140°, cupreine is converted into apoquinine, without formation of methyl chloride.

The conversion of cupreine into quinine is described on page 195.

Cupreine yields 2 classes of *salts*. Those of the general formula $\overline{BA}$ are sparingly soluble, and the aqueous solutions have a yellow colour, though their alcoholic solutions are perfectly colourless. The salts of the formula $\overline{BA}_2$ are, as a rule, pretty freely soluble, and their aqueous solutions are colourless.

Cupreine sulphate, $B_2H_2SO_4+6H_2O$, crystallises in minute white needles, very difficultly soluble in cold water, and insoluble in a saturated solution of sodium sulphate. $BH_2SO_4+H_2O$ crystallises in prisms sparingly soluble in cold water. *Cupreine tartrate* forms delicate efflorescent needles, very sparingly soluble in cold water. *Cupreine thiocyanate* is produced on adding potassium thiocyanate to a hot solution of the monohydrochloride. The liquid becomes turbid and gradually deposits acicular crystals of the salt. It is very sparingly soluble in, and is precipitated in an oily form by, an excess of the precipitant.

Homoquinine.—When molecular proportions of quinine and cupreine are dissolved in dilute acid, and the solution precipitated by ammonia and shaken with ether, the solvent deposits on evaporation characteristic crystals[2] of a molecular compound of quinine and cupreine containing $C_{20}H_{24}O_2N_2,C_{19}H_{22}O_2N_2+4H_2O$. The same substance is readily obtainable by precipitating a solution of sodium cupreinate with one of quinine hydrochloride:

$$C_{20}H_{24}O_2N_2,HCl+C_{19}H_{21}ON_2.ONa=$$
$$NaCl+C_{20}H_{24}O_2N_2,C_{19}H_{21}ON_2.OH.$$

This remarkable compound was discovered and described simultaneously by Howard and Hodgkin (*J. Chem. Soc.*, 1882, **41**, 66), Paul and Cownley (*Pharm. J.*, 1881 [*iii*], **12**, 497), and W. G.

[1] **Diacetyl-cupreine,** $C_{19}H_{20}(C_2H_3O)_2O_2N_2$, was obtained by Hesse by heating cupreine with acetic anhydride to 85° for a few hours It forms hexagonal plates m p 88°, and is soluble in alcohol, chloroform, and ether The alcoholic solution is strongly alkaline, gives no colour with ferric chloride, but is turned dark green by chlorine and ammonia By alkali hydroxides, the base is hydrolysed in a few minutes with formation of cupreine and acetic acid.

[2] Homoquinine is deposited from ether in very thin prismatic laminæ, having characteristically-shaped ends terminated with two oblique planes.

Whiffen under the name of homoquinine, prior to the isolation of cupreine by Paul and Cownley (*Pharm. J.*, 1884 [*iii*], **15**, 221). It forms salts, having different characters from those either of quinine or cupreine, and is only resolved into its constituents by precipitating the solution with excess of sodium hydroxide, when the quinine may be shaken out with ether, while the cupreine remains in the alkaline liquid as sodium cupreinate.

The analytical differences between homoquinine and cupreine have been fully described by Paul and Cownley (*Pharm. Jour.*, 1884 [*iii*], **15**, 402).

In concluding this section the reviser desires most gratefully to acknowledge the help given him by Mr. David Howard, who has kindly read through the original and revised work and made many suggestions and corrections. His wide scientific knowledge of the subject and his fifty years' practical experience in the testing of cinchona barks and alkaloids he has placed freely at my disposal, a fact which has materially lessened the labour of revision. Thanks are also due to Messrs Howards & Sons, of Stratford, who kindly gave me the use of their complete library of literature on the subject for purposes of reference.

BERBERINE AND ITS ASSOCIATES.

By EDWARD HORTON, B. Sc.

BERBERINE.

Berberine is an alkaloid occurring in a very large number of plants, in many cases in association with one or more of the alkaloids, berbamine, oxyacanthine, hydrastine, canadine, etc. It is the only natural basic colouring matter receiving practical application as a dye.

The principal sources of berberine and the associated alkaloids are the roots of the following plants:

Plant	Alkaloids, etc.
Berberis vulgaris (Barberry)[1]	Berberine, oxyacanthine, berbamine, and at least two other alkaloids (Hesse)
Berberis aquifolium	Berberine, 2 35%, oxyacanthine, 2 82%
Coptis trifolia	Berberine, 4%
Coptis teeta (India)	Berberine, 8 5%, coptinine (crystallisable, Gross)
Hydrastis Canadensis (Golden seal)	Berberine, 1 3 to 1 8%, hydrastine, 1 5%; canadine, etc Also meconin and phytosterin.
Jateorhiza Calumba or Cocculus palmatus (Calumba root)	Columbic acid, and the neutral principle columbin
Menispermum Canadense	Berberine, oxyacanthine, menispermine, menispine

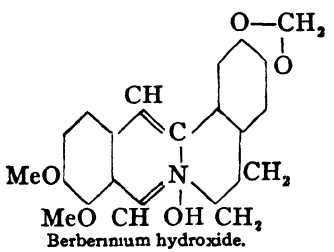
Berberine. $C_{20}H_{17}O_4N,H_2O$, or $C_{18}H_{11}(O.CH_3)_2O_2N,H_2O$.

Berberine was discovered in 1826 by Chevallier and Pelletan (*J. Chim. médicale*, 1826, **2**, 314), who found it in *Xanthoxylum clava-Herculis*. It has also been isolated from *Berberis Vulgaris* (Buchner, *Annalen*, 1837, **24**, 228; Rudel, *Arch. Pharm.*, 1891, **229**, 631), *Cœlocline polycarpa* (Stenhouse, *Annalen*, 1855, **95**, 108; 1858, **105**, 360),

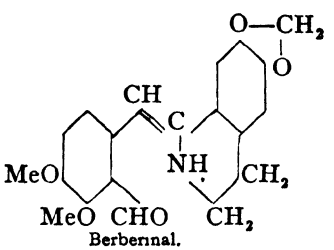
[1] A concentrated liquid extract of barberry root still receives a limited application for dyeing silk and leather yellow In America, the root-bark is commonly used, but in Europe the entire root is generally employed

the wood of *Coscinium fenestratum* and *Xanthorhiza apiifolia* (Perrins, *Annalen*, 1852, **83**, 276), *Hydrastis Canadensis* (Mahla, *Sill. Amer. J.*, 1862 [*ii*], **33**, 43), *B. aristata*, *Caulophyllum thalictroides*, *Podophyllum pellatum*, and *B. aquifolium* (Pommerehne, *Arch. Pharm.*, 1895, **233**, 127; Rudel, *loc. cit.*), and *B. Oetnensis* (A. G. Perkin, *Chem. Soc. Trans.*, 1897, **71**, 1198). Boedecker (*Annalen*, 1849, **66**, 384; **69**, 40) states that berberine occurs in *Cocculus palmatus*, but this has been contradicted by Gordin (*Arch. Pharm.*, 1902, **240**, 146). The latter author finds also that *Pereira brava*, *Menispermum Canadense* and *Jeffersonia diphylla* do not contain this alkaloid. Schlotterbeck (*Amer. J. Pharm.*, 1902, **74**, 584) has shown that the yellow colouring matter of *Chelidonium majus* and of *Styllophorum diphyllum* is berberine.

The constitution of berberine has been elucidated mainly through the work of W. H. Perkin, Jr. (*Chem. Soc. Trans.*, 1889, **55**, 63; 1890, **57**, 992; 1910, **97**, 305) and of Gadamer (*Arch. Pharm.*, 1901, **239**, 648; *Chem. Zeit.*, 1902, **26**, 291; *Arch. Pharm.*, 1905, **243**, 31). The latter showed that when a solution of the acid sulphate of berberine is treated with the equivalent amount of barium hydroxide solution a dark brownish-red strongly alkaline solution results, which on addition of excess of sodium hydroxide gives a bright yellow precipitate. It is highly probable that the dark-coloured alkaline solution contains berberinium hydroxide from which the salts of berberine are derived, whilst the yellow precipitate "berberinal" is the aldehyde form in which the solid alkaloid exists.



Berberinium hydroxide.



Berberinal.

Berberine is isolated from the root of *Hydrastis Canadensis* by boiling with water, evaporating the decoction to an extract, and exhausting with strong alcohol. One-fourth of its volume of water is added to the filtered alcoholic solution, the alcohol distilled off, and the residue treated with dilute sulphuric acid. Berberine sulphate crystallises out, and is decomposed by freshly precipitated lead

hydroxide (Merril, *J*, 1864, 452) The alkaloid may also be converted into the sparingly soluble nitrate or hydrochloride instead of the sulphate

L Wolff recommends a previous treatment of the root with light petroleum to remove fixed oil.

Berberine may be isolated from barberry root by exhausting the material with alcohol, evaporating off the spirit, taking up the residue with water, and treating the filtered solution with excess of hydrochloric acid, when berberine hydrochloride crystallises out The salt may be purified by re-solution in alcohol and precipitation by ether [1]

Gaze (*Zeit Naturwiss Halle*, 1890, **62**, 399) proposed to purify berberine through the acetone derivative by boiling the latter with chloroform and alcohol, but it has been shown by Gordin (*Arch Pharm*, 1901, **239**, 626) that the product consists not of berberine itself, but of its hydrochloride

Berberine crystallises with difficulty in small, concentrically grouped prisms, or bright yellow, silky needles [2] When air-dried, the crystals appear to contain $5\,5H_2O$ (W H Perkin, Jr), of which $3H_2O$ is driven off at 100° At this temperature the crystals lose their lustre and become yellowish-brown, at 110° the change is very rapid, and above 160° total decomposition occurs Fleitmann gives 120° as the m p of berberine, but Perkin considers this figure too low [3] When warmed, it emits a faint but peculiar odour resembling that of quinone.

Berberine has a persistent, very bitter taste, and is employed medicinally in doses of 2 to 5 grains 60 grains have been taken by man without injury, but the alkaloid is poisonous to dogs and other of the lower animals

Berberine dissolves in 4 5 parts of water at 21°, giving a solution neutral to litmus It is easily soluble in hot water and alcohol, and dissolves in 100 parts of cold alcohol (Proctor, *J*, 1864, 453) The alkaloid is slightly soluble in chloroform and benzene, and insoluble in ether (separation from oxyacanthine and hydrastine) and petroleum

[1] Berberine may also be prepared by precipitating an aqueous decoction of barberry root with lead acetate, and treating the concentrated filtrate with excess of sulphuric acid The precipitate of berberine sulphate is washed with cold water, and separated from lead sulphate by solution in boiling water, which on cooling deposits the salt in yellow needles

[2] An orange colour or other shade darker than bright yellow, is indicative of impurity

[3] E Schmidt has obtained some evidence that berberine prepared from the commercial sulphate is occasionally a mixture of berberine with methylberberine He obtained pure berberine by converting the alkaloid into the acetone compound, B,C_3H_6O, from which the free base was liberated by heating in alcoholic solution Thus obtained, berberine contained $6H_2O$, all of which was lost at 100° The anhydrous alkaloid scarcely began to darken below 150°.

spirit. It is said to be taken up with difficulty from its acidified solutions by amyl alcohol, chloroform, and benzene.[1]

Reactions and Detection.

When treated with sodium hydroxide solution berberine is coloured brown, and on boiling a resinous mass separates. On distilling berberine with milk of lime, quinoline is formed. Fusion with potassium hydroxide produces berberic acid, $C_8H_8O_4$, and an acid of the composition $C_8H_8O_5$.

When boiled with excess of fuming hydriodic acid, two methyl groups are eliminated and a salt of berberoline, $C_{18}H_{11}(OH)_2O_2N$, formed. On rendering the diluted liquid slightly alkaline by ammonia, an intense blackish-blue colouration is obtained, probably owing to oxidation. Nitric acid gives, with berberoline, a magnificent violet colouration, which on standing or warming changes to a deep reddish-brown.

Concentrated nitric acid dissolves berberine to a dark, reddish-brown liquid, which on dilution with water gives a yellow flocculent precipitate partly soluble in ammonia. If the dark solution of berberine in strong nitric acid be warmed, oxidation rapidly occurs with formation of berberonic acid (a pyridine-tricarboxylic acid), oxalic acid, and other products.

Potassium permanganate in presence of potassium carbonate oxidises berberine with formation of hemipinic acid, $C_{10}H_{10}O_6$, and other products (W. H. Perkin, *Chem. Soc. Trans.*, 1889, **55**, 71; see also Schmidt and Schilbach, *Arch. Pharm.*, 1887, **225**, 164).

By the action of nascent hydrogen, berberine is reduced to hydroberberine, $C_{20}H_{21}O_4N$.

Berberine dissolves in concentrated sulphuric acid with orange-yellow colour, changing to olive-green on warming. On adding potassium dichromate, or other oxidising agent, a black colour changing to violet (or brown-violet changing to brownish-yellow) is obtained. Frohde's reagent gives a brown or green colour with berberine; or,

[1] According to E Schmidt (*Pharm Zeit*, 1887, **32**, 542), berberine has a remarkable tendency to combine with neutral solvents, such as alcohol, ether, acetone, and chloroform, to form crystalline compounds. When berberine and chloroform are mixed in molecular proportions, they unite to form a beautiful crystalline substance, permanent at 100° This does not appear to be a mere additive product, since it is not decomposed by acids simply into berberine and chloroform, but yields decomposition-products of the latter Berberine can also combine with a second molecule of chloroform, but this behaves like water of crystallisation. Schmidt has also described a compound of berberine with acetone, of the formula $C_{20}H_{17}O_4N,C_3H_6O$

according to Hirschhausen, an immediate yellow, changing through dark brown to violet-brown. Sulphovanadic acid is stated to give a fine violet colouration.

Berberine is also characterised by the insolubility of many of its salts (*e. g.*, the chromate, picrate, hydriodide, platinichloride, aurichloride), and the sparing solubility of others in presence of excess of mineral acid.

On adding chlorine-water (avoiding excess) to a solution of berberine strongly acidified with hydrochloric or sulphuric acid, a zone of bright red colour is formed at the junction of the liquids, and is still recognisable as a pink colouration in a dilution of 250,000.[1] The reaction is destroyed by reducing agents.

On cautiously adding iodised potassium iodide (avoiding excess) to a solution of a berberine salt, BHI_3 is thrown down as a difficultly soluble red-brown precipitate, which crystallises from strong alcohol in red needles, or on adding water in green iridescent scales which completely polarise light.

Mayer's reagent gives with berberine solutions a precipitate of the approximate composition $B_2H_2HgI_4$, containing, after drying at 100°, from 50 to 52% of the alkaloid.

Perkin states that even very dilute solutions of berberine give a yellow precipitate with bromine-water, which darkens on standing and is probably the hydrobromide.

Berberine does not combine with hydroxylamine or phenylhydrazine and is apparently not attacked by phosphorus oxychloride or pentachloride.

According to Jaworowski (*Pharm. Zeit. Russ.*, 1896, **35**, 326) a solution containing 0.01—0.002% of berberine is precipitated by the reagent prepared by dissolving 0.3 grm. of sodium vanadate in 10 c.c of hot water, cooling the solution, adding 0.3 grm. of crystallised copper sulphate and 8 drops of glacial acetic acid, and filtering the liquid. 1 c.c. of the reagent obtained by mixing a 30% solution of hydrogen peroxide with 10 times its volume of pure sulphuric acid gives a red to purple colouration with 0.005—0.01 grm. of berberine (Schaer, *Arch. Pharm.*, 1910, **248**, 458).

Gordin describes (*Arch. Pharm.*, 1902, **240**, 146) the following method of detecting berberine in plants. 5 to 20 grm. of the

[1] Brucine gives a similar indication with chlorine-water, but the original solution is colourless, and the colour less permanent than with berberine

powdered product are extracted with hot alcohol, the extract evaporated, treated with 20–40 c.c. of water and filtered, with addition of a little powdered talc if necessary. A small portion of the clear filtrate is mixed with a small quantity of a 10% solution of potassium iodide. If no precipitate is formed, no appreciable amount of berberine is present, but if a precipitate is deposited 10 c.c. of the original filtrate are mixed with 1–2 c.c. of a 10% solution of sodium hydroxide, filtered if necessary, heated to 50°, treated with 5 c.c. of acetone and allowed to stand. If after two hours no crystals have formed, 30 c.c. of water are added and the solution kept in a cool place overnight. In this time crystals will separate if the solution used (10 c.c.) contained not less than 0 01 grm. of the alkaloid. The crystals may be identified by dissolving in dilute hydrochloric acid and testing portions of the solution with potassium iodide, potassium dichromate, picric acid and chlorine-water. When no crystals form although potassium iodide has given a precipitate with the aqueous solution of the extract, 10 or 20 c.c. of the latter are mixed with excess of a 20% solution of potassium iodide. The precipitate formed is collected on a filter and washed first with dilute potassium iodide solution, and then with water. The filtrate and washings are concentrated to about 2 c.c., treated with a few drops of sodium hydroxide solution and 1 c.c. of acetone, and, after standing some hours, diluted with an equal bulk of water. Good crystals of berberine-acetone will be deposited within 24 hours if 0.001 grm. of the alkaloid is present.

A microchemical method of detecting berberine has been described by Bauer (*Pharm. Zeit.*, 1908, **53**, 618). A section of the plant tissue is floated in a few drops of water on a microscope slide, allowed to macerate for a few seconds, warmed with 1 or 2 drops of sodium hydroxide solution (10%) and then treated with 4–5 drops of acetone and covered with a micro-cover glass. The growth of characteristic crystals of berberine-acetone is observed under the microscope in some cases in 5 minutes, whilst in others several hours are required.

Estimation. Volumetric Methods.

The following method of estimating berberine in the root of *Hydrastis Canadensis* has been described by Gordin and Prescott (*Arch. Pharm.*, 1899, **237**, 441). 10 grm. of the powdered root are stirred into

a paste with a few c.c. of a mixture of alcohol, concentrated ammonia and ether (1:1:6), and kept in a stoppered jar for several hours (overnight). The mixture is then dried in a current of air, until all the ammonia has volatilised, and afterwards in a vacuum over sulphuric acid for 5–6 hours. The dry substance is transferred to a Soxhlet extractor, the jar being rinsed with powdered barium nitrate, and extracted with 40–50 c.c. of absolute ether until the residue from the evaporation of a few drops of the extract gives no reaction with either Mayer's or Wagner's reagent. The ethereal extract contains the hydrastine. A current of air is passed through the Soxhlet tube until all the ether has evaporated, and the residue extracted with 40–50 c.c. of alcohol until the extract is colourless. The alcoholic extract is washed into an evaporating dish together with a little hot water and dilute acetic acid, and evaporated with addition of water until all the alcohol is expelled. A further small quantity of dilute acetic acid is added, the liquid cooled, filtered into a 300–400 c.c. Erlenmeyer flask and the filter washed. The filtrate and washings are treated with 6–8 c.c. of acetone, and a 10% solution of sodium hydroxide added drop by drop until the precipitate first formed no longer disappears on shaking, and the liquid acquires a strongly alkaline reaction. The flask is closed with a stopper, shaken in a circular direction for 10–15 minutes, and then allowed to stand for 2–3 hours. The crystals of berberine-acetone formed are washed until the washings are colourless, returned to the flask and treated with 4–5 c.c. of 5% sulphuric acid and water up to 100–200 c.c. The flask is heated in hot water until the precipitate has dissolved, when the solution is transferred to a long-necked flask and boiled gently for 1 5 to 2 hours, water being added when necessary. The liquid is cooled, mixed with 100 c.c. of $N/20$ potassium iodide solution, the mixture diluted to 1 litre and allowed to stand overnight. The liquid is filtered, 500 c.c. of the filtrate mixed with 50 c.c. of $N/20$ silver nitrate solution, nitric acid added, and the mixture diluted to 1 litre. The liquid is well shaken, filtered, and 500 c.c. of the filtrate titrated with $N/40$ ammonium thiocyanate solution using ferric alum as indicator. Twice the number of c.c. of ammonium thiocyanate solution used is equal to the number of c.c. of $N/20$ potassium iodide solution consumed by the berberine in 10 grm. of the root. The number of c.c. of $N/20$ potassium iodide solution consumed multiplied by 0.167125 gives the percentage of anhydrous berberine.

An absolute alcoholic solution of berberine or one of its salts, when treated with alcoholic sulphuric acid, gives a precipitate of the acid sulphate $C_{20}H_{17}O_4N,H_2SO_4$, thus the precipitate formed in Thompson's process (*Amer. J. Pharm.*, 1893 [*iv*], **23**, 371) is this sulphate, not the hydrochloride. On this behaviour Gordin has based the following method for the estimation of berberine in absolute alcoholic solutions. The solution is precipitated with excess of alcoholic sulphuric acid, diluted with an equal volume of ether and allowed to stand overnight. The precipitate is filtered off, washed with a mixture of equal volumes of alcohol and ether and the total volume of filtrate and washings noted. The precipitate is transferred to a 200 c.c. graduated flask, 20 c.c. of a 20% potassium iodide solution added, the mixture diluted to 200 c.c. and well shaken. Berberine hydriodide is precipitated, one equivalent of free acid remaining in solution for each molecule of berberine precipitated. The liquid is filtered and 100 c.c. of the filtrate titrated with $N/40$ potassium hydroxide solution which has been previously standardised against $N/40$ sulphuric acid under the same conditions. Each c.c. of the standard alkali required corresponds with 0.008378 grm of berberine. A correction has to be applied on account of the alkaloid dissolved in the alcohol-ether filtrate and washings; this amounts to 0.0000526 grm. per c.c.

According to Troger and Linde (*Arch. Pharm.*, 1900, **238**, 6) berberine is completely precipitated from solutions of its hydrochloride by additions of excess of a solution of potassium naphthalene-β-thiosulphonate, $C_{10}H_7SO_2SK$. A 0.33% solution of the latter salt is used and is titrated against $N/100$ iodine solution. The precipitate formed with the alkaloid is filtered off, washed, and the filtrate and washings titrated with $N/100$ iodine solution. A molecule of berberine is precipitated by a molecule of the thiosulphonate, and a molecule of the latter reacts with an atom of iodine. Accordingly each c.c. of an $N/100$ thiosulphonate solution used by the alkaloid corresponds with 0.003351 grm. of berberine.

Gravimetric Method.

For the estimation of berberine in aqueous-alcoholic solutions, Gordin recommends the following process (*Arch. Pharm.*, 1901, **239**, 638). The berberine is precipitated with excess of a 10% solution of potassium iodide, the precipitated hydriodide washed with a 2%

solution of the same salt, and transferred with a little water into a flask. After heating to 60–70°, acetone is added to the extent of one-third the volnme of the water aud the mixture shaken for 10 minutes. 5 c.c. of a 10% solution of sodium hydroxide are then added and the liquid shaken (heating at 50–60° if necessary) until the yellow hydriodide has disappeared. After cooling the solution is diluted to three times its bulk with water and allowed to stand overnight. The berberine-acetone is filtered off, dried first under reduced pressure and then at 105° and weighed. 1 grm. of the acetone compound corresponds with 0.853 grm. of berberine. To correct for the berberine-acetone dissolved in the mother-liquor 0.0000273 grm. is added per c.c.

Salts of Berberine.

Berberine is a weak base, but forms definite and readily crystallisable salts with acids. The salts have a bitter taste, and are mostly very sparingly soluble, the pyrophosphate and acetate being exceptions.

Berberine nitrate, B,HNO_3, separates in fine yellow needles on acidifying a warm aqueous or alcoholic solution of berberine with nitric acid. It is soluble in about 500 parts of cold water, more readily in hot, and almost insoluble in alcohol or water strongly acidified with nitric acid. It does not darken or undergo other change at 100°. It turns dark brown on heating with sulphuric acid.

Berberine Hydrochloride, $B,HCl+2H_2O$,[1] is precipitated in golden-yellow needles on adding hydrochloric acid to a warm aqueous solution of the alkaloid. It requires about 500 parts of cold water for solution, and is almost insoluble in alcohol or dilute hydrochloric acid. The salt is with difficulty decomposed by bases, the liberated alkaloid being apt to retain chlorine. Prolonged digestion with litharge fails to decompose it completely, but silver oxide readily decomposes the solution. Berberine hydrochloride darkens to an orange colour when heated to about 60°, but regains its original colour on cooling. By prolonged exposure at 100° the colour changes permanently, and much of the salt becomes readily soluble in cold water, with red colour.

Reichard describes a number of tests characteristic of berberine hydrochloride (*Pharm. Centr. Halle*, 1906, **47**, 473). Thus this salt dissolves to a yellow liquid in a solution of stannous chloride, which is not affected by heating, and to a brownish-black liquid when heated with bismuth chloride solution. When heated with a drop of a strong

[1] According to Schmidt the rochhydloride contains $2H_2O$ when crystallised from dilute alcohol.

solution of potassium thiocyanate the hydrochloride becomes green, with sulphuric acid dark green. If previously mixed with ammonium persulphate or mercurous nitrate, it is blackened by sulphuric acid, but in the latter case the solution becomes yellowish-red on exposure to air. The mixture of the salt with potassium iodate becomes graphite colour when treated with a drop of hydrochloric acid and then yellow on adding potassium hydroxide solution, whilst if ammonium meta-vanadate be substituted for the iodate, a brown deposit is produced. If treated with a solution of α-naphthol in a 40% solution of potassium hydroxide, the hydrochloride turns dark reddish-brown but the solution is not affected. The salt becomes dark green when mixed with picric acid and treated with sulphuric acid, or when mixed with α-nitroso-β-naphthol and treated with potassium hydroxide solution.

The *aurichloride*, $B,HAuCl_4$, is amorphous, brown, and quite insoluble in water. It crystallises from boiling dilute alcohol in chestnut-brown needles, unchanged at 100°. The *platinichloride*, B_2,H_2PtCl_6, forms a yellowish precipitate, almost insoluble in all the ordinary solvents. It may be crystallised from boiling amyl alcohol, in which it is slightly soluble.

Berberine hydriodide, B,HI, obtained by precipitation, forms minute yellow needles, nearly insoluble in cold water or potassium iodide solution. It does not darken or suffer other change at 100.° BHI_3 is precipitated on cautiously adding iodised potassium iodide (carefully avoiding excess) to a solution of a berberine salt in hot spirit. It is quite insoluble in cold water. When recrystallised from hot alcohol, the smaller crystals have the property of completely polarising light (compare Herepathite).

Berberine sulphate, B,H_2SO_4, is met with in commerce both in the amorphous state and crystallised. The latter form, which is considerably the higher priced, can be prepared by dissolving 15 grm. of the amorphous preparation in a boiling mixture of 250 c.c. of alcohol with 8 of acetic acid, when on cooling the crystallised salt separates out. It has an orange colour, and is permanent in the air when free from impurity. It is soluble in about 100 parts of water. According to J. U. Lloyd (*Amer. Drug.*, Sept., 1884), the yellow crystalline powder obtained by heating commercial berberine sulphate with ammonia and shaking with ether is not the free alkaloid, as commonly assumed, but a neutral sulphate, $B_2H_2SO_4$, which is readily soluble in water.

The *chromate* B,H_2CrO_4 is obtained in orange-yellow needles on adding potassium dichromate to a boiling and very dilute solution of a salt of berberine. The salt separates entirely on cooling, and is insoluble in cold water or an excess of the precipitant.

Berberine picrate requires 45,000 parts of cold water for solution. As a consequence, on mixing aqueous solutions of berberine and picric acid in equivalent proportions and filtering, a liquid is obtained free from yellow colour or bitter taste.

Berberine acetate is prepared by adding berberine sulphate to a solution of potassium acetate in rectified spirit, and heating gently till the yellow salt has dissolved. After cooling, the liquid is filtered from the potassium sulphate, evaporated to a syrup, and shaken with ether, when berberine acetate, $B(C_2H_4O_2)_2$, is precipitated as a crystalline orange powder. It is readily soluble in water and alcohol, nearly insoluble in ether, and loses acid on exposure to air.

Shedden (*Pharm. J.*, 1900 [*iv*], **11**, 89) has prepared *berberine phosphate*, $B,2H_3PO_4,1\ 1/2H_2O$, by adding excess of phosphoric acid to berberine-acetone. It is a bright yellow non-deliquescent crystalline substance soluble in 14.3 parts of water at 16°. The anhydrous salt dissolves in 15 parts of water at 15–16°. When prepared by Parsons and Wrumpelmeier's method (*Proc. Amer. Pharm. Assoc.*, 1879, 514) the phosphate contains $1H_2O$.

Oxyacanthine, $C_{19}H_{21}O_3N$. This base is contained in *Berberis vulgaris*, and remains in the mother-liquor, from which the berberine has been separated as hydrochloride.

The alkaloid has been isolated by Rudel (*Arch. Pharm.*, 1891, **229**, 636) from *Berberis aquifolium* and *B. vulgaris* by the following method: The ground-up roots are extracted in a Soxhlet apparatus first with alcohol acidified with acetic acid and then with dilute aqueous acetic acid. The combined extracts, after concentration, deposit berberine acetate and resin. These are filtered off and more resin precipitated from the filtrate by adding 3–4 volumes of water. The liquid is filtered, evaporated to half its bulk, and treated with sodium carbonate solution so long as a precipitate is formed. The latter is collected on a filter, washed, dissolved in dilute sulphuric acid, the solution filtered from resin and reprecipitated with sodium carbonate solution. The precipitate is redissolved in dilute sulphuric acid and the solution saturated with sodium sulphate at 50°. On cooling, a resinous mass containing oxyacanthine separates. This is filtered off, washed with

the minimum quantity of water, dissolved in a large quantity of the same solvent and reprecipitated with sodium carbonate solution. The precipitate is dried and extracted with ether in a Soxhlet apparatus, the ethereal extract evaporated and the brown resinous residue dissolved in a mixture of equal parts of alcohol and dilute sulphuric acid. The solution, on evaporation, deposits white wart-like crystals of oxyacanthine sulphate. On decomposing the solution of oxyacanthine sulphate with ammonia the free alkaloid is precipitated in flocks, which, after drying at 100°, melt at 138–150°; but when crystallised from alcohol or ether it forms anhydrous needles. The m. p. of the latter is stated by Hesse (*Ber.*, 1886, **19**, 3190) to be 208–214°, by Rüdel 188–198°, and by Pommerehne (*Arch. Pharm.*, 1895, **233**, 131) 208–210°.

From Rudel's analyses, which have been confirmed by Pommerehne, the composition of the alkaloid is represented by the formula $C_{19}H_{21}O_3N$, and contains one or two methoxyl groups.

Oxyacanthine is dextro-rotatory having $[\alpha]_D^{20}+174.5°$. Oxyacanthine is readily soluble in chloroform and benzene, but only sparingly so in petroleum spirit. It may be separated from berberine by extracting the ammoniacal solution with ether or chloroform. From its acidified solutions it is not extracted by petroleum spirit or benzene, and only sparingly by chloroform.

Both Hesse and Rudel found that the amorphous alkaloid is more soluble than the crystalline variety.

Reactions—Oxyacanthine dissolves in concentrated nitric acid to a yellowish-brown solution, which is unchanged by sulphuric acid. In the latter acid it gives a colourless solution, which on addition of nitric acid becomes faint yellow, then reddish-brown and finally reddish-yellow. Erdmann's reagent gives a faint reddish-yellow colour, Frohde's reagent a strong violet colour which turns dirty green, becoming fainter and finally yellow. Sulphovanadic acid gives a faint dirty violet colour which becomes reddish-violet. Free oxyacanthine is not precipitated by mercuric or zinc chloride, but the solutions of its salts give a white precipitate immediately on adding these chlorides. A solution of the alkaloid is coloured yellow by chlorine-water and on adding potassium dichromate a yellow precipitate is formed. Bromine-water gives a yellow precipitate. Oxyacanthine liberates iodine from a solution of potassium iodide in dilute sulphuric acid, and reduces a solution of ferric chloride and potassium ferricyanide with the

formation of Prussian blue. The solution of the alkaloid in concentrated sulphuric acid gives a dark colour with basic bismuth nitrate.

Oxyacanthine closely resembles narcotine. Like morphine, it reduces iodic acid.

Oxyacanthine hydrochloride, $BHCl+2H_2O$, forms small colourless needles, the 2% aqueous solution of which shows $[\alpha]_D = +163.6°$. Hot strong solutions are coloured green by ferric chloride.

The *hydrobromide*, $B,HBr,2H_2O$, forms white silky needles, the *hydriodide*, $B,HI,2H_2O$, small wart-like crystals. The latter salt is far less stable than the hydrochloride or hydrobromide.

The *nitrate*, $B,HNO_3,2H_2O$, forms small glistening white wart-like crystals easily soluble in hot but difficultly soluble in cold water.

The *sulphate*, $B_2H_2SO_4,4H_2O$, crystallises in white warts or fine needles.

The *platinichloride*, $B_2H_2PtCl_6,5H_2O$, is an amorphous yellow salt, whilst the *aurichloride*, $B,HAuCl_4,4H_2O$, is a golden-yellow amorphous substance.

Oxyacanthine forms a non-crystalline *benzoyl* derivative, $C_{19}H_{20}BzO_3N$, of which the *platinichloride*, $(C_{19}H_{20}BzO_3N,HCl)_2PtCl_4,8H_2O$, is a yellowish-white flocculent precipitate, whilst the *aurichloride*, $C_{19}H_{20}BzO_3N,HAuCl_4,2H_2O$, forms reddish-yellow amorphous flocks.

When heated with potassium hydroxide and a little water, oxyacanthine melts to a brown mass which floats on the fused alkali. This consists of the *potassium* derivative of β-oxyacanthine, a substance probably differing from the parent alkaloid by the elements of water. A similar change occurs very readily even at the ordinary temperature, by the action of alcholic potash or baryta on α-oxyacanthine. Ether fails to extract the β-modification from the alkaline solution. Hydrochloric acid precipitates β-oxyacanthine, which is soluble both in alkalis and excess of acid. With much acid, α-oxyacanthine hydrochloride is precipitated. β-Oxyacanthine changes back to the α-form on drying in the air.

Berbamine, $C_{18}H_{19}O_3N$, the second *Berberis* alkaloid soluble in ether, was obtained by Hesse (*Ber.*, 1886, **19**, 3190) by adding sodium nitrate to the liquid from which oxyacanthine had been thrown down as sulphate. The precipitated berbamine nitrate when decomposed by ammonia yields a crystalline precipitate of the free base, which crystallises from alcohol in small plates containing $2H_2O$.

Rüdel afterwards studied this alkaloid (*Arch. Pharm.*, 1891, **229**, 631) and found that its composition is represented by the formula $C_{18}H_{19}O_3N$, and that it melts at 197–210° (Hesse gave 156°). It is insoluble in light petroleum, and may therefore be separated from oxyacanthine which is difficultly soluble in this medium.

Reactions.—Berbamine dissolves in concentrated sulphuric acid to a colourless solution, turning greenish-yellow and becoming reddish on addition of nitric acid. When dissolved in nitric acid it gives a brownish-red solution unchanged by sulphuric acid. Sulphovanadic acid gives a pale violet colour gradually becoming brown. Zinc and mercuric chlorides give white precipitates immediately. The reactions with Frohde's reagent, with a mixture of ferric chloride and potassium ferricyanide solutions, with basic bismuth nitrate and with a solution of potassium iodide in dilute sulphuric acid are similar to those of oxyacanthine.

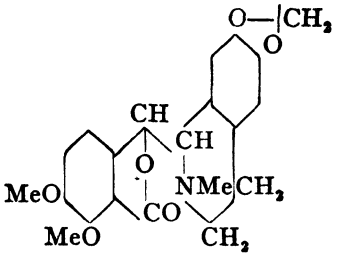
Berbamine sulphate, $B_2H_2SO_4,4H_2O$, crystallises in small leaflets or needles. The *hydrochloride*, $B,HCl,2H_2O$, described by Pommerehne (*Arch. Pharm.*, 1895, **233**, 156) forms small wart-like crystals very similar to the corresponding salt of oxyacanthine.

The *platinichloride*, $(B,HCl)_2PtCl_4,5H_2O$, is a yellow powder which is only slightly soluble in water; the *aurichloride*, $B,HAuCl_4,5H_2O$, is a golden-yellow amorphous substance.

Hydrastine. $C_{21}H_{21}O_6N$; or $C_{19}H_{15}(O.CH_3)_2O_2N$ (see also page 565).—This alkaloid was discovered in 1851 by von Durand (*Amer. Pharm. J.*, 1851, **23**, 112) in *Hydrastis Canadensis* or Golden Seal. Perrins found 1.5% in the dried root, but the yield in manufacture is from 0.25–0.75%. It also occurs in *Stylophorum diphyllum*. Hydrastine differs from berberine in being colourless, but commercial medicinal preparations of berberine from *Hydrastis* are not unfrequently called hydrastine.[1]

Hydrastine has been studied very largely by Freund and his pupils, Will, Lachmann, Rosenberg, Heim, Philips, and Dormeyer (*Ber.*, 1886, **19**, 2797; 1887, **20**, 80, 2400; 1889, **22**, 456, 1156, 2322, 2329; 1890 **23**, 404, 416, 2897, 2910; 1891, **24**, 2730, 3164; *Annalen*, 1892, **271**, 311) and more recently by Schmidt (*Arch. Pharm.*, 1893, **231**, 541). From the work of these authors it is probable that the constitution of the alkaloid is represented by the formula

[1] The root of Golden Seal is a bitter tonic analogous to calumba It is exhibited in the form of powder and in doses of 8 to 24 grains The hydrochlorides of the mixed alkaloids of golden seal are sometimes sold under the name of "hydrastine"



Hydrastine has been extracted from *Hydrastis Canadensis* by F. Wilhelm (*Arch. Pharm.*, 1888, **226**, 329) in the following way: The coarsely powdered root is digested with boiling water acidified with acetic acid. The extract is evaporated to a syrup and treated with excess of dilute sulphuric acid. The berberine sulphate which crystallises on standing is filtered off, and the filtrate neutralised with ammonia. The precipitate formed contains much hydrastine, and on again filtering and adding excess of ammonia to the filtrate, a second precipitate is produced which is said to contain canadine. Both precipitates when boiled with ethyl acetate give solutions which on cooling deposit large crystals of hydrastine, those from the second precipitate being more nearly pure than those from the first.

Eberhardt describes the following method of purifying the alkaloid. The freshly precipitated hydrastine is dissolved in the minimum quantity of boiling chloroform, the solution filtered through glass wool and poured into excess of alcohol. On shaking the liquid vigorously for some minutes the hydrastine separates as a fine crystalline precipitate. The process is repeated and the product then recrystallised from boiling alcohol.

Hydrastine forms colourless or milk-white four-sided prisms, melting at 132° and decomposing at a higher temperature with an odour of phenol.

Free hydrastine is tasteless and odourless, but the salts have an acrid taste. It is poisonous in large doses, 3 grains being fatal to a frog in 4 minutes. It resembles strychnine in causing death by arresting the respiratory movements in a tonic spasm.

Hydrastine is insoluble in water, and nearly insoluble in alkaline solutions. It dissolves in 120 parts of alcohol, in 1.75 parts of chloroform, in 16 of benzene, and in 83 of ether. It is quite insoluble in petroelum spirit.

According to the *United States Pharmacopœia* hydrastine dissolves in 135 parts of alcohol, 124 parts of ether, and 2 parts of chloroform at 25°, in 4,000 parts of water at 80°, 17 parts of alcohol at 60°, and easily in benzene. The solubility of hydrastine in ether may be utilised to separate it from berberine. Hydrastine is lævo-rotatory, $[\alpha]_D$ in chloroform solution (1.2759 grm. in 50 c.c.) being $-67.8°$.[1]

Hydrastine is a feeble base, and is completely extracted by chloroform from solutions freely acidified with hydrochloric acid. In part, however, it is dissolved as hydrochloride, which salt is very soluble in chloroform.

Reactions.—Hydrastine is not attacked by acetic anhydride, but reacts with acetyl chloride. The acetyl derivative crystallises in beautiful yellowish-green needles the solution of which has a bluish-green fluorescence.

Hydrastine solutions give no colour change with chlorine-water. With iodised potassium iodide they yield a deep brown flocculent precipitate.

Picric acid forms in hydrastine solutions a yellow amorphous precipitate of the picrate, $BA+4H_2O$, which is deposited in splendid yellow needles from its solution in boiling alcohol.

Solutions of hydrastine are precipitated by potassium dichromate. On touching the separated precipitate with a drop of strong sulphuric acid, it *instantly* becomes bright red, the colour fading in a few seconds. This behaviour distinguishes hydrastine from strychnine and gelsemine (page 448).

If a solution of hydrastine be acidified with sulphuric acid, and a few drops of an $N/10$ solution of potassium permanganate added, the colour of the reagent is instantly discharged, and an intense blue fluorescence is developed. A single drop of a 1% solution of hydrastine when treated in this way renders a large test-tube of liquid strongly fluorescent (A. B. Lyons, *Pharm. J.*, 1885–6 [*iii*], **16**, 880). Excess of permanganate must be avoided, or both the alkaloid and fluorescent product will be destroyed. The fluorescent substance differs from æsculin in not being extracted from either acid or alkaline solutions by chloroform or ether, and in not having the fluorescence intensified by addition of alkali.[2]

[1] The figure for specific rotation given in the text is that of Pfeund and Will. Eijkman gives -57.5 ($c=3.042$ in 100 c.c.) F. B Power (*Pharm J.*, 1884–5 [*iii*], **15**, 298) gives the widely different figure $-170°$

[2] The same fluorescent oxidation-product is sometimes developed in solutions of hydrastine by mere exposure to air Neither pure hydrastine nor any ready-formed constituent of *Hydrastis* root appears to be fluorescent.

According to Dunstan and Carr (*Pharm. J.*, 1896 [*iv*], **2**, 122) hydrastine gives a pink-purple precipitate with potassium permanganate.

The colour-reactions of solid hydrastine have been re-investigated by A. B. Lyons (*Pharm. J.*, 1885–6 [*iii*], **16**, 880) with the following results: Concentrated sulphuric acid dissolves the pure alkaloid with faint yellow colour, changing to a deep blue-purple on heating. If the reagent contains a trace of nitric acid a yellow colour is produced, and with a larger proportion (1:1,000) the colour is orange-red. Pure nitric acid produces a permanent orange solution, which on adding water deposits an insoluble substance, and yields a liquid exhibiting an intense blue fluorescence.

With sulphuric acid and oxidising agents hydrastine produces some well-defined colour indications. With manganese dioxide an orange colour is first developed, changing to a rich cherry-red, and passing through carmine to a yellowish shade of red, which after a time changes rather suddenly to a pale orange-yellow. This test distinguishes hydrastine from strychnine and gelsemine, whilst berberine dissolves in sulphuric acid with yellow colour, changing on addition of the oxidising agent to violet, then to chocolate-brown, and finally becoming orange-red. (The intermediate chocolate-brown stage distinguishes the berberine reaction from that given by strychnine.) Potassium permanganate gives with hydrastine and sulphuric acid the same colourations as manganese dioxide, but the changes are more rapid. A violet tint is sometimes produced *after* the red is developed, the contrary order being characteristic of strychnine.

Frohde's reagent gives with hydrastine a sage-green colour, slowly changing to brownish, and then gradually fading. This succession of tints is very characteristic. Sulphovanadic acid gives a rose-red colour, which fades slowly.

Vitali (*L'Orosi*, 1892, **14**, 405) observed that if the brownish-yellow solution formed by dissolving a fragment of hydrastine in sulphuric acid and adding a fragment of nitre, is treated drop by drop with a solution of stannous chloride, a magnificent reddish-violet colour is developed, the intensity of which depends on the amount of alkaloid present. The colour is not destroyed by the addition of water.

It is stated by Labat (*Bull. Soc. Chim.*, 1909 [*iv*], **5**, 742, 745) that if 0.1 c.c. of a 0.33% solution of hydrastine or of a 1% solution of hydrastinine, in 10% sulphuric acid is added to 2 c.c. of pure sulphuric

acid and the mixture treated with 0.1 c.c. of a 5% alcoholic solution of gallic acid and heated on a water-bath, an intense emerald green colour is produced, which gradually becomes Fehling blue, and violet if diluted with glacial acetic acid. With a solution of guaiacol or catechol the colour is of a red-currant shade becoming violet. The reaction with gallic acid is the most sensitive, 1 part of hydrastine in 40,000, or 1 part of hydrastinine in 50,000 of water being recognisable by it. Berberine gives the reaction with gallic acid but not those with the other two phenols.

Norton and Newman (*J. Amer. Chem. Soc.*, 1897, **19**, 838) found that hydrastine gradually dissolves when triturated with a cold saturated solution of mono-calcium phosphate, the amount dissolved increasing with the duration of the trituration, until after six weeks the product in solution has the composition represented by the formula $2Ca(H_2PO_4)_2,3C_{21}H_{21}O_6N$.

When hydrastine is heated to fusion (220°) for some minutes ammonia is evolved and if the cooled liquid is poured into water meconin is precipitated (Beckurts and Frerichs, *Arch. Pharm.*, 1903, **241**, 259).

On treating an acid solution of hydrastine with oxidising agents (*e.g.*, manganese dioxide and sulphuric acid), it splits up into opianic acid and hydrastinine, a base closely resembling cotarnine. If the oxidation be effected in alkaline solution, the action proceeds further, the chief products being hemipinic and nicotinic acids.

Estimation. Volumetric Methods.

Hydrastine may be approximately estimated by titration with Mayer's reagent, but the precipitating power of the solution is materially affected by the dilution of the liquid.

For the volumetric estimation of hydrastine both Beckurts (*Apoth. Zeit.*, 1896, **11**, 552) and Maben (*Chem. and Drug.*, 1901, **59**, 234) recommend the dissolution of the alkaloid, after isolation, in excess of standard acid and estimation of the excess by titration with standard alkali. Maben's method is as follows: 10 grm. of the finely powdered *Hydrastis Canadensis* are exhausted with hot alcohol, the percolate cooled and diluted to 100 c.c. 25 c.c. of the latter are treated with 1.5 c.c. of hydrochloric acid (32%), 0.25 c.c. of pure sulphuric acid and 125 c.c. of ether. The mixture is cooled, well shaken and kept at

0° for 24 hours. The separated berberine hydrochloride is filtered (on a tared filter if it is desired to estimate the berberine), and washed with a mixture of equal volumes of alcohol and ether. The filtrate and washings are made nearly neutral, evaporated almost to dryness on a steam-bath and the residue extracted with small quantities of hot water until the extract ceases to react with the ordinary reagents for alkaloids. The extraction of the alkaloid from the resinous mass is facilitated by adding a few drops of alcohol to the hot water and afterwards evaporating it. The hot aqueous solution is filtered into a separating funnel, made alkaline with ammonia and extracted repeatedly with ether. The ethereal extract is evaporated, the hydrastine dissolved out of the residue by treatment with successive quantities of 5% sulphuric acid, the solution made alkaline with ammonia and the alkaloid again extracted with ether. The extract is evaporated to dryness, the residue dissolved in excess of $N/20$ acid and the solution titrated back with $N/100$ alkali, using cochineal as indicator. 1 c.c. of $N/100$ acid corresponds with 0.00383 grm. of hydrastine. (compare also Thompson, *Amer. J. Pharm.*, 1893 [*iv*], **23**, 371).

If a solution of iodine in potassium iodide is added to a solution of hydrastine, iodides of varying composition are precipitated. If, however, the hydrastine solution is added to a large excess of the iodine solution, the dark brown hydriodide iodide, $C_{21}H_{21}O_6N,HI,I_5$, is precipitated. This compound loses 5I when treated with sodium thiosulphate solution. On this behaviour Gordin and Prescott (*Arch. Pharm.*, 1899, **237**, 439) have based the following method of estimating hydrastine. 10 grm. of the powdered root of *Hydrastis Canadensis* are extracted with 40–50 c.c. of ether as described (page 556) under these authors' method of estimating berberine. The ethereal extract is washed into an evaporating basin with 2% sulphuric acid, evaporated to dryness, the residue dissolved in acidified water and the solution diluted to 100 c.c. 20 c.c. of the latter (equivalent to 2 grm. of the root) are measured into a 100 c.c. graduated flask, containing 20–30 c.c. of standard iodine solution (about 1%), the mixture diluted to 100 c.c. and shaken until all the pentiodide has separated. The latter is filtered off and the excess of iodine in the filtrate determined by titration with standard sodium thiosulphate solution.

One part of iodine is equivalent to 0.60403 parts of hydrastine.

Elvove proposes to estimate alkaloids by the application of Volhard's method of estimating chlorine (*J. Amer. Chem. Soc.*, 1910, **32**, 132).

About 0.2 grm. of the substance containing hydrastine is accurately weighed and dissolved in 20 c.c. of 4% hydrochloric acid and the solution evaporated on a water-bath. The residue is evaporated twice with 5 c.c. of alcohol, then dissolved in 10 c.c. of water and the solution titrated against standard alkali using phenolphthaleïn as indicator. This titration will give approximately the amount of acid combined with the alkaloid. The liquid is filtered and if necessary the precipitate washed free from chlorides. The filtrate and washings are acidified with nitric acid and excess of standard silver nitrate solution added. After filtering off the precipitate the excess of silver in the filtrate is determined by titration with standard thiocyanate solution using ferric alum as indicator.

Gravimetric Methods.

The gravimetric methods described by Gordin and Prescott (*loc. cit.*), Hegland (*Ned. Tijd. Pharm.*, 1896, **8**, 197), Puckner (*Pharm. Rev.*, 1908, **26**, 132), Roeder (*Apoth. Zeit.*, 1908, **23**, 583), Rupp (*Apoth. Zeit.*, 1909, **24**, 922) and that in the *United States Pharmacopœia* are all very similar and depend on the extraction of the alkaloid and weighing it as such. Puckner states that the following methods are preferable to those described in the *United States Pharmacopœia.*

5 grm. of the powdered rhizome of *Hydrastis Canadensis* are treated with 50 c.c. of ether and, after standing 10 minutes, 2 c.c. of ammonia are added and the mixture allowed to stand for 30 minutes with frequent agitation. The mixture is then filtered and the residue extracted with a second 50 c.c. of ether. The combined ethereal extracts are first shaken with a mixture of 2 c.c. of dilute hydrochloric acid and 18 c.c. of water, then with 10 c.c. of water containing 5 drops of dilute hydrochloric acid and finally with 10 c.c. of water. The combined aqueous solution is made alkaline to litmus with ammonia and extracted three times with 20 c.c. of ether. The ethereal extract is evaporated at ordinary temperature, the residue dried at 98–100°, and weighed.

In the case of the fluid extract 5 c.c. are well shaken with an equal volume of a 20% solution of potassium iodide and 25 c.c. of water, filtered and the precipitate washed twice by stirring with 5 c.c. of a 1% solution of potassium iodide, the liquid being passed through the filter, and then twice with the same solution on the filter. The filtrate and

washings are extracted three times with 20 c.c. of ether, then with 5 c.c. of ether, the ethereal solution filtered through cotton-wool, evaporated, the residue dried at 95–98°, and weighed.

Matthes and Rammstedt (*Arch. Pharm.*, 1907, **245**, 112) for the assay of nux vomica, hydrastis and jaborandi, recommend the precipitation of the alkaloids with picrolonic acid. In the case of the hydrastis rhizome, 6 grm. of the powdered substance are macerated with a mixture of 50 grm. of ether, 10 grm. of light petroleum and 6 grm. of ammonia solution, the whole being well shaken for half an hour. 6 c.c. of water are added and the mixture shaken until the drug aggregates leaving a clear supernatant liquid. 50 grm. of the latter (equivalent to 5 grm. of the drug) are filtered off, evaporated to one-half the volume, treated with 5 c.c. of an $N/10$ solution of picrolonic acid in alcohol and kept in a cool place for 24 hours. The precipitated hydrastine picrolonate is collected on a Gooch crucible, washed with 2 c.c. of a mixture of alcohol and ether (1:3), dried for 30 minutes at 110°, and weighed. The molecular weight of hydrastine picrolonate is 647, hence the weight of the latter multiplied by 0.59198 gives the weight of hydrastine.

In the case of the liquid extract of hydrastis, 15 grm. are evaporated to 5 grm. which are dissolved in 10 c.c. of water and then extracted with a mixture of ether, light petroleum and ammonia as above, 40 grm. of the ethereal extract (equivalent to 10 grm. of the liquid extract) being used for precipitation.

Of the tincture of hydrastis, 50 grm. are evaporated to one-fifth of this weight and the residue treated as with the fluid extract.

With the exception of the picrate, the salts of hydrastine are generally uncrystallisable, or are obtainable in crystals by special means only. Most of them, except the tannate and picrate, are soluble in water, the solutions having an acid reaction.

Hydrastine hydrochloride and sulphate are used in medicine.[1] B,HCl is best prepared by passing dry hydrogen chloride over the surface of a solution of hydrastine in anhydrous and alcohol-free ether. After drying over sulphuric acid the precipitate forms a micro-crystalline powder easily soluble in water and chloroform (Schmidt and Kerstein, *Arch. Pharm.*, 1890, **228**, 49).

The *hydrobromide* is similar in appearance but less soluble in water. The *hydriodide* is a yellowish-brown micro-crystalline powder much

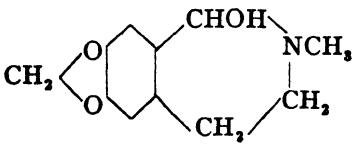
[1] The *crystallised* sulphate of hydrastine advertised by some manufacturers is simply sulphate of berberine, to which the name hydrastine is persistently misapplied.

less soluble in water than either the hydrochloride or hydrobromide. The *sulphate* B,H_2SO_4 is similarly obtained by cautiously adding a solution of strong sulphuric acid in ether to an ethereal solution of hydrastine. The salt readily takes up water and forms a gummy mass. Beckurts (*Arch. Pharm.*, 1890, **228**, 347) describes the *ferrocyanide* as a white amorphous sparingly soluble powder.

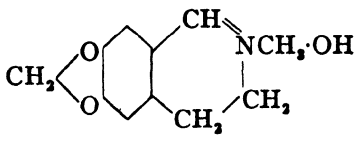
A *tartrate*, $B,C_4H_6O_6,4H_2O$, has been prepared by Merck (*Chem. Zeit. Rep.*, 1893, **17**, 30). It crystallises in white needles, easily soluble in hot, but sparingly soluble in cold water. When dried at 105° it loses 12% in weight and leaves a yellowish fused mass.

Hydrastinine, $C_{11}H_{13}O_2N$, produced together with opianic acid by the action of oxidising agents on hydrastine, forms white crystals, melting at 116–117°, or at 100° after heating for some time at that temperature. It dissolves in water to form a strongly alkaline and very bitter solution. It is also soluble in ether, ethyl acetate, benzene, and light petroleum, and crystallises from each of these solvents with $1H_2O$, which, however, is eliminated in the salts, a fact probably due to the formation of a closed ring.

The solution in water or alcohol is yellow and exhibits fluorescence, but it dissolves in anhydrous alcohol-free ether or chloroform to a colourless solution. Dobbie and Lauder have found that the absorption spectra of the coloured and colourless solutions are entirely different (*Chem. Soc. Trans.*, 1904, **85**, 1005) and from a study of these they conclude that the solid substance and that in solution in ether or chloroform has the carbinol constitution (I), whilst in aqueous or alcoholic solution, hydrastinine has the quaternary ammonium constitution (II) from which the salts are derived.



I



II

Reactions and Detection.—When hydrastinine is oxidised in dilute alkaline solution with a cold saturated solution of potassium permanganate, it is converted almost quantitatively into oxyhydrastinine, $C_{11}H_{11}O_3N$. Excess of the oxidising agent and slight heating carries the oxidation to hydrastinic acid, $C_{11}H_9O_6N$, a substance crystallising

in flat needles melting at 164°, soluble in alcohol and ether, and yielding no precipitate with silver, barium or lead salts.

Hydrastinine, when treated with potassium hydroxide solution, yields hydrohydrastinine, $C_{11}H_{13}O_2N$, and oxyhydrastinine, $C_{11}H_{11}O_3N$. The latter is a feeble base, melting at 97–98° and distilling above 350°, and forms crystallisable salts. The former base is also formed by the action of reducing agents on hydrastinine. It forms white crystals melting at 66°, and yields crystallisable salts.

When warmed with acetic anhydride in benzene solution, hydrastinine gives an *acetyl* derivative, $C_{11}H_{12}O_3AcN$, which crystallises in needles m. p. 105°, whilst the corresponding *benzoyl* derivative can be prepared by the Schotten-Baumann method. The latter compound melts at 98–99°.

Von Bunge states that 1 part of hydrastinine in 100,000 of water can be detected by means of Mayer's reagent. The reactions observed by Labat and described under *Hydrastine* are also given by hydrastinine.

The *hydrochloride*, $C_{11}H_{13}O_3N,HCl$, crystallises in feebly coloured needles, soluble in water and alcohol. The aqueous solution is optically inactive and feebly fluorescent.

According to Merck (*Zeit. Osterreich. Apoth. Ver.*, 1892, **30**, 107) hydrastinine hydrochloride is always coloured light lemon-yellow. Its degree of purity can be ascertained as follows: 0.2 grm. is dissolved in 6 c.c. of water and 6 drops of sodium hydroxide solution (1:5) added. Each drop of alkali produces a white precipitate which redissolves on shaking. The base crystallises out if the clear solution is stirred. If the salt is pure the precipitate is quite white and the supernatant liquid clear and colourless. Addition of hydrochloric acid effects the dissolution of the base and gives lemon-yellow solution. Samples of the hydrochloride, which, when treated as above, give a precipitate not entirely soluble in hydrochloric acid, or leave a turbid or coloured mother-liquor after the base has crystallised, are contaminated with foreign matter.

Jorissen observes that the blue fluorescent aqueous solution of hydrastinine hydrochloride has the property of instantly reducing cold Nessler solution with the deposition of mercury. The same reaction is given by morphine, apomorphine and picrotoxin (*Ann. Chim. Anal.*, 1903, **8**, 126).

The *sulphate*, B,H_2SO_4, forms yellow crystals showing a green fluorescence, and is soluble in alcohol.

Canadine, $C_{20}H_{21}O_4N$, is an alkaloid accompanying berberine and hydrastine in golden seal root. Until recently there was some doubt as to its actual existence, Lloyd having failed to detect it in the extract from a very large quantity of the root; but F. Wilhelm and E. Schmidt have independently isolated the alkaloid, which is identical with Lerchen's Xanthopuccine (*Jahresber* 1878, 144).

Schmidt prepared it from crude hydrastine (*Arch. Pharm.*, 1894, **232**, 136). This was dissolved in acetic acid and precipitated with ammonia. The precipitate after washing and pressing was dissolved in dilute sulphuric acid, the filtered solution treated with nitric acid and allowed to stand for one or two days. The precipitate was dissolved in hot water and the base reprecipitated with ammonia. This process was repeated until nitric acid gave a white crystalline precipitate becoming yellow on exposure to light. The alkaloid is then recrystallised from boiling light petroleum and finally from alcohol.

Canadine forms white needles, m.p. 132.5°, insoluble in water, readily soluble in alcohol, ether, chloroform and benzene, difficultly soluble in light petroleum. The alcoholic solution is neutral to litmus and phenolphthaleïn.

It has been established by Gadamer (*Arch. Pharm.*, 1901, **239**, 648) that canadine is identical with *l*-tetrahydroberberine, and thus has $[\alpha]_D - 298.2°$ in 1% chloroform solution.

Reactions.—Canadine dissolves in sulphuric acid to a yellow solution, gradually turning red. Nitric acid also gives a yellow solution. Erdmann's and Fröhde's reagents give a transient olive-green colour rapidly becoming brownish-red. The alkaloid dissolves in sulphovanadic acid to an olive-green solution changing to brownish-black. It liberates iodine from iodic acid, and reduces a solution of potassium ferricyanide and ferric chloride, giving Prussian blue. The solution of canadine in pure sulphuric acid is turned brownish-black by basic bismuth nitrate.

When heated in alcoholic solution with iodine it forms berberine hydriodide.

The salts are difficultly soluble in water, particularly in the presence of excess of the corresponding acids.

The *hydrochloride*, $C_{20}H_{21}O_4N,HCl$, is a white crystalline powder more easily soluble in hot than in cold water, giving a neutral solution.

It turns yellow on exposure to light. The *platinichloride* is a yellow amorphous precipitate, and the *aurichloride* a reddish-brown flocculent precipitate.

The *nitrate*, B,HNO_3, forms small white glistening leaflets, less soluble than the hydrochloride in water.

The *sulphate*, B,H_2SO_4, crystallises in large colourless plates easily soluble in water, associated with varying quantities of yellow needles, which probably consist of the hydrated salt.

The *methiodide* forms pale yellow crystals, m. p. 228–232°.

Calumba, or *Columba*, is the root of *Jateorhiza Calumba* or *Cocculus palmatus*, a herbaceous climbing plant occurring in the forests of East Africa.

The calumba of commerce consists of dried transverse slices of the root. It possesses mild, bitter tonic properties, and the tincture, extract, and infusion are official preparations. The roots of bryonia and *Frasera Walteri* have been occasionally sold as calumba.

Calumba has been studied by Gadamer (*Arch. Pharm*, 1902, **240**, 450; 1906, **244**, 255; *Chem. Zeit.*, 1906, **30**, 924), Gunzel (*Arch. Pharm.*, 1906, **244**, 257) and Feist (*ibid.*, 1907, **245**, 586). These authors have isolated from the root three distinct alkaloids, *Jateorrhizine*, *Columbamine* and *Palmatine*. Berberine is not present in *Calumba*.

Jateorrhizine, $C_{17}H_8(OCH_3)_3(OH)_2N$, is the most soluble of the three. It forms a *hydriodide*, B,HI,H_2O, which crystallises in reddish-yellow needles m. p. 208–210°, a *hydrochloride* which crystallises from water with $1/2H_2O$ in light yellow needles m. p. 206°, and from alcohol with $1H_2O$ in copper-coloured needles m. p. 206°.

The *sulphate*, B_2,H_2SO_4, forms brownish-yellow prisms, and the *nitrate*, B,HNO_3, glistening golden-yellow needles.

Columbamine, $C_{17}H_8(OH)(OCH_3)_4N$, is the methyl ether of jateorrhizine.

The *hydriodide*, B,HI, forms orange needles m. p. 224°, difficultly soluble in water and in cold methyl and ethyl alcohols, and having an intensely bitter taste.

The *hydrochloride*, B,HCl, crystallises with $2.5H_2O$ in yellow needles. m. p. 194°, and with $4H_2O$ in brown prisms m. p. 184°.

The *hydrogen sulphate*, B,H_2SO_4, melts at 220–222°. The *nitrate*, $B,HNO_3,2\,1/2H_2O$, forms lemon-yellow needles, m. p. 232°. The *platinichloride*, $B_2H_2PtCl_6$, is a yellow powder, m. p. 238°

(decomp.), whilst the *aurichloride*, $B,HAuCl_4$, forms slender yellow needles, m. p. 220°.

Palmatine, $C_{17}H_9O_2(OCH_3)_4N$. This, the least soluble of the three alkaloids, closely resembles berberine.

The *hydriodide*, B,HI, crystallises in slender yellow needles, m. p. 238–240° (decomp.). The *aurichloride* forms small cinnamon-brown crystals. The *nitrate*, $B,HNO_3,1.5H_2O$, forms slender lemon-yellow needles, m. p. 238–240°.

Columbin, or Calumba Bitter, $C_{28}H_{30}O_9$, exists in calumba root to the extent of 0.34 to 0.40% To extract it, the material is exhausted with boiling alcohol, the extract evaporated to dryness, the residue taken up with hot water, and the filtered liquid shaken with ether; or the tincture is evaporated to a syrup, and shaken with chloroform. The chloroform solution is filtered, evaporated, and treated with 60% alcohol, which dissolves most of the colouring matter. The residue is dissolved in strong alcohol, the solution decolourised with animal charcoal, and the columbin crystallised.

Hilger (*Apoth. Zeit.*, 1896, **11**, 73) recommends boiling with ether to extract columbin from the root.

From analyses and molecular weight determinations made by Ulrich (*Annalen*, 1907, **351**, 363) columbin seems to have the formula $C_{28}H_{30}O_9$, whilst Frey has shown (*ibid.*, 372) that the substance contains two hydroxyl groups, since it forms a *diacetyl* derivative, and a lactone ring. Accordingly the formula can be written $C_{27}H_{26}O_5(OH)_2\begin{smallmatrix}O\\|\\CO\end{smallmatrix}$

The diacetyl compound crystallises in white needles, m. p. 218°.

Columbin is an intensely bitter, inodorous, neutral substance. It melts at 182°, and crystallises from acetic acid solution in colourless trimetric prisms, very slightly soluble in cold water, more freely in hot.

Columbin is sparingly soluble in cold alcohol, and in 40 parts of the boiling solvent. It dissolves with difficulty in cold, more readily in hot ether.

The solution of columbin is intensely bitter; it is not precipitated by tannin or any metallic salts.

Columbin dissolves in strong sulphuric acid with orange colour, changing to deep red; on adding water brown flakes are deposited. Columbin dissolves in aqueous alkalis, and is reprecipitated by acids. On heating with alkali hydroxide an acid substance is formed. Accord-

ing to Houdé, columbin produces vomiting and diarrhœa. 0.10 grm. was fatal to a fowl, death being preceded by digestive disturbance and frequent evacuations (*Pharm. J.*, 1885–6 [*iii*], **16**, 838).

Columbic acid, $OHC_{27}H_{30}O_7.CO_2H$, is prepared by treating the dried alcoholic extract of calumba root with lime-water, and precipitating the solution with hydrochloric acid.

It has been prepared by Frey (*loc. cit.*) by boiling a solution of columbin in strong potassium hydroxide solution, in a current of hydrogen. Thus prepared, Columbic acid crystallises in rosettes, m. p. 220°. It is somewhat less bitter than columbin, nearly insoluble in water, slightly soluble in ether, more readily in acetic acid and easily in alcohol. The alcoholic solution gives a yellow precipitate with lead acetate.

CAFFEINE, TEA AND COFFEE.

By J. J. FOX and P. J. SAGEMAN.

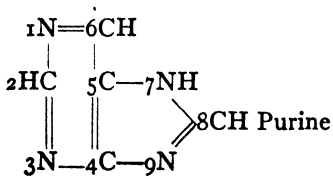
CAFFEINE AND ITS ALLIES.

Caffeine, the characteristic alkaloid of *coffee,* was obtained pure in 1821, when is was prepared almost simultaneously by Runge, Pelletier and Caventon, and Robiquet. In 1827, Oudry discovered a similar principle in *tea,* and named it theine. Berzelius suggested the identity of this with caffeine, and this was afterwards established, as also was that of the alkaloid of *guarana,* called by Martius *guaranine. Maté,* or Paraguay tea, and *Kola nuts* contain the same alkaloid, while *cocoa* contains the alkaloid theobromine (a lower homologue of caffeine) in addition to small quantities of caffeine.

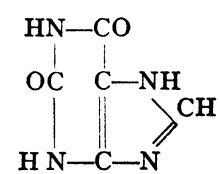
Unlike the majority of the alkaloids hitherto described, theobromine and caffeine are not related to pyridine or quinoline. They are respectively the di- and tri-methyl-derivatives of xanthine, $C_5H_4O_2N_4$, a weak base forming the chief constituent of certain rarely-found urinary calculi, and existing constantly to a minute extent in normal urine and in most of the organs of the human body.

Xanthine is closely related to uric acid, $C_5H_4O_3N_4$, and the whole of these bases are classed together as derivatives of "purine," $C_5H_4N_4$. The synthesis and constitution of purine and its derivatives have been worked out by E. Fischer and his co-workers (*Ber.,* 1897, **30,** 549, 1839, 2226, 2604; 1899, **32,** 435; *Untersuchungen in der Puringruppe,* 1907).

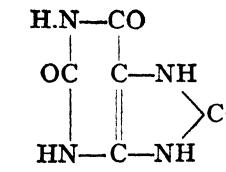
The following formulæ show the relationship existing between the various purine derivatives:



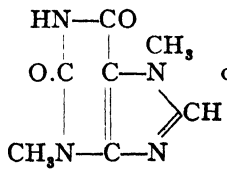
Purine



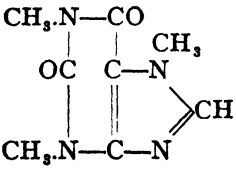
Xanthine or 2.6-dioxy-purine



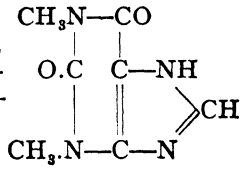
Uric acid, or 2: 6: 8-trioxypurine



Theobromine, or 3.7-dimethylxanthine



Caffeine, or 1.3.7-trimethylxanthine



Theophylline, 1.3-dimethylxanthine

The close relationship between xanthine and caffeine is established by the synthesis of the latter from the former. Xanthine readily dissolves in hot aqueous ammonia and from this solution silver nitrate precipitates a silver salt, $C_5H_2O_2N_4Ag_2,H_2O$. A similar lead salt may be obtained and this on being treated under pressure with methyl iodide at 100° is converted into theobromine, $C_5H_2(CH_3)_2O_2N_4$. The silver derivative of theobromine is then heated at 160° with methyl iodide and yields trimethylxanthine or caffeine. Fischer has shown that uric acid may be readily methylated by treatment of its aqueous alkaline solution with methyl iodide whereby tetramethyluric acid is formed. Under the action of phosphorus oxychloride this is converted into chlorocaffeine; and when chlorocaffeine is reduced by nascent hydrogen, caffeine results.

Xanthine is a white amorphous powder, occurring in all animal tissues, in beet roots and to a small extent in tea extract, from which it has been isolated by A. Baginsky and Kossel. Baginsky extracted the material with diluted sulphuric acid, treated the clear liquid with barium hydroxide solution in excess, and then passed carbon dioxide to precipitate the excess of barium hydroxide. After filtering and evaporating ammonia and silver nitrate were added, and the resultant precipitate of xanthine-silver nitrate was crystallised from its solution in dilute nitric acid to which some urea had been added. The xanthine-silver nitrate so obtained contained 33.6% of Ag, or very nearly the amount required by the formula $C_5H_4O_2N_4,AgNO_3$. The weight of xanthine obtained from 1 lb. of tea was only 0.1567 grm. (*Pharm. J.*, 1888 [*iii*], 19, 41). It is probable that the xanthine obtained in this way was somewhat impure.

Xanthine is only slightly soluble in cold water, alcohol and ether,

and is not readily dissolved by diluted mineral acids. It disolves readily in alkali hydroxides, and in hot aqueous ammonia. On careful evaporation with hydrochloric acid and a crystal of potassium chlorate, and treatment of the residue with gaseous ammonia, a reddish-violet colour results.

Xanthine is dealt with more fully in Vol. 7.

Caffeine, Theine, or 1:3:7-Trimethylxanthine, $C_5H(CH_3)_3O_2N_4$.

The constitution and synthesis of caffeine have already been described (see page 579).

Caffeine exists naturally in the following sources, all of which are employed for food or preparing beverages:

a. Coffee; the dried seed of *Coffea Arabica.*

b. Tea; the prepared and dried leaves of *Camellia Thea.*

c. Maté or Paraguay tea; the dried leaves and twigs of *Ilex Paraguayensis.*

d. Guarana or Brazilian chocolate; the dried pulp of the seed of *Paullinia Cupana.*

e. Kola; the seeds or nuts of the Kola tree (*Cola* or *Sterculia acuminata*) of West Central Africa.

Caffeine is found in other parts of these plants besides those commonly used for food, and also occurs in small quantity, together with theobromine, in cocoa.

Caffeine is now prepared on a considerable scale from damaged tea. Several methods have been employed for the purpose, one of the simplest being to exhaust the tea with boiling water, boil the decoction with litharge or acetate of lead, and concentrate the filtered solution till the alkaloid crystallises out on cooling. The product can be purified by sublimation, or by crystallisation from hot water.

Caffeine forms long, white, silky, flexible needles, which readily felt together to form light fleecy masses. When deposited slowly from an aqueous or chloroform solution, the crystals of caffeine present a characteristic appearance under a magnifying power of 100 to 300 diameters.

The m. p. of caffeine is given as 233–4° (Strecker); Allen gives 231.5°; *German Pharmacopœia* 230.5, and *United States Pharmacopœia* 236.8° after drying. The sublimation-point is 180° (*German Pharmacopœia*) or 178° (*United States Pharmacopœia*). Caffeine crystallises from aqueous solution with 1 molecule of water, but commercial caffeine usually contains rather less than this quantity. The *British Pharmacopœia* states that crystalline caffeine should contain 8.49% of

water; Allen found only 7.05 and 7.10% in two commercial specimens and this is more nearly the quantity present in ordinary samples, probably owing to efflorescence. The water of crystallisation is lost by prolonged exposure over sulphuric acid at ordinary temperatures.

On heating crystallised caffeine to 100° the crystals become opaque and friable, owing to the loss of water, the residue consisting of anhydrous caffeine and dissolving without turbidity in chloroform. According to Mulder, caffeine is deposited in anhydrous crystals from alcohol or ether, and under certain conditions from water also.

The effect of heat on caffeine is of considerable importance from the point of view of its estimation. The alkaloid undergoes only a slight loss of weight at 100°; above 100° the loss is greater and at 120° is gradual but continuous, and at 178° the caffeine sublimes in long silky needles. According to A. Wynter Blyth caffeine sublimes distinctly at 79°; and W. A. Puckner (*Amer. J. Pharm.*, 1905, **77**, 488) states that caffeine can be dried without loss at 95° in a flask, but not in an open dish and that the caffeine does not become anhydrous at 100°. G. E. Smith, C. M. Caines and G. S. A. Caines working with Allen, however, obtained results which undoubtedly demonstrated that caffeine can be estimated by drying in a water oven and that any loss of alkaloid sustained by this method is practically negligible. They further show that on heating at 120° a steady and continuous loss of alkaloid is sustained. Moreover no loss resulted in evaporating a solution of caffeine in chloroform, or on repeatedly evaporating to depress with water (A. H. Allen, *Pharm. J.* 1892, [*iii*], **23**, 213.)

The present writers confirm Allen's statement that caffeine can be repeatedly evaporated with water at 100° in an open beaker, without more than the slightest loss of caffeine.

Caffeine is odourless, but has a bitter taste. It has a marked physiological action, and in excessive doses possesses decided poisonous properties. Administered to frogs, it produces tetanus and rigor of the voluntary muscles. A cat was killed in 35 minutes by administering 0.25 grm. of alkaloid. In all experiments with caffeine on the lower animals there has been increased frequency of the heart's action, and repeated emptying of the bladder and intestines. After death, the alkaloid has been detected in the blood, the bile, and the urine. In man, caffeine increases the heart's action, by stimulating the cardiac muscles, and excites the nervous system, and it has been stated to be an antidote in nicotine poisoning.

The *British Pharmacopœia* gives from 0.07 to 0.35 grm. as the medicinal dose of caffeine; the *German Pharmacopœia* states the maximum single dose at 0.5 grm., and the daily maximum dose at 1.5 grm.

The physiological action of infusions of tea and coffee is in part due to the caffeine, but is largely modified by the other constituents—notably the tannin, extractive matter, and possibly the essential oil of tea, and the *caffeol* or essential oil of coffee.

The solubility of caffeine is given in the following tables:

Solvent	Parts of solvent required for 1 of caffeine					
	A Commaille (*Compt Rend*, 1875, 81, 817)			Göckel (*Chem Centr*, 1897,2,401)		U S P
	At 15° to 17°		At b p of solvent	18°	B p	25°
	Hydrated	Anhydrous	Anhydrous			
Water	68	74	2 2 (at 65°)	.....	....	45 6
Rectified spirit	40	44		.....	.....	.. .. .
Absolute alcohol	. .	165	32		....	53.2
Commercial ether.	476	526	.	. .		. ..
Pure anhydrous ether		2288	277	839	339	375
Chloroform . . .		7 7	5 25	8 5	6 4	8
Carbon disulphide		1709	220			. . .
Petroleum spirit .. .	..	4000				. ....
Carbon tetrachloride . .	. . . .			1123	142.4	.
Benzene .. .	.	.	.	109 8	18 9	.. .

The *United States Pharmacopœia* states that 1 part dissolves in 5.2 of water at 80° and 17.1 of alcohol at 60°.

A systematic study of the solubility of caffeine was made by A. Seidell (*J. Amer. Chem. Soc.*, 1907, **29**, 1091) with the object of finding a method of estimating caffeine in mixtures. His results are given in the table:

Solvent	Sp gr of solvent	Temperature of solution	Solubility grm caffeine per 100 grm of saturated solution	Sp gr of saturated solution
Water.... . .	0 997	25	2 14	.. . . .
Ether .	0 716	25	0 27	. . . .
Chloroform . ..	1 476	25	11.0	. . . .
Acetone	0 809	30–1	2 18	0 832
Benzene	0 872	30–1	1 22	0.875
Benzaldehyde .	1 055	30–1	11.62	1.087
Amylacetate..	0 860	30–1	0.72	0.862
Aniline . . .	1 02	30–1	22 89	1 080
Amyl alcohol..... ...	0 814	25	0.49	0.810
Acetic acid..... . . .	1.055	21 5	2.44	. . ..
Xylene.....	0 847	32 5	1.11	0 847
Toluene..... . . .	0 862	25	0.57	0 861

In headache powders consisting of mixtures of salol, acetanilide and caffeine, Seidell obtained a fair approximate separation by digestion first with toluene and then with amyl alcohol, most of the caffeine being insoluble.

Chloroform and benzene dissolve out the alkaloid even from its acidified aqueous solutions, but the agitations must be several times repeated to effect complete extraction.

1 c.c. of conc. sulphuric or conc. nitric acid should dissolve 0.1 grm. of caffeine and give colourless solutions (*German Pharmacopœia*). Hydrochloric acid has no action on caffeine below 200°, but when heated under pressure with concentrated hydrochloric acid to 250° for 6 to 12 hours caffeine yields ammonia, methylamine, sarcosine, carbon dioxide, and traces of formic acid. The volume of methylamine produced is double that of the ammonia, which proves the presence of three NMe groups in caffeine, and establishes the following equation for the action: $C_8H_{10}O_2N_4 + 6H_2O = NH_3 + 2N(CH_3)H_2 + C_3H_7O_2N + CH_2O_2 + 2CO_2$ (E. Schmidt, *Annalen*, 1883, **217**, 270).

When caffeine is warmed with dilute alkali hydroxide or boiled with concentrated barium hydroxide solution, it at first assimilates the elements of water and is converted into an acid, $C_8H_{12}O_3N_4$. On further treatment, this substance splits up with great facility into carbon dioxide and the base caffeidine, $C_7H_{12}ON_4$. On still further boiling with the alkali this is again decomposed with formation of carbon dioxide, formic acid, ammonia, methylamine, and sarcosine (methylaminoacetic acid).

Caffeidine-carboxylic acid, $C_8H_{12}O_3N_4$, or $C_7H_{11}ON_4.COOH$, is best prepared by digesting finely-divided caffeine for some hours at 30° in a dilute solution of potassium or sodium hydroxide, neutralising with acetic acid, adding cupric acetate (avoiding excess), and decomposing the resultant precipitate by hydrogen sulphide. The liberated acid obtained on evaporation of the filtrate *in vacuo* at the ordinary temperature, may be purified by solution in chloroform and precipitation with benzene, and is thus obtained in the form of a thick oil, which on exposure to the air solidifies to a yellowish-white, semi-crystalline mass, very soluble in water to a strongly acid liquid. It is soluble in alcohol and chloroform, but insoluble in benzene. On boiling the aqueous solution of caffeidine-carboxylic acid, carbon dioxide is evolved and a reddish oil remains, which when stirred up with a small quantity of sulphuric acid and treated with alcohol solidifies to a white

crystalline mass of *caffeidine sulphate*. The reaction affords a ready method of preparing caffeidine. It is merely necessary to decompose the copper salt with hydrogen sulphide, evaporate the filtrate rapidly, and treat it with strong sulphuric acid. The *copper salt* of caffeidine-carboxylic acid, $Cu(C_8H_{11}O_3N_4)_2$, is a pale blue crystalline powder, nearly insoluble in water and wholly so in alcohol. The barium, calcium, zinc, cadmium, and magnesium salts are nearly insoluble in water, but the *lead* salt is soluble. $\overline{KA}$ is a yellow oil. On adding mercuric chloride to the solution of a soluble caffeidine-carboxylate, a copious white precipitate is obtained which contains $(C_8H_{11}O_3N_4)_2Hg,2HgCl_2$. If this be suspended in water and decomposed with hydrogen sulphide the filtered liquid leaves caffeidine hydrochloride on evaporation.

Caffeidine, $C_7H_{12}O_4N$, may be obtained as above described, or may be prepared by boiling caffeine with a solution of 10 parts of crystallised barium hydroxide for half an hour, or until ammonia and methylamine begin to be evolved. From the product of the action, *caffeidine sulphate*, BH_2SO_4, is obtained by acidifying the filtered liquid with dilute sulphuric acid, and evaporating the filtrate to a thin syrup, when the salt is deposited in readily soluble needles. The free base is an oily, strongly alkaline liquid, readily soluble in water, alcohol and chloroform, but with difficulty in ether. It reduces silver oxide, even in the cold, and decomposes very readily into ammonia, methylamine, and cholestrophane (dimethylparabanic acid), $CO\begin{<}\begin{matrix}NMe.CO\\|\\NMe.CO\end{matrix}$

Caffeidine nitrate, hydrobromide, and hydrochloride crystallise well. $B_2H_2PtCl_6$ crystallises from water in large orange-yellow needles, containing either 2 or 4 H_2O.

Allen has proved that caffeine readily undergoes decomposition when boiled with lime-water, a fact which has a practical bearing on several of the published processes for its estimation. When caffeine is boiled with magnesia and water, the decomposition is insignificant, and with litharge there is no change; heated with soda-lime at 180°, ammonia is evolved, and carbonate and a large quantity of cyanide formed. According to Rochleder this last product distinguishes caffeine from piperine, morphine, quinine, and cinchonine. When caffeine is ignited with excess of soda-lime, the nitrogen is evolved as ammonia, any cyanide formed as an intermediate product at a lower temperature being decomposed

in the usual manner; but in order to ensure complete conversion of the nitrogen into ammonia, it is better to mix the caffeine with about twice its weight of cane-sugar (A. H. Allen).

When caffeine is treated with bromine-water, avoiding excess, and the liquid evaporated to dryness at 100°, a yellowish residue is left, which becomes crimson-red on further heating, and is turned a magnificent purple by ammonia. The test is very delicate, and is not affected by a considerable excess of ammonia. On adding sodium hydroxide complete and instant decolorisation occurs.

Another modification of the test consists in treating a minute quantity of the solid substance (such as a residue of caffeine left on evaporation) in a porcelain dish with a few drops of strong hydrochloric acid and a minute crystal of potassium chlorate, and evaporating the liquid to dryness at 100°. When cold, the reddish-yellow or pinkish residue is cautiously moistened with ammonia, avoiding an excess, when the characteristic purple colouration is produced; or, preferably, it is exposed to ammoniacal vapours by inverting the dish bearing the residue over another containing strong ammonia.

The products of the oxidation of caffeine include amalic acid, which by subsequent treatment with ammonia is converted into murexoïn; the reactions being identical to the eye and parallel in chemical change to those yielded by uric acid under like conditions. Thus:

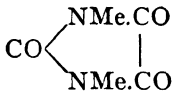
	Uric acid yields	Caffeine yields
With the oxidising agent,	Alloxantin.	Amalic acid.
	$C_8H_6O_8N_4$	$C_8H_2(CH_3)_4O_8N_4$
On adding ammonia,	Murexide.	Murexoïn.
	$NH_4.C_8H_4O_6N_5$	$NH_4.C_8(CH_3)_4O_6N_5$

Strong nitric acid may be substituted for the bromine-water or hydrochloric acid and potassium chlorate; but the reaction is in that case for less distinct and easy to regulate, and excess of ammonia must be carefully avoided. O. Hehner pointed out that, if the nitric acid used be perfectly pure, caffeine fails to give the murexoïn reaction, but that in presence of a minute trace of hydrochloric acid the colour is readily developed.

Theobromine and xanthine give similar reactions to caffeine with an oxidising agent and ammonia. The purple colourations due to caffeine and theobromine are decolourised by adding alkali hydroxide solution, but that due to uric acid is changed to blue.

If caffeine solution is boiled with a strong solution of nitric acid and potassium ferricyanide a precipitate of prussian blue is formed on dilution. This test is delicate, but applies to uric acid also.

When caffeine is heated with a large excess of nitric acid, it is converted into cholestrophane or dimethylparabanic acid,



a compound which crystallises in pearly laminæ, m. p. 145·5°, b. p. 275–277°, and difficultly soluble in cold water and alcohol. It is decomposed with great facility by alkalies into symmetrical dimethyl carbamide (melting at 97–100°) and oxalic acid. Hence on adding ammonia and calcium chloride to its aqueous solution, and warming the liquid, calcium oxalate is precipitated.

Cholestrophane is also produced (35·4 to 41·8%) by oxidising caffeine with chromic acid mixture, the main action being:

$$C_5H(CH_3)_3O_2N_4 + O_3 + 2H_2O = C_3(CH_3)_2O_3N_2 + NH_2(CH_3) + NH_3 + 2CO_2.$$

Caffeine is very imperfectly precipitated by the usual alkaloidal reagents. No reactions result with neutral iodised potassium iodide and Mayer's solution, which behaviour distinguishes caffeine from nearly all other alkaloids except theobromine and colchicine. From an acid solution however caffeine is quantitatively precipitated by a solution of iodine and potassium iodide. Potassio-bismuth iodide percipitates caffeine after a time from moderately dilute solutions (1:3,000). Phosphomolybdic acid produces a yellowish precipitate, soluble in warm sodium acetate solution, the liquid depositing free caffeine on cooling. $(C_8H_{10}O_2N_4.HCl)_2PtCl_4$ is obtained on adding hydrochloric acid and platinic chloride to a highly concentrated solution of caffeine, as an orange precipitate soluble in 20 parts of cold and an even smaller quantity of warm water, crystallising again on cooling.

A solution of caffeine in 200 parts of water gives an immediate and abundant precipitate on adding a saturated solution of mercuric chloride. With a more dilute solution (1:1,000) crystals appear in a few minutes, and in an hour or two an abundant crop of large acicular crystals is obtained. With a solution of caffeine in 4,000 parts of water

crystals appear after a few days. The precipitate contains $C_8H_{10}O_2N_4$-$HgCl_2$, and is much less soluble in excess of the reagent than in pure water. Hence the best results are obtained by adding an equal volume of a concentrated solution of mercuric chloride to the liquid to be tested. The compound is soluble in about 260 parts of cold water, and more readily in hot, crystallising out again on cooling and it also crystallises from hot alcohol. The compound is not sufficiently insoluble to be applicable to the quantitative precipitation of caffeine (R. H. Davies, *Pharm. J.*, 1890 [*iii*], **21**, 253).

Gallotannic acid precipitates moderately dilute solutions of caffeine, the precipitate being somewhat soluble in excess of the reagent. A difference of a few degrees in temperature greatly alters the solubility, and hence a solution of properly adjusted strength may be perfectly limpid at one temperature, and become completely opaque from separation of amorphous caffeine gallotannate on cooling a few degrees. A similar separation of caffeine tannate is the cause of an infusion of tea becoming turbid on cooling.

Salts of Caffeine.—Caffeine is a very feeble base. Its aqueous and alcoholic solutions have no action on litmus, and it is extracted from aqueous liquids by benzene and chloroform, even in presence of a free mineral acid. This behaviour is doubtless due to the facility with which the majority of caffeine salts are hydrolytically dissociated on dilution. They are decomposed by alcohol and ether as by water, and the salts with volatile acids (*e. g.*, acetic) are decomposed on exposure to air. The hydrochloride leaves merely free caffeine after heating at 100°. Allen found that on adding free caffeine to hot water containing a trace of sulphuric acid and coloured with methyl orange, the red colour of the liquid was immediately destroyed, proving neutralisation of the acid; but an acid reaction was re-established when standard acid had been added equivalent to only about 1/20 of the caffeine present. Owing to these facts, certain devices have to be employed for the preparation of the majority of the salts of caffeine. The *oxalate* and *salicylate* are sparingly soluble, and can be readily prepared by mixing equivalent quantities of the acid and alkaloid in aqueous solution. The *citrate* is best obtained by mixing a chloroform solution of caffeine with an alcoholic solution of citric acid, and evaporating the mixture to a syrup.

The salts of caffeine with acids are so readily dissoated by water that in order to obtain the neutral salts considerable excess of acid is

necessary. With an excess of acid, and at a sufficient degree of concentration, the alkaloid will momentarily dissolve to a clear solution, and then almost immediately crystallise out as salt.

H. W. Snow (*Pharm. J.*, 1890 [*iii*], **21**, 1185) gives the following as the composition of the principal salts of caffeine:

Caffeine hydrochloride,	$B,HCl+2H_2O$
Caffeine hydrobromide,	$B,HBr+2H_2O$
Caffeine nitrate,	$5(B,HNO_3)+H_2O$
Caffeine sulphate (normal),	B,H_2SO_4
Caffeine oxalate,	$B_2,H_2C_2O_4$
Caffeine salicylate,	$B,HC_7H_5O_3$

Caffeine hydrochloride crystallises in colourless prismatic needles. It loses the whole of its acid at 75°. The *sulphate* is deposited from a hot alcoholic solution in shining needles unchanged at 100°. Caffeine *nitrate* forms fine transparent crystals, which when dropped into water become opaque, and are converted into pesudomorphs consisting of microscopic needles of free caffeine.

Caffeine gives rise to a number of perhaloids (Gomberg, *J.*, *Amer. Chem. Soc.*, 1896, **18**, 347) which are noteworthy from their insolubility in water. Thus Gomberg showed that if to a solution of caffeine acidified with hydrochloric acid an aqueous solution of iodine and potassium iodide were added, a precipitate of *caffeine periodide*, $C_8H_{10}O_2N_4HI.I_4$, resulted. The substance crystallises from methyl alcohol in dark blue prisms. Similar salts are the *di-iodo-hydriodide*, $C_8H_{10}O_2N_4HI.I_2,1.5H_2O$, consisting of almost black prismatic iridescent crystals and the *dibromo-hydrobromide*, $C_8H_{10}O_2N_4HBr.Br_2$, consisting of orange-coloured crystals.

Caffeine citrate is official in the *British Pharmacopœia* of 1898, where the formula $C_8H_{10}O_2N_4,C_6H_8O_7$ is ascribed to it. The *British Pharmacopœia* article is regarded as an indefinite, unstable, inaccurately described, and superfluous preparation (*Pharm. J.*, 1888 [*iii*], **19**, 252). Free caffeine has not unfrequently been sold as the citrate. The proportion of acid can be directly ascertained in the citrate and other caffeine salts by titrating the solution with a standard alkali hydroxide (preferably barium hydroxide) and phenolphthaleïn; and the total caffeine can be isolated by agitating the neutralised or original aqueous solution with chloroform. On treating

the dry substance with cold chloroform, only the uncombined caffeine, if any, will be dissolved out (J. U. Lloyd).

A strong and stable solution of caffeine can be readily prepared by dissolving it in benzoate, cinnamate, or salicylate of sodium or ammonium. Such solutions are employed for hypodermic injections; caffeine phenate and phthalate have been applied to the same purpose. Caffeine sodium salicylate should contain about 40% of caffeine.

The extraction and estimation of caffeine are fully dealt with in the section on tea and coffee (page 233), but the following observations by A. H. Allen[1] (*Pharm. J.*, 1892 [*iii*], **23**, 215) may be conveniently summarised here as they are of considerable importance for judging the value of the processes for estimating caffeine which have been proposed from time to time.

1. Aqueous solutions of caffeine may be concentrated and evaporated to dryness at 100°, the resulting product being practically anhydrous caffeine. No appreciable loss of weight occurs in this operation.

2. Caffeine can be completely extracted from its acidified or slightly ammoniacal aqueous solutions by repeated agitation with chloroform. In Allen's experiments, from a solution slightly acidified with sulphuric acid, the first treatment with chloroform extracts from 70 to 85% of the total alkaloid.

3. Charcoal cannot be employed for decolourising caffeine solutions, without a considerable adsorption of alkaloid, which is retained with extreme persistency.

4. Caffeine remains completely unchanged by heating to 100° with strong hydrochloric acid, or with sulphuric acid diluted with 1/3 of its volume of water.

5. Caffeine is readily decomposed by alkalies. By warming with dilute sodium hydroxide, it easily undergoes change, and by boiling with lime it is partly decomposed, with formation of ammonia and methylamine (see page 224).

6. When commercial caffeine is treated with ignited magnesia and water, and the mixture distilled, a slight but distinct formation of ammonia is observed, apparently accompanied with traces of volatile amines. But the volatile bases are found chiefly in the first fractions of the distillate, the latter portions being quite free from alkaline reaction; and when carefully purified caffeine is employed, the forma-

[1] Worked out by C. M. Caines, G. S. A. Caines and G. E. Scott-Smith, in Allen's Laboratory.

tion of ammonia and other volatile bases is reduced to a minute trace. Hence their formation is more probably due to the decomposition of some impurity present in small quantity than of the caffeine itself.

7. If a mixture of caffeine with magnesia be made into a paste with water and dried, the alkaloid can be wholly extracted from the mixture by prolonged treatment with chloroform.

8. When 1 part of caffeine is dissolved in hot water, and a solution of 2 parts of gallotannic acid or tannin prepared from tea is added, the caffeine can be accurately estimated by precipitating the solution with lead acetate and extracting the concentrated filtrate with chloroform. If however the mixture be concentrated to a syrup, mixed with ignited magnesia, and dried at 100°, the whole of the alkaloid cannot be extracted by boiling the powdered mass with dry chloroform.

9. When a decoction of tea is substituted for the foregoing artificial mixture of caffeine with excess of tannin a precisely similar result is obtained. Whether sand or magnesia be used, the alkaloid is only partially extracted, even after prolonged boiling with chloroform or ether.

10. When finely-powdered tea is mixed with slaked lime, ignited magnesia, or sand, made into a paste with hot water, and the mixture thoroughly dried at 100°, only a fraction of the total alkaloid can be extracted with chloroform.

11. By prolonged boiling with litharge a decoction of tea becomes completely decolourised, but the process is tedious. If after a time a small addition of lead acetate be made, clarification occurs in a few minutes.

From the foregoing statements it is evident that the estimation of caffeine when in a state of solution presents no great difficulty, though the plan of evaporating the liquid with sand and lime or magnesia, and extracting the dried mixture with chloroform or ether, may give gravely inaccurate results.

Theobromine, 3.7-Dimethyl-xanthine,

$$C_7H_8O_2N_4.$$

The constitution and synthesis of theobromine have already been described (page 580). It is the lower homologue of caffeine, to which alkaloid it presents a close general resemblance, but differs consider-

ably from it in its solubilities. Theobromine is isomeric with theophylline and paraxanthine.

Theobromine exists naturally in cocoa, the seed or bean of *Theobroma cacao;* and together with caffeine in the kolanut (*Sterculia acuminata*). An alkaloid apparently identical with theobromine was found by Zoller in a specimen of Himalayan tea.

Theobromine forms a white, crystalline powder, which under the microscope appears as trimetric needles and club-shaped groups. When heated to about 290° it sublimes without decomposition or previous fusion. but melts at 329° in a sealed tube.

Theobromine has a very bitter taste, which is only slowly developed. Its physiological action is similar to that of caffeine, but more powerful. In large doses it produces well-defined poisonous effects.[1] It is eliminated by the kidneys, and can be detected in the urine.

Theobromine requires 1,700 parts of cold or 600 of boiling ether for solution, dissolves in 105 parts of boiling chloroform, is soluble in amyl alcohol, dissolves slightly in benzene, and is insoluble in petroleum spirit. Gockel (*Chem. Centr.*, 1897, **2**, 1401) states that theobromine dissolves in 4,703 parts of boiling carbon tetrachloride and 3,125 parts of boiling anhydrous ether.

Eminger (*Forschurgsber. Lebensmittel*, 1896, **3**, 275; *Jahresber. Pharm.*, 896, 746) gives the following data:

1 part theobromine dissolves in 736.5 parts water at 18°.
1 part theobromine dissolves in 136 parts water at 100°.
1 part theobromine dissolves in 5,399 parts 90% alcohol at 18°.
1 part theobromine dissolves in 440 parts boiling 90% alcohol.
It dissolves more readily in 80% than in 90% alcohol.

Theobromine dissolves in acids, and is precipitated from the solution by alkalies, but is soluble in excess of ammonia or alkali hydroxides. It is wholly extracted from its solution in sodium hydroxide by agitation with chloroform.

Theobromine is a weak base, its salts being readily decomposed by water with separation of the alkaloid (compare Caffeine, page 588). The *hydrochloride*, $BHCl+H_2O$, and *nitrate*, $BHNO_3$, lose all their acid at 100°. The *platinichloride*, $B_2H_2PtCl_6+2H_2O$, crystallises in oblique prisms, which effloresce in the air and become anhydrous at 100.° The *aurichloride*, $BHAuCl_4$, forms tufts of yellow needles.

[1] Veley and Waller (*Proc. Roy. Soc.*, 1910, 38a, 568) have estimated the relative toxicity of theobromine to caffeine as 1.8:1.

An aqueous solution of theobromine forms with mercuric chloride a white crystalline precipitate, sparingly soluble in water and alcohol.

One of the most definite and insoluble compounds of theobromine is that with silver nitrate. When a very dilute aqueous solution of theobromine nitrate is treated with silver nitrate, silver-white needles containing silver form after a short time. The compound is only sparingly soluble in water, and may be dried without change at 100°. If a solution of theobromine in ammonia be treated with silver nitrate, a gelatinous precipitate is obtained which dissolves easily in warm ammonia, and on boiling the solution for some time, hydrated silver theobromine, $C_7H_7O_2N_4Ag$, separates as a granular nearly insoluble precipitate. Monthulé (*Ann. Chim. Anal. Appl.*, 1911, **16**, 137) utilises the formation of insoluble silver theobromine as a method of separating theobromine from caffeine.

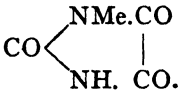
Theobromine reacts with alkalies like a weak acid and forms definite salts. Thus the *sodium salt* is obtained by adding theobromine to sodium hydroxide until a portion remains undissolved after long standing, and evaporating the filtrate *in vacuo*. The product is destitute of crystalline structure, is extremely soluble in water, has a strong alkaline reaction, and absorbs carbon dioxide from the air. The *barium salt*, $(C_7H_7O_2N_4)_2Ba$, separates on adding theobromine to barium hydroxide solution as a mass of microscopic needles, and is obtainable as a snow-white felt of silky needles by slowly cooling its solution in hot water. If the solution be rapidly cooled, it solidifies to a stiff jelly.

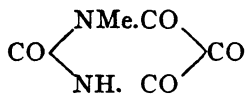
Theobromine yields no product similar to caffeidine when boiled with concentrated barium hyroxide solution or alkali hydroxides. By such treatment, as also when heated with hydrochloric acid under pressure to 240°, theobromine gives the same products as caffeine (page 584).

The best precipitant of theobromine is a solution of sodium phosphotungstate which should be added to a solution strongly acidified with sulphuric or nitric acid. The yellow precipitate should be mixed with sodium carbonate or magnesia, dried, and the mixture exhausted with hot chloroform, which dissolves the theobromine.

When theobromine is heated with dilute sulphuric acid and lead dioxide, carbon dioxide is evolved. Once started, the action continues without further application of heat, and if excess of the oxidising agent and too long heating be avoided the filtered liquid is

colourless, but evolves ammonia on treatment with an alkali hydroxide, separates sulphur from hydrogen sulphide, colours the skin purple-red, and immediately turns blue when treated with a moderate quantity of magnesia. Excess of magnesia destroys the colour, which may be restored by cautious addition of sulphuric acid.

By oxidation with chromic acid mixture, theobromine yields carbon dioxide, methylamine, and methyl-parabanic acid, 

Aqueous chlorine converts it into methyl-urea, $NHMe.CO.NH_2$, and methyl-alloxan, 

while treatment with hydrochloric acid and potassium chlorate oxidises it to dimethyl-alloxantin, $C_8H_4(CH_3)_2O_8N_4$. Theobromine gives with oxidising agents and ammonia the same colour-reactions which characterise caffeine (page 586).

For **isolation and estimation of theobromine,** see under Cocoa.

Diuretin.—Under this name a preparation has been introduced into medicine having the constitution of a combination of sodium-theobromine and sodium salicylate, and the formula $C_7H_7O_2N_4Na$,-$C_6H_4(OH).COONa$.

Diuretin is colourless, odourless, slightly soluble in cold water, and insoluble in chloroform or ether, but readily soluble in hot water or warm dilute alcohol. It is decomposed by carbon dioxide. The physiological action of diuretin is said to be quite distinct from that of the analogous compound of caffeine. It is stated to be much more readily absorbed than simple theobromine, and to be devoid of any toxic properties, or of the peculiar excitant influence on the central nervous system exerted by caffeine.

Owing to the high price of theobromine as compared with caffeine, substitution of the former by the latter alkaloid is possible, and hence G. Vulpius (*J. Chem. Soc.*, 1890, **58**, 1475) has proposed the following method for the assay of diuretin: 2 grm. of the sample is dissolved in 10 c.c. of water in a porcelain dish, the solution acidified with hydrochloric acid, and then rendered faintly alkaline with ammonia. The liquid is kept for 3 hours at the ordinary temperature, and frequently stirred. The separated theobromine is then collected on a tared filter, the filtrate being used to transfer the last portions from the dish.

Gentle suction is used to remove the last of the mother-liquor, and the theobromine is then washed twice with 10 c.c. of cold water, dried at 100°, and weighed. By this method, Vulpius recovered from 41 to 41.5% of theobromine from pure diuretin, 6.5% remaining in the filtrate and washings. Making this allowance, the theobromine should not be less than 46.5%, and that isolated should melt when carefully heated, be completely volatile, and dissolve readily in sodium hydroxide solution. From the filtrate from the theobromine, the salicylic acid can be isolated by acidifying with hydrochloric acid and agitating with chloroform. The separated chloroform is washed with water to remove mineral acid, a little water and a drop of phenolphthaleïn solution added, and the liquid then titrated with $N/10$ alkali hydroxide. Each c.c. of $N/10$ alkali required for neutralisation represents 0.0138 grm. of salicylic acid. Diuretin should contain 38.5% of salicylic acid. The titration completed, the chloroform may be separated and evaporated, when the residue will represent the 6.5% of theobromine not previously separated, together with any caffeine the preparation may have contained. To prove the absence of caffeine in diuretin, Vulpius recommends that 1 grm. of the sample should be dissolved in 5 c.c. of water, and the solution neutralised with hydrochloric acid, when the theobromine will form a milky precipitate readily soluble in soda solution. If the mixture be shaken with its own volume of chloroform, not more than 0.005 grm. of residue should remain on evaporating the separated chloroform.

Other compounds of theobromine are also on the market; such are sodium-theobromine-citrate and sodium-theobromine-sodium-acetate, known also as *Agurin*.

Derivatives of caffeine, such as hydroxycaffeine and caffeine ethylenediamine, have come into use for medicinal purposes. Hydroxycaffeine, $C_8H_{10}O_3N_4$, has been prepared by the action of alcoholic potassium hydroxide on chlorocaffeine, and subsequent treatment with hydrochloric acid (Starkenstein, *Pharm. Centr.*, 1907, **47**, 618; *Pharm. J.*, 1908 [*iv*], **1**, 630). It crystallises from water in needles, m. p. 345°. It is stated to be a powerful diuretic and to be free from toxicity.

Theophylline, 1:3-Dimethylxanthine, $C_5H_2(CH_3)_2O_2N_4$, a base existing in minute quantity in tea, together with xanthine, hypoxanthine $C_5H_4ON_4$, and adenine. It is isomeric with theobromine and paraxanthine (occurring in human urine). According to A. Kossel (*Ber.*, 1888, **21**, 2164; *Zeit. Physiol. Chem.*, 1889, **13**, 298),

theophylline crystallises with $1H_2O$, which it loses at 110°. It melts at 264°. It is easily soluble in warm water, but sparingly in cold alcohol, and is extremely soluble in very dilute ammonia. It forms a crystalline hydrochloride, nitrate, platinichloride, aurichloride, and mercurichloride, and combines with sodium hydroxide to form a readily soluble compound. When evaporated with chlorine-water, theophylline yields a scarlet residue, changed to violet on addition of ammonia. The *silver-derivative*, $C_7H_7O_2N_4Ag$, is obtained as an amorphous precipitate on adding silver nitrate to an aqueous solution of theophylline. It crystallises from hot ammonia, and dissolves readily in nitric acid. The methyl-derivative, $C_7H_7MeO_2N_4$, prepared by heating the last substance with methyl iodide and methyl alcohol, is identical with caffeine.

For the isolation of theophylline Kossel extracts tea-leaves with alcohol and evaporates the tincture to a syrup, when most of the caffeine crystallises out on cooling. The filtrate is diluted with water, acidified with sulphuric acid, filtered after a considerable time, made alkaline with ammonia, and precipitated with nitrate of silver. After standing 24 hours the precipitate is filtered off and warmed with nitric acid; on cooling the liquid, the silver nitrate compounds of *adenine* and *hypoxanthine* (sarcine) crystallise out. The acid filtrate is treated with ammonia, and the precipitate suspended in water acidified with nitric acid and decomposed by hydrogen sulphide. On concentrating the filtrate, *xanthine* first crystallises, and subsequently theophylline. The mother-liquor is precipitated with mercuric nitrate, the free acid being nearly neutralised with sodium hydroxide. The precipitate is then separated, suspended in water, and decomposed by hydrogen sulphide, and the theophylline recovered from the filtrate.

Theophylline is used as a diuretic, for which purpose it is much stronger than either caffeine or theobromine, although it acts on the heart to a less extent than caffeine. A preparation with sodium acetate, used medicinally, is *sodium theophylline sodium acetate*, $C_7H_7O_2N_4Na,C_2H_3O_2Na$, containing upward of 60% of theophylline. It is sold under the name of *Theocin*.[1]

Adenine or 6-Aminopurine, $C_5H_5N_5$, occurs to a small extent in tea extract, and was isolated by Krüger from tea (*Zeitsch. Physiol. Chem.*, 1895, **21**, 274). Krüger precipitated the adenine as the silver derivative

[1] The alkaloid is prepared commercially from 8-chlorocaffeine by the action of phosphorus oxychloride and chlorine which yield dichlorocaffeine, this is heated with water and the resulting 8-chlorotheophylline reduced to theophylline (Eng. Pat. 5901 of 1903).

from the ammoniacal solution of the extract; and then from an aqueous solution of the silver adenine and other alkaloids obtained insoluble adenine *picrate*, $C_5H_5N_5,C_6H_2(NO_2)_3OH$. The adenine silver was finally purified by recrystallisation from hot nitric acid. According to Krüger, hypoxanthine does not occur in tea extract, but is probably derived from the action of the nitrous and nitric acid during the recrystallisation of adenine silver.

Adenine crystallises from aqueous solution in long needles with $3H_2O$. It gives no colour reaction on evaporation with nitric acid and treatment of the residue with alkali; but if some of the crystals are warmed to 53° they become opaque at this temperature, probably owing to loss of water of crystallisation (see Vol. 7).

TEA.

The tea of commerce is the prepared leaf of *Thea chinensis* (and perhaps allied species), a shrub-like plant belonging to the genus *Camellia*. It occurs native in the Himalayas and Assam, has long been cultivated in China and Japan, and is now grown largely in British India, Ceylon, Brazil, etc. *T. assamica* is native to Assam.

It was formerly believed that green and black teas were the product of distinct plants, but it is now known that the difference is due to the method of preparation, black tea having undergone a certain amount of fermentation, whereas in green tea this change is carefully prevented.

"For *black teas*, the leaves are withered a little, rolled to liberate the juices, left in balls for the proper state of fermentation, then sun-dried and subjected to a careful firing in a furnace. For *green teas*, the fresh leaves are first withered in hot pans, then rolled to free the juices, slightly roasted in the pans, sweated in bags, and returned to the pans for a final slow roasting, with stirring, for 8 or 9 hours, beginning at the temperature of 69°, and falling to 49° at the close" (A. B. Prescott). The methods of preparing tea vary materially in different countries. In India, the manufacturing processes are very much simplified, being reduced to five, instead of the twelve practised in China. In addition, the work is nearly all accomplished by machinery, so that the leaves are not touched by the labourers, except in picking. This is partially true also of Japanese tea, whereas Chinese tea was formerly manipulated almost entirely by hand, except when the feet were employed. A detailed description of the method of preparing Japanese tea has been given by J. Takyama (*Chem. News*, 1884, **50**, 299).

The leaves are gathered from the plants four times a year, and are distinguished according to their age. Each leaf is at first a "Flowery Pekoe" leaf, which is the name applied to the leaf-bud. This becomes in succession "orange Pekoe," "Pekoe," "Souchong 1st," "Souchong 2nd," "Congou," and finally "Bohea." In some cases the leaves are classified simply as Pekoe, Souchong, and Bohea. The first and second pickings of the season furnish the finest teas; but the quality of the product depends on the age of the tree as well as the age of the leaf; the finest teas being produced from the young leaves of young plants, while old leaves, and the leaves of old wood, are deficient both in flavour and extract. O. Kellner (*Land. Versuchs-Stat.*, 1887, **33**, 370) has published analyses of the leaves of the same tea-plant during six months (May to November). His figures show a decrease in the proportion of total nitrogen, and almost entire disappearance of amino-nitrogen in the older leaves. The caffeine fell from 2.85 to 1.00 (estimated by evaporating the infusion to dryness with magnesia, and extracting with ether), and the tannin rose from 8.53 to 12.16. The hot-water extract remained practically stationary, while the ether-extract rose from 6.48 to 22.19. The ash increased from 4.69 to 5.04 only, but in July fell to 4.29, and in September reached 5.11. All the ash determinations are probably low, and suggest ignition at too high a temperature. Such an error would vitiate the potash estimations, which showed a variation from 49.06 in May to 17.31 in November. The manganese (Mn_3O_4) ranged from 1.21 to 2.48, and the chlorine from 1.04 to 1.56% of the ash.

Besides the foregoing distinctions, based on the age of the leaf, there are other classifications based on the the district of growth and the method of preparation. Thus among the chief commercial varieties of *black* tea are Assam, Ceylon, Japan, Kaisow, Moning, and Oolong; and those of *green* tea, Gunpowder, Hyson, Young Hyson, Imperial, and Twankay. Green tea has much declined in popularity of late years.

The colour of black tea has been shown by K. Asō to be due to the action of an oxydase on tannin, the preliminary treatment in green tea destroying the oxydase.

Mann (*J. Asiatic Socy. Bengal*, 1901, **70**, 154) proved that the oxydase in tea leaves is most active below 55° and can be destroyed at 80°. The enzyme was found to occur in the unopened tip leaf of the shoots and in the stems; it was very sensitive to acids and alkalies and

diminished as the leaf aged. It was found that more highly flavoured teas resulted from increased enzymic action; and the enzyme increased during the withering of the leaves. The starch persisted during withering but disappeared on fermentation.

Very few complete analyses of tea have been published; and, indeed, they have but a limited interest or practical value, since the tea is not consumed as a whole, but invariably infused, and the infusion contains the tea-constituents in very different proportions from those in which they exist in the leaf.

König gives the following percentage results as the average of 158 analyses of various kinds of tea:

	Water	Nitrogenous matter	Caffeine	Essential oil	Ethereal extract
Maximum . . .	12 0	38 7	4.7	1.0	15 2
Minimum	3 9	18 2	1.1	0 5	3 6
Average. . .	8 5	24 1	2 8	0 7	8 2

	Tannin	Various nitrogen-free substances	Crude fibre	Ash		
				Sol in water	Insol in water	Aqueous extract
Maximum..... . .	25 2	.. . .	15 5	5 0	5 6	55.7
Minimum	4 5	. .	8 5	1 6	1 3	27.5
Average.	12 4	30 3	10 6	3 0	3.0	38.8

These figures are calculated from analyses by different observers and some of the constituents have therefore been estimated by methods which are now superseded by more accurate processes; but the mean values are in fair agreement with the most recent work.

J. M. Eder has published estimations (*Dingl. Polyt. J.*, 1879, **231**, 445 and 526) which differ somewhat from those of other observers and are probably low in tannin and in soluble mineral substances. Eder's results include 0.1% of Boheic acid obtained as stated by Rochleder; but it has been shown by Hilger and Tretzel that boheic acid is merely an oxidation product of the tannin of tea and does not exist as such in tea. Polstorff (*Chem. Zentr.*, 1909, **1**, 2014) has detected *choline* in tea leaves. He obtained 3 grm. of choline from 10 kilogrm. of tea.

Some idea of the variations of the most important constituents in

teas of different origin may be obtained from the following table (numbers are percentages):

Observer	Kind of tea	Moisture	Caffeine	Tannin	Ash			Aqueous extract	Number of samples
					Sol.	Insol	Sand		
J Zolcinski (*Zeit. Analyt. Chem*, 1898, 37, 365)	Cheap Chinese.	10 6	1.55	..	. ..	5 9	....	29.7	10
P Dvorkovitch (*Ber.*, 1891, 24, 1945).	Chinese.	8 2	2 9	9.6	..	.....	.....	33.3	29
J. F. Geisler....	Oolong.	5.9	2 3	16 4	3 2	2.6	0.5	43 3	13
J. F. Geisler.. .	Congo..	8 4	2 4	11 5	3.1	2.7	0.4	34.4	.....
J. F. Geisler....	Indian .	5.8	2 7	14.9	3 5	2 1	0.2	42 9	6
J. F. Geisler .	Japan .	4.0	2 5	14.7	3 5	2.0	0.4	50 4	2
R. R. Tatlock & R. J. Thomson (*Analyst*, 1910, 35, 103)	Indian.	6 8	3 5	14.3	3 5	2 3	0 2	46 4	10
R. R. Tatlock & R. J. Thomson (*Analyst*, 1910, 35, 103)	Ceylon .	6 8	3 3	12.3	3 0	2.3	Traces	44.1	5
R R Tatlock & R. J Thomson (*Analyst*, 1910, 35, 103)	Chinese	7 8	3.5	9 5	3 2	3 3	0 8[1]	43.1	6
R. R Tatlock & R. J. Thomson (*Analyst*, 1910, 35, 103)	Java ...	7 5	3.4	14.5	3.5	2 4	0 1	44.8	1

The following analyses by Y. Kozai (*Bulletin* No. 7, Imperial College of Agriculture, Japan) have a special value owing to the author's knowledge of tea manufacture. Special precautions were taken in sampling the leaves to ensure strictly parallel specimens being taken. The figures refer to the moisture-free leaves in each case:

	Unprepared leaves	Green tea	Black tea
Caffeine	3.30	3.20	3.30
Ether-extract	6 49	5 52	5.82
Hot-water extract	50 97	53 74	47 23
Tannin (as gallotannic acid)	12 91	10.64	4.89
Other nitrogen-free extract	27.86	31 43	35 39
Crude protein	37.33	37 43	38.90
Crude fibre	10.44	10.06	10 07
Ash	4 97	4 92	4 93
Albuminoid nitrogen	4 11	3 94	4.11
Caffeine nitrogen	0 96	0 93	0.96
Amino-nitrogen	0 91	1.13	1.16

The proportion of ash found by Kozai is remarkably low, but it seems not impossible that this is characteristic of Japanese teas, since some analyses by J. Takayama (*Chem. News*, 1884, 50, 299) show the same peculiarity.

[1] High result due to "gunpowder" with 2.7% sand.

An analysis of the so-called "flower of tea," consisting of the hairs of the leaf-buds of the tea-plant, has been published by T. B. Groves (*Year-book Pharm.*, 1876, 610).

Dried tea flowers have been introduced into Paris from Tonquin and according to Perrot and Goris (*Rev. Sci. L'Union Pharm.*, 1907, **48**, 301, and *Pharm. J.*, 1907 [*iv*], **2**, 381) contain 2% of caffeine and fair quantities of manganese and iron. There are apparently two kinds—green and black—probably owing to difference in drying. The infusion prepared from the flowers of tea is stated to possess a very delicate aroma and to be sweeter than ordinary tea infusion

James Bell (*Foods*, **1**, 6) gives the following figures as illustrating the composition of fair representatives of black and green teas of commerce:

	Congou (black) %	Young Hyson (green) %
Moisture	8 20	5 96
Caffeine	3 24	2.33
Albumin, insoluble	17.20	16.83
Albumin, soluble	70	.80
Extractive by alcohol, containing nitrogenous matter	6 79	7.05
Dextrin or gum	...	.50
Pectin and pectic acid	2 60	3 22
Tannin	16 40	27.14
Chlorophyll and resin	4 60	4.20
Cellulose and insoluble colouring matter	34 00	25 90
Ash	6 27	6 07
	100 00	100.00

Bell's values for tannin are probably too high for ordinary tea.

The following figures are given by J. P. Battershall (*Food Adulteration*, page 28) as the results of the analysis by American chemists of samples representing 2,414 packages of Indian tea, a class remarkable for their general strength, high quality, and freedom from adulteration:

	Maximum, %	Minimum, %	Average, %
Moisture	6 32	5.83	5.94
Insoluble leaf	55 87	47.12	51.91
Extract	40 35	37.80	38 84
Tannin	18 87	13.04	15 32
Caffeine	3 24	1 88	2 74
Ash.—Total	6.02	5.05	5.61
Soluble in water	4 28	3.12	3 52
Insoluble in acid	0.30	0 12	0 18

A. A. Besson (*Chem. Zeit.*, 1911, **35**, 813) has examined 86 samples with the following results:

Kind	Moisture	Stalk	Ash	Caffeine
Chinese green tea	6 00– 7 69	0 4– 5.3	4 88–7 46	2 13–3 22
Foochow	6 29– 9 06	4 1–17 5	4.80–5.73	2.23–3.64
Hankow	6 48– 8.33	8 6–17.1	4 95–5.65	2 65–3.64
Ceylon	4 57– 8 12	5 8–43 4	4 54–5 65	2.80–4 10
Indian	4 00– 8 08	11 5–37.4	4 72–5 64	3 31–4.15
Java.	8 22–10.50	11.4–29 9	5 53–7.32	2 22–4 54

Besson found very little relation between the price and quality, as determined by the tasting test, and the amount of stalk present in the tea.

E. Sage (*Pharm. J.*, 1898, [*iv*], **7**, 106) has furnished the following figures for the analysis of Natal teas:

	Moisture	Caffeine	Tannin	Total ash	Sol ash	Water extract	Alkalinity of ash as K_2O
Flowery Pekoe	8 2	3 0	8 1	5 3	3 4	39 9	1 4
Golden Pekoe	8 0	2 8	10 8	5.3	3.3	41 6	1.3
Souchong.	9 2	3 3	10 7	5 5	3 6	38 3	1 5
Pekoe	9 4	2 9	8 9	5 6	3 7	41 2	1 4
Broken Pekoe	9 6	3 1	6 7	5 3	3 4	36 8	1.2

Similar figures are given by C. H. Caines and also in the *Bull. Imp. Inst.* (1908, [*vi*], **1**, 2).

Natal tea must not be mistaken for the so-called "Cape tea" and "Bush tea," consisting of the dried leaves and twigs of certain species of *Cyclopia*. According to H. G. Greenish (*Pharm. J.*, 1880 [*iii*], **11**, 549, 569, 832), Cape tea is destitute of caffeine or other alkaloid, but contains a glucosidal substance called cyclopin, $C_{25}H_{28}O_{13}$, similar to cinchona-novatannic acid, and yielding, when boiled with dilute acid, dextrose and a substance of the formula $C_{19}H_{22}O_{10}$, closely resembling cinchona-nova-red. Greenish also found a crystalline substance exhibiting a green fluorescence in alkaline solutions, and probably identical with the cyclopic acid previously described by A. H. Church (*Chem. News*, 1870, **22**, 2); and likewise a third substance analogous to cyclopin, and apparently an oxidation-product of that compound. Cape tea yielded Allen 30% of extract, and on ignition left 3.7% of an ash containing manganese.

Analysis of Tea.—The most important analytical data which it is usually desirable to obtain are moisture, ash, caffeine, tannin, aqueous extract, detection of facing and detection of foreign leaves. The above estimations together with qualitative tests are usually sufficient to furnish a means of judging of the adulteration of tea.

Before analysis the sample should be powdered so that the whole passes through a sieve with meshes of 0.5 mm. breadth.

The moisture (determined at 100°–105°) contained in commercial tea varies within somewhat wide limits (4.2 to 10.8%); but the range is far less when teas of the same class are compared. Thus G. W. Wigner (*Pharm. J.*, 1875, [*iii*], **6**, 261, 281, 402) found that hyson and gunpowders, both of which are highly-dried teas, contained the smallest proportions of moisture (4.84 to 6.55%), and, after drying at 100°, absorbed from 6.04 to 6.98% of water on exposure to air. Congou teas showed in their original condition an average of 8.50% of moisture, and never wholly regained their original weight on exposure to air after drying at 100°. The average proportion of moisture in commercial tea is about 7.7%, and the usual range between 7 and 9%.

Ash.—For the detection of *mineral adulterants*, the tea should be ignited, and the proportions of ash soluble in water and acid determined. In practice, this is best effected by igniting 5 grm. of the tea, in platinum, at as low a temperature as possible. When the carbon is burnt off, the ash will have a distinct green colour, owing to the formation of manganate. The ash is weighed and boiled with water, the solution filtered, and the residue washed, ignited, moistened with ammonium carbonate, very gently ignited, and weighed. The difference between the weight now found and that of the *total ash* gives that of the *ash soluble in water*. The *insoluble ash* is next boiled with strong hydrochloric acid, the solution diluted with hot water, filtered, and the *insoluble residue* washed, ignited, and weighed. It consists of extraneous siliceous matter, such as sand, fragments of quartz, etc., and insoluble silicates, such as steatite from the facing of gunpowder tea. If titanic iron sand be present, some of it will almost certainly remain undissolved, and present the appearance of jet-black magnetic particles. The adulteration of tea with magnetic matter, formerly very common, is now obsolete, a clear proof that the mineral admixtures were not due to accidental causes. Magnetic matter is best detected by reducing 10 grm. of the tea to powder and spreading it in a thin layer on a sheet of smooth paper. A magnet is then applied to the under-side of the paper and moved laterally, with its poles in contact with the paper. Any magnetic matter may thus be readily drawn out and separated from the tea.

The weighing of the matter actually extracted by a magnet is far

more satisfactory than the estimation of the iron existing in the tea. Tea naturally contains a small proportion of iron, but it only amounts to about 3% of the weight of the ash, or about 0.18% of the entire tea. Of course the iron in this form is not affected by a magnet, the use of which has the advantage of extracting the iron in the form in which it actually exists.

The solution of the ash soluble in water should be titrated with methyl-orange or litmus and standard acid, the volume used being calculated to its equivalent of potassium oxide (1 c.c. of $N/10$ acid = 0.00471 grm. of K_2O).

The analyses of a very large number of teas show that the proportion of soluble ash and its alkalinity vary with the age of the leaves, the figures yielded being highest with young leaves and teas of high quality. The total ash of absolutely pure tea rarely, if ever, exceeds 6%, but some licence must be allowed in dealing with commercial samples.

If tea contains more than 8% of ash calculated on the dry tea, adulteration is probable. The soluble ash should amount to at least 50% of the total and is frequently nearer 60%. R. R. Tatlock and R. J. Thomson (*Analyst*, 1910, **35**, 103) have recently published analyses of various teas and they find that the total ash varies from 5.14 to 6.65% and in one sample of *gunpowder* they obtained 8.87% of ash. This sample, however, showed 2.74% of sand. The soluble ash in these teas varied from 2.76 to 3.91%, the proportion of sand being much less than 1% except in the one case mentioned.

G. W. Wigner (*Pharm. J.*, 1875 [*iii*], **6**, 261, 281, 402) obtained the following average results by the analysis of 68 samples of commercial tea taken from the original chests. The samples embraced 41 of ordinary character, 18 special teas of high price, and 9 samples of caper.

	Results of analysis of ash, %			
	Total	Siliceous matter	Soluble in water	Alkalinity as K_2O
Samples in commercial state:				
Maximum	7.02	1 67	3.88	1.96
Minimum	5 17	0 04	2 64	1 08
Average	5.78	0 46	3.15	1.45
Samples after drying at 100°:				
Maximum	7.42	1.76	4.16	2.11
Minimum	5 57	0.04	2.94	1.26
Average	6.33	0 50	3.45	1.54

The ash of these 68 samples of tea had the following average composition:

	Including silica, etc., %	Exclusive of silica, etc., %
Siliceous matter	7 96	
Soluble in acid	37 54	40 79
Soluble in water	54 50	59 21
	100 00	100 00
Alkalinity of soluble ash, as K_2O	25 09	27 26

James Bell (*Foods*, **1**, 29, 31) has published figures agreeing with those of Wigner. The proportion of soluble ash in genuine teas analysed by Bell ranged from 2.8 to 4.2% (calculated on the moisture-free tea), the proportion being usually between 3.2 and 3.6%. In one instance only did the soluble ash fall below 3%, and in that case the deficiency was very trifling, the proportion being 2.97%. The alkalinity of the soluble ash of the teas examined by Bell ranged from 1.30 to 1.91% of K_2O. In only one case did the total ash reach 8%, while the insoluble siliceous matter exceeded 1% in a few instances only. Bell's results are fairly in accordance with the experience of Allen (see *Chem. News*, 1874, **29**, 167, 189, 221), and other observers.

In certain cases a high soluble ash does not indicate a high quality of tea. This happens when the ash contains a notable proportion of sodium chloride, owing to the tea having been damaged by sea-water and re-dried. The ash of pure tea contains only a trifling proportion of sodium, less than 2%, and the chlorine does not exceed 1.1%, equivalent to about 1.8% of sodium chloride, representing 0.108% of the weight of the tea. Wigner (*Pharm. J.*, 1875 [*iii*], **6**, 403) found 3.08% of sodium chloride in tea which had been a fortnight under sea-water and completely soaked, and 0.17% and 0.23 in samples which had been slightly moistened.

The following is the composition of the ash of tea, and it will be seen that two of the most important constituents are the potassium and phosphoric acid. Manganese always occurs in about the proportions shown.

	Bell (*Foods* 10)[1]			P van Romburgh and Lohmann
	Maximum, %	Minimum, %	Mean, %	
Total ash	8 29	5 99	6 99	. . .
Sand	13 37	1 51	5 38	. . . . .
Silica	9 27	2 51	6 26	64
Chlorine	1 12	97	1 05	1 08
Potassium oxide (K_2O)	37 71	26 83	32 63	50 62
Sodium oxide (Na_2O)	1.27	.34	79	.65
Iron oxide	2 82	57	1 59	.55
Alumina	5 55	1 54	3 52	1.55
Oxide of manganese (Mn_3O_4)	2 11	1 37	1 73	2 57
Lime	9 54	8 19	8 84	9.27
Magnesia	6 52	2 12	4 32	8 55
Phosphoric anhydride	18 54	12 69	15 00	16 60
Sulphuric anhydride	6 68	5 39	6 24	8 08
Carbonic anhydride	13 42	9 58	11 50	. . . . .

Bell's results were the means of 7 teas, some of the highest figures being due to a sample of Moning. Romburg and Lohmann's values are the mean of 3 Java teas (*Zeitsch. Nahr. Genussm.*, 1899, **2**, 290).

Isolation and Estimation of Caffeine. The *isolation* of caffeine in a state of purity presents little difficulty. Provided that the caffeine isolated be well crystallised, colourless, free from acid or alkaline reaction to litmus, completely soluble in chlorofrom, exerts no reducing action on Fehling's solution, and leaves no ash on ignition, it may be regarded as pure. The extraction and estimation of the total caffeine in tea (or coffee), however, is by no means a simple matter and has received considerable attention in recent years. The methods proposed may be divided roughly into four groups.

a. Extraction of the caffeine by means of boiling water and subsequent treatment of the infusion with lime, magnesia, litharge or basic lead acetate to render tannin, etc., insoluble.

b. Treatment of the material with lime and magnesia or ammonia and extraction with chloroform.

c. Extraction of caffeine in the material directly by means of aqueous sodium benzoate or salicylate with or without an alkali and subsequent treatment of the alkaline liquid with chloroform.

d. Gomberg's method whereby the caffeine is extracted by means of water and the alkaloid is precipitated in acid solution as a periodide.

In the writers' experience Stahlschmidt's method (*Chem. Centr.*, 1861, **6**, 396) as modified by Allen, and Keller's method to be described below, both give satisfactory results; but it is desirable in the latter

[1] Bell assumed that the chlorine was combined as potassium chloride and gives potassium chloride as a constituent; the iron according to Bell should be calculated as ferrous oxide, FeO. van Romburgh and Lohmann calculated the Manganese as Mn_3O_4.

case that the nitrogen in the product should be estimated by Kjeldahl's method. Provided the details are adhered to, these mehods are convenient and require comparatively little attention, although the individual experiments occupy considerable time.

a. Allen's Modification of Stahlschmidt's Method.—6 grm. of finely powdered tea are treated in a flask with 500 c.c. of water, which is then kept boiling under a reflux condenser. No Soxhlet extractor or similar arrangement is so effective or rapid as actual boiling with the water. After 6 or 8 hours' boiling, the decoction may be filtered, the residue washed with hot water on the filter, and the filtrate made up with water to 600 c.c. It is then heated nearly to boiling, and about 4 grm. of lead acetate in powder added, a reflux condenser attached, and the liquid boiled for ten minutes. If on removing the source of heat the precipitate does not curdle and settle readily, leaving the liquid colourless, or nearly so, a further addition of lead acetate must be made and the boiling repeated. When clarification is effected, the liquid is passed through a dry filter. 500 c.c. of the filtrate (= 5 grm. of tea) is then evaporated to about 50 c.c., when a little sodium phosphate is added to precipitate the remaining lead or the lead is removed by hydrogen sulphide. The liquid is filtered, the precipitate washed, and the filtrate further concentrated to about 40 c.c., when the caffeine is extracted by repeated agitations with chloroform, at least 4 treatments with which are necessary to ensure the complete extraction of the alkaloid. In the great majority of cases the chloroform separates readily. Should an obstinate emulsion be formed, the best plan is to place the mixture in a flask, distil off the chloroform, treat the residual liquid with a few drops of basic lead acetate, filter, and agitate the filtrate again with chloroform. The separated chloroform solutions are mixed, and distilled in a tared flask immersed in boiling water. The last traces of chloroform are removed while the flask is still hot by a current of air, and the residual alkaloid is weighed.

The process can be shortened by boiling the tea with 600 c.c. of water in the first place, and adding lead acetate without previously filtering from the exhausted tea. This modification becomes necessary in the case of certain teas (*e.g.*, gunpowder), the aqueous decoctions of which filter very slowly.

The following results by the above process were obtained by C. M. Caines (*Pharm. J.*, 1892, [*iii*], **23**, 218). In some instances the caffeine extracted by half an hour's boiling was determined, in

addition to the total amount obtained by 6 hours' boiling with water. The results refer to the moisture-free teas, which were representative commercial samples:

Description of tea	Tannin; by lead acetate	Caffeine	
		Extracted in 30 minutes	Total, extracted in 6 hours
	%	%	%
Ceylon, whole leaf (Pekoe)	13 01	3 40	3.85
Ceylon, broken leaf	12 31		4 03
Assam, whole leaf (Pekoe)	10 08		4.02
Assam, broken leaf	11 33		4.02
Java Pekoe	12 93		3.75
Kaisow, red leaf	11 35		3 41
Moning, black leaf	11 76	3 44	3.74
Moyume Gunpowder	12 95	2 76	2 89
Natal Pekoe-Souchong	9 90	2 71	3 08

The foregoing process is applicable to the determination of the caffeine in *coffee*, of which 12 grm. may be conveniently employed. In the presence of chicory the extracted alkaloid is liable to be strongly coloured, in which case is should be redissolved in water, a few drops of sodium hydroxide added, and the liquid again exhausted with chloroform.

Dvorkowitsch's method (*Ber.*, 1891, **24**, 1945) has been recommended by the *Association of Official Agricultural Chemists* (U. S. A.) in the provisional methods for the analysis of tea (1907) and is as follows:

Digest 10 grm. of the powdered tea with 200 c.c. of boiling water for 5 minutes and decant the solution; repeat the treatment twice and boil the residue twice with 200 c.c. of water.[1] Make up the combined solutions to 1,000 c.c. and extract a portion with petroleum spirit to remove the fat, etc. To 600 c.c. of the fat-free solution (= 6 grm. tea) add 100 c.c. of a 4% solution of barium hydroxide, mix and filter. To 583 c.c. of the filtrate (= 5 grm. tea) add 100 c.c. of a 20% solution of sodium chloride and extract 3 times with chloroform. Distil the greater part of the chloroform from the combined extracts, place the residue in a tared dish, evaporate the remainder of the chloroform, dry at 100° and weigh. The caffeine is usually of sufficient purity to render a nitrogen estimation unnecessary.

Hilger and Juckenack (*J. Pharm.*, 1897, [*vi*], **6**, 184, and *Forschungsber. Lebens*, 1897, **4**, 49, 145) have modified the above process by using basic

[1] In our experience the time of boiling recommended is generally insufficient to ensure complete extraction of the caffeine.

aluminium acetate and sodium hydrogen carbonate to precipitate the tannin, and evaporating the filtrate with aluminium hydroxide and extracting the dried residue with carbon tetrachloride. The method has been adopted by the German States. Gadamer states (*Arch. Pharm.*, 1899, **237**, 58) that Hilger and Juckenack's method gives low results in all cases as the caffeine is not extracted thoroughly by the hot water in an hour and a half; and Lendrich and Murdfield (*Zeit. Nahr. Genussm.*, 1908, **16**, 647) recommend moistening the dried mass with steam before extraction with carbon tetra chloride; otherwise only 60 to 70% of the caffeine is recovered.

Tatlock and Thomson (*Analyst*, 1910, **35**, 105) boil 2 grm. of tea with 800 c.c. of water for 1 hour and evaporate the aqueous filtrate to small bulk; this is treated with sodium hydroxide solution and extracted with chloroform and the chloroform evaporated to dryness.[1]

b. An accurate method is that due to Keller (*Ber. Deut. pharm. Ges.*, 1897, **7**, 105, and *Chem. Centr.*, 1897, [*i*], 1134) as modified by Katz (*Ber. Deut. pharm. Ges.*, 1902, **12**, 250, and *Chem. Centr.*, 1902 [*ii*], 1526). 10 grm. of the tea are treated with 5 grm. of ammonia solution and then shaken for half an hour with 200 grm. of chloroform. 150 grm. of the solution is filtered off and evaporated to dryness; the residue is treated with 5 c.c. of ether and then with 20 c.c. of hydrochloric acid (0.5% HCl). The solution is gently warmed to drive off the ether and is then filtered warm through a wet filter, and the filtrate is extracted 6 times by means of chloroform. The chloroform solution is evaporated to dryness and the caffeine weighed. The caffeine from tea tends to retain some colouring matter and in this case a nitrogen estimation is desirable.

c. Virchow (*Chem. Zeit.*, 1910, **34**, 1037) modifies this method (for coffee) by adding a little paraffin wax to the chloroform extract, whereby the fat is more conveniently filtered from the aqueous caffein solution.

J. Burmann (*Bull. Soc. Chim*, 1910, [*iv*], **5**, 239) has recently used a similar process for coffee. He recommends that the chloroform extract should be placed in a test-tube constricted at two points, and evaporated to dryness. An asbestos plug is inserted in the lower constriction and the tube is placed in a bath at 210 to 240° as far as the

[1] We have found that the boiling in this case is insufficient in some teas for complete extraction of the caffeine and the final product is not so clean as that obtained by Allen's method, although Tatlock and Thomson claim that the method if carefully carried out gives results identical with the process involving the use of tannin precipitants.

lower constriction and the heating continued for 3 hours to sublime the caffeine. The lower portion of the tube is then cut off and the caffeine is removed by a suitable solvent and weighed.

The use of ammonia in these methods is essential, as chloroform alone fails to extract all the caffeine from dried tea as shown by the experiments by G. E. Scott Smith working with Allen. Petit and Terrat (*Ann. Chim. Anal.*, 1896, 1, 228) have shown that all methods which depend upon the extraction of the dry material with organic solvents give low results.

The following experiments were made by G. E. Scott Smith. 50 grm. of commercial black tea of medium quality were powdered and boiled with water for 30 minutes. The solution was filtered and made up to 1000 c.c. after cooling Aliquot parts of the solution were then treated in the following manner.

A. 100 c.c. (= 5 grm. of tea) was evaporated to a syrup and mixed with 5 grm. of ignited magnesia. The mixture was dried thoroughly at 100°, powdered, and boiled with ether free from alcohol and water.

Caffeine extracted by 6 hours' treatment,	0.059 grm.
Caffeine extracted by 4 hours' further treatment,	0.009 grm.
Caffeine extracted by 3 hours' further treatment,	0.001 grm.
Total, 13	0.069 = 1.38%

On subsequently boiling the residue with alcohol an additional 0.0605 grm. of caffeine was extracted, making 2.59% in all.

B. Was conducted like A, but dry chloroform was substituted for ether. The total caffeine extractable by chloroform was 1.54%.

C. Conducted like A, but rectified spirit was employed at once. It extracted 2.81% of brownish caffeine, which was reduced to 2.78% by re-solution in water and extraction with chloroform.

D. Conducted like B, but sand was substituted for magnesia. Treatment with dry chloroform extracted successively 0.0365, 0.0175, 0.0135 and 0.0010 grm. of caffeine during 9 hours' treatment. On subsequent treatment with alcohol much tannin and colouring matter was extracted. This was precipitated by lead acetate, and the concentrated filtrate shaken with chloroform. Additional yield, 0.070 grm. making a total yield of 2.77%

E. 100 c.c. (= 5 grm. tea) was heated to boiling, treated with solid lead acetate, filtered, and an aliquot part of the filtrate concentrated,

freed from lead, and shaken repeatedly with chloroform. Caffeine was recovered equivalent to 2.63% of the tea.

The determination of caffeine in *tea* as carried out by Paul and Cownley (*Pharm. J.*, 1887 [iii], **18**, 417), is as follows: 5 grm. of finely-powdered tea is well mixed in a mortar with 2 grm. of ignited magnesia, the mixture thoroughly moistened with hot water, again triturated, and then dried at 100°. It is next extracted with boiling alcohol, and the resultant liquid evaporated nearly to dryness. The residue is boiled with 50 c.c. of water, and treated with a few drops of dilute sulphuric acid. When cold, the liquid is filtered and repeatedly shaken with chloroform until exhausted. The united chloroform solution is then agitated with a very dilute solution of sodium hydroxide, which removes a little colouring matter, so that on subsequently distilling off the chloroform in a weighed flask, the caffeine is obtained perfectly pure and colourless, or at most with a faint green tinge.

Paul and Cownley by this method found that Indian and Cingalese teas contained a much larger percentage of caffeine than, owing to the faulty methods of analysis employed up to that time was commonly supposed.

Paul and Cownley have also employed the foregoing method of determining caffeine for the assay of *coffee* (*Pharm. J.*, 1886 [*iii*], **17**, 565, 648). The caffeine obtained by evaporation of the chloroform is liable to contain a small quantity of a brownish waxy or resinous impurity, and hence should be purified by re-solution in boiling water, and recovered by evaporating the filtered solution and drying the residual alkaloid at 100°. By this process they found the proportion of caffeine in coffee-berries to vary within comparatively narrow limits, and not to be materially affected by roasting. Hence they recommend the estimation of the alkaloid in commercial coffee as a means of ascertaining the proportion of chicory or other admixture present.

c. Georges (*J. Pharm. Chim.*, 1896 [*vi*], **4**, 58) considers the following process satisfactory. 0.5 grm. of the sample is mixed with sand and extracted 2 or 3 times by means of a hot 1% solution of sodium salicylate, in which caffeine is readily soluble. The united extracts are mixed and evaporated to a volume of about 50 c.c. and the solution is then shaken in a separator with chloroform. The chloroform extracts are evaporated to dryness and the caffeine weighed. In this process it is better to add some ammonia or sodium hydroxide before

extracting with chloroform on account of the hydrolytic dissociation of the sodium salts, especially if sodium benzoate is used.

d. Gomberg's process (*J. Amer. Chem. Soc.*, 1896, **18**, 331) was devised for the estimation of caffeine in drugs. It depends upon the fact that when a solution of caffeine containing hydrochloric acid is treated with a solution of iodine and potassium iodide the whole of the caffeine is precipitated as periodide, $C_8H_{10}O_2N_4,HI,I_4$. The process as modified for tea by Spencer (*J. Amer. Chem. Soc.*, 1897, **19**, 279) is as follows: 5 grm. of tea are boiled for half an hour with 400 c.c. of water and then digested for another half hour with an excess of ferric hydroxide (freshly precipitated). The liquid is cooled and made up to a suitable volume. A definite volume of this solution is filtered, acidified with diluted hydrochloric acid, the caffeine precipitated by a known volume of standardised iodine solution and the precipitate allowed to settle after being made up to a convenient volume. An aliquot part of the clear supernatant liquid is drawn off and the excess of iodine is determined as usual. The amount of caffeine is obtained from the value 1 part iodine=0.3834 caffeine or 1 c.c. $N/10$ sodium thiosulphate=0.00485 caffeine. The most accurate results are obtained when the iodine solution is used in large excess.[1]

Modifications of the first two classes of methods involving the use of lime have also been suggested, but as it is known that alkali hydroxides decompose caffeine to some extent (page 585) the results obtained by this means are probably low.

[(*Note by American Editor.*) I have found the following method of estimating caffeine (and theobromine) very satisfactory. It is based upon characteristic reactions. (See page 588.)

As part of the naturally occurring caffeine is combined as tannates, glucosides, etc., it is necessary to adopt some preliminary procedure and if possible one that is more complete in its action in all cases than the method described on pages 608 to 611. Both in extracting the samples and in purifying the extract it is very desirable to have a form of apparatus that can be used for extraction of liquids with immiscible solvents heavier than water and for the percolation of solids, etc. The form used by the writer is known as "The Landsiedl Extraction Apparatus" and can be obtained from the larger supply houses. The extract

[1] Caffeine is sometimes a constituent of headache powders, and W A. Puckner has suggested its separation from acetanilide by extraction of both substances with chloroform in the presence of sulphuric acid. The caffeine is precipitated as periodide and the precipitate decomposed by sodium sulphite.

tions can be made in a Soxhlet apparatus and separatory funnels, but not nearly so well, due to the considerable solubility of caffeine in water, etc.

In the case of tea leaves, ground kola nuts, ground coffee, etc., add enough 5% sulphuric acid to thoroughly moisten (and what is necessary to penetrate to all parts when thoroughly soaked). The size of the sample used for analysis will vary from 2 to 28 grm. due to the probable caffeine content. Heat this moist mass in a covered beaker immersed in a steam bath or boiling water for about 2 hours so as to break up the complex molecules by hydrolysis. Then mix with clean sand if the wet mass is too dense to allow penetration of the chloroform. Place a plug of cotton in the bottom of the inner tube "A" of the extraction apparatus "D" (see Fig. 2) and fill in the moist sample and cover with a few thicknesses of filter paper. Pour enough pure chloroform into the bottom of "D" to cover to the extent of 0.5 to 0.75 in. Connect with condenser which fits with ground-glass surfaces. Heat over water-bath so that the chloroform boils freely and drips steadily from the condenser upon the sample. Continue heating 4 hours.

Transfer the chloroform solution to a flask and drive off the chloroform by means of a water-bath, until in the case of an Erlenmeyer flask, the bottom is just about covered. Evaporate the balance of the chloroform at very low heat, which is easily done by adding a little pure ether, and driving this off at just below its boiling-point.

The caffeine (theobromine) at this stage is generally contaminated with resins, fatty oils, terpenes, etc. Add a mixture of 15 c.c. of water and 5 c.c. conc. sulphuric acid and agitate with 25 c.c. of ether. Separate the layer in a small funnel, washing with only a few cubic centimeters of water. Neutralise the aqueous layer with 20% sodium hydroxide (a more dilute solution may result in too large a volume for the inner tube of the extractor). When alkaline add dilute sulphuric acid until barely acid and then make it alkaline (a few drops excess) with ammonium hydroxide. Use inner tube "C" which must be absolutely clean and dry each time, and fill about to dotted line with chloroform. Add the aqueous solution and water until chloroform begins to run from the exit tube. Have a layer of about 0.5 in. of chloroform in the bottom of the extractor "D." Place the inner tube in "D" so that the upper projecting edge rests on projections that are blown in the glass to support it. Connect up and boil the chloroform for about 2 hours. This will extract the chloroform in a high degree of purity from tea. That extracted from coffee may have traces of colour, but when the nitrogen

is estimated it has been found by the writer to be close to the theoretical A discoloured caffeine is due to some of the aqueous layer being mechanically carried over with the chloroform. If the operation o refining is repeated, it will be white and free from appreciable impurity The chloroform is removed as in the previous case.

With infusion of tea or coffee already made proceed as follows.

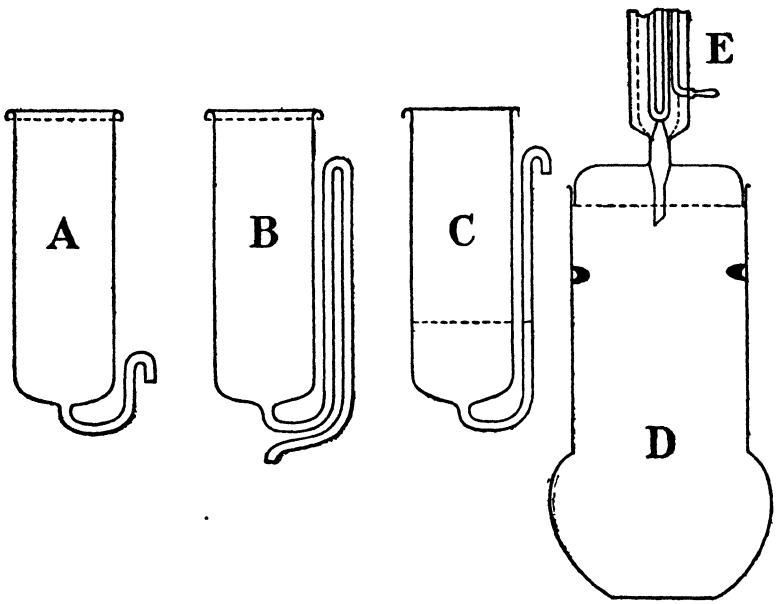


FIG. 2.

Take 100 c.c. or less if desirable, of the liquid, add enough hydrc chloric acid to make 1% HCl and boil with reflux condenser for a hal hour. Place in inner tube "C" and extract for about 4 hours. Refin as previously described, after making faintly alkaline.

Caffeine is advantageously separated from theobromine because c its ready solution in benzene, while theobromine is only very slightl soluble.

In Fig. 2 "B" is used as a regular Soxhlet tube when desired.]

Tannin.—The *tannin* of tea is described in Vol. 5. *The estima tion of tannin in tea* affords valuable information respecting th

probable presence of *previously infused leaves* or *extraneous tannin matters*, such as catechu. This is best effected in the aqueous decoction obtained by exhausting the sample with boiling water, as required for the determination of the extract.

The tannin may be estimated by H. R. Proctor's modification of the Löwenthal process described in Vol. 5. The process as recommended by the A. O. A. C. (*U. S. Dept. Agric.*, 1907, *Bull.* 107) is suitable for this estimation. The following reagents are required:

1. A solution containing 1.33 grm. of potassium permanganate per litre; 2. an $N/10$ solution containing 6.3 grm. of crystallised oxalic acid per litre; 3. a solution containing 6 grm. of indigo-carmine (free from indigo blue) and 50 c.c. of concentrated sulphuric acid per litre; 4. a solution prepared by soaking 25 grm. of gelatin for 1 hour in a salurated solution of sodium chloride, heating until the gelatin is dissolved, cooling and making up to 1,000 c.c.; 5. 975 c.c. of saturated sodium chloride solution mixed with 25 c.c. of concentrated sulphuric acid; 6. powdered kaolin.

Estimation.—Obtain the value of the permanganate solution in terms of oxalic acid. Boil 5 grm. of tea for half an hour with 400 c.c. of water; cool, and make up to 500 c.c. To 10 c.c. of the infusion (filtered if not clear) add 25 c.c. of indigo-carmine solution and about 750 c.c. of water in a large porcelain basin. Add gradually from a burette the potassium permanganate solution, with stirring, until the colour changes to light green; then cautiously, drop by drop until the colour changes to bright yellow, or further to a faint pink at the rim. The number of c.c. of permanganate solution used furnishes the value a of the formula given below. Mix 100 c.c. of the clear tea infusion with 50 c.c. of gelatin solution, 100 c.c. of salt acid solution, and 10 grm. of kaolin, and shake for several minutes in a corked flask. After settling decant through a filter. Mix 25 c.c. of the filtrate (corresponding to 10 c.c. of the original infusion) with 25 c.c. of indigo-carmine solution and about 750 c.c. of water, and titrate with permanganate as before. The number of c.c. of permanganate solution used gives the value b; $a-b=c$; c equals the amount of permanganate required to oxidise the tannin. Assume that 0.04157 grm. of tannin (gallotannic acid) is equivalent to 0.063 grm. of oxalic acid.

This factor is based on the assumption that the whole of the tannin in tea is gallotannic acid but the assumption is unsatisfactory.

The process of fermentation to which black tea has been subjected

undoubtedly causes modification of the tannin. Allen found that a tincture of green tea precipitated tincture of ferric chloride bluish-black, like nut-galls, while the tincture of black tea gave a green colour with iron, just as catechu does.

Some observers, including Allen, regard the tannin of tea as quercitannic acid. Dvorkowitch, on the other hand, takes a factor one-half that for quercitannic acid (see his process below). In our experience the factor varies with the nature of the tea and lies somewhere between those for gallotannic and quercitannic acids. The following are a few of the results of experiments made by the writers and indicate the differences to be expected between the volumetric and gravimetric methods:

Kind of tea	Volumetric (AOAC) method · Result calculated as		Gravimetric method
	Gallotannic acid	Quercitannic acid	
Ceylon .	8 3	12 5	10 5
Java .	11 8	17 7	12.3
Indian .	9 8	14 7	10 0
China. .	6 1	9 1	8 1

The gravimetric method used was that due to Tatlock and Thomson (page 619), the quinine being estimated and its weight deducted from that of the total precipitate. The above differences cannot be attributed to gallic acid since quinine does not precipitate it and only a very small quantity, if any, is thrown out of solution by the salt acid and gelatine reagents. (See also Vol. 3, page 529.)

It would thus appear that the volumetric processes cannot be relied upon to furnish a determination of the absolute amount of tannin in tea.

A modification of the permanganate process, which appears to possess some advantages for the examination of tea, has been described by P. Dvorkovitch (*Ber.*, 1891, **24**, 1945), who aims not only at estimating the tannin but also the proportion of *fermentation-products* formed in the process of fermentation to which black tea has been subjected. For this purpose he treats 10 grm. of finely-powdered tea with 3 successive quantities of 200 c.c. of boiling water, 5 minutes being allowed for each digestion. The residue is then boiled twice with 200 c.c. of water, or until the last extract is almost, if not entirely, free from colour, when the decoction is diluted to 1 litre. 40 c.c.

of this solution is then diluted to 500 c.c. with water, and treated with 25 c.c. of indigo-carmine solution and 25 c.c. of dilute sulphuric acid (200 grm. of H_2SO_4 per litre). The liquid is then titrated with a standard solution of potassium permanganate (containing approximately 2.6 grm. per litre), and of such strength that 130 c.c. are equivalent to 100 c.c. of $N/10$ oxalic acid. The mode of adding the permanganate is important, and Dvorkovitch recommends that in the titration of the indigo-carmine 18 c.c. should be added at the rate of 2 to 3 drops per second, and the remainder at the rate of 1 drop per second, and that, in the titration of the tea solution mixed with indigo-carmine, 23 c.c. of the permanganate should first be run in, the addition continued at the rate of 2 to 3 drops per second, and finally 1 drop per second added unil the reaction is complete. If more than 38 c.c. be required, a smaller quantity of tea infusion should be used. To estimate the *fermentation-products*, 80 c.c. of the tea infusion are mixed with 20 c.c. of barium hydroxide solution (containing 4 grm. of barium hydroxide per 100 c.c.), the liquid filtered, and 50 c.c. of the filtrate (representing 0.4 grm. of the tea) diluted with 500 c.c. of water, mixed with 25 c.c. of dilute sulphuric acid and 25 of the indigo-carmine solution, and titrated with permanganate. 18 c.c. should be run in first of all, then 2 or 3 drops per second added, and finally 1 drop per second till the end of the action. The volume of permanganate required less that reduced by the indigo solution, represents that required for the oxidation of the fermentation-products of 0.4 grm. of tea. According to Dvorkovitch, the joint weight of tannin and fermentation-products is obtained by multiplying the weight of oxalic acid equivalent to the measure of permanganate required for their oxidation by 31.3, since 63 grm. of oxalic acid correspond, according to Dvorkovitch's experiments, to 31.3 grm. of tea-tannin (as compared with 62.3 of quercitannic acid!). Employing this process, he found from 8.84% to 10.55% of tannin, and from 0.90 to 1.88 of fermentation-products, in teas of the first crop of 1890; and he concludes that the higher the proportion of caffeine to the total amount of tannin and fermentation-products, the more valuable is the tea.

Allen and Fletcher's Process.—The Löwenthal process distinguishes the tannic acid from the small quantity of gallic acid also present in tea, but as the astringent character of the infusion is due to both these substances, a method which will estimate the total amount of astringent matter, without distinction of its nature, is in some respects preferable

to a process that gives merely the amount of tannin, while ignoring the gallic acid. Such a process was devised by F. W. Fletcher and Allen (*Chem. News*, 1874, **29**, 167, 189), and was based on the precipitation of the tea infusion by lead acetate, and the use of an ammoniacal solution of potassium ferricyanide to indicate the complete precipitation of the astringent matters. In practice, 5 grm. of neutral lead acetate should be dissolved in distilled water, and diluted to 1 litre, and the solution filtered after standing. The indicator is made by dissolving 0.050 grm. of pure potassium ferricyanide in 50 c.c. of water, and adding an equal bulk of strong ammonia solution. This reagent gives a deep red colouration with gallotannic acid, gallic acid, or an infusion of tea. 1 drop of the solution will detect 0.001 mgrm. of tannin, or 0.001 grm. dissolved in 100 c.c. of water. In carrying out the process, 3 separate quantities of 10 c.c. each of the standard lead solution should be placed in beakers, and each quantity diluted to about 100 c.c. with boiling water. A decoction made from 2 grm. of powdered tea in 250 c.c. of water (the same as is used for determining the extract) is added from a burette, the first trial quantity receiving an addition of 12, the second 15, and the third 18 c.c.; or if green tea be under examination, 8, 10, and 12 c.c. may be preferably employed. Portions (1 c.c.) of these trial quantities are passed through small filters, and the filtrates tested with ammoniacal ferricyanide solution.

The approximate volume of tea decoction required is thus easily found, and after repeating the test nearly the requisite volume can be at once added. In this case about 1 c.c. of the liquid should be removed with a pipette, passed through a small filter, and drops of the filtrate allowed to fall on to spots of the indicating solution previously placed on a porcelain slab. If no pink colouration is observed, another small addition of the tea decoction is made, a few drops of the liquid filtered and tested as before, and this process repeated until a pink colour is observed. The greatest delicacy is obtained when the drops of filtered solution are allowed to fall directly on to the spots of the indicator, instead of observing the point of junction of the liquids.

The volume of tea solution it is necessary to add to 100 c.c. of pure water, in order that a drop may give a pink reaction with the indicator, should be subtracted from the total amount run from the burette.

The foregoing process is simple, and gives very concordant results; but the repeated filtrations requisite for the observation of the end-

point are apt to be tedious. It is difficult to obtain pure tannin for standardising the lead solution, and hence it is preferable to abandon the attempt and make pure lead acetate the starting-point. Allen found that 10 c.c. of the lead solution would precipitate 0.010 grm. of the purest gallotannic acid he could obtain. Hence, if all the weights and volumes above mentioned be adhered to, the number of c.c. of tea decoction required, divided into 125, will give the percentage of tannin and other precipitable matters in the sample. The proportion found in undried black tea by Fletcher and Allen ranged from 8.5 to 11.6% with an average of 10%. A sample of brown catechu tested by the lead process gave a result corresponding to the presence of 119% of tannin (*sic*). (See also page 615.)

Another simple method for the *determination of tannin* is that of J. M. Eder (*Dingl. Polyt. J.*, 1878, **229**, 81), which consists in treating the boiling decoction of 2 grm. of tea with excess of a 5% solution of cupric acetate. The precipitate is separated by filtration, washed, dried, and ignited. The resultant cupric oxide, CuO, can be moistened with nitric acid, re-ignited and weighed as such; or re-ignited with sulphur in a closed crucible, and thus converted into an equal weight of non-hygroscopic cuprous sulphide, Cu_2S. The weight obtained, multiplied by 1.305, gives that of the tannin precipitated. The method is said to give results correct to within 0 2 to 0.3%; but any pectous substances should be previously separated, if present in quantity, by precipitation with alcohol. By this method Eder found an average of about 10% of tannin in 25 samples of black tea, and 12 to 12.5% in green and yellow tea. S. Janke, by the same process, found from 6.9 to 9.1% of tannin in black tea (18 samples), and 8.6 to 9.9 in green. Cupric acetate may be extemporised by mixing a solution of cupric sulphate with excess of sodium acetate, but it is necessary to acidify the solution faintly with acetic acid.

C. M. Caines (page 607) obtained results by Eder's method closely agreeing with those yielded by the same samples with the lead process, and hence the proportion of gallic acid in tea is probably very insignificant.

The formation of an *insoluble quinine tannate* has been used by Tatlock and Thomson (*loc. cit.*) to estimate tannin in tea. In brief, the process consists of cooling the aqueous extract (400 c.c. in volume) of 1 grm. of tea to 15–16° and adding to the liquid a solution of 1 grm. of basic quinine sulphate dissolved in a mixture of 25 c.c. of water

and 2.5 c.c. of *N* sulphuric acid. The solution is stirred well and allowed to settle for 15 minutes when the quinine tannate settles as a buff coloured flocculent mass. This is decanted through a tared filter and finally washed on to the filter with the filtrate. Tatlock and Thomson state that the precipitate contains "on an average" 75% of tannin,[1] but it would be better in all cases to estimate the quinine in the weighed precipitate. By this method Tatlock and Thomson found the following proportions of tannin:[2]

	Maximum, %	Minimum, %	Average, %	Number of samples
Indian tea . . .	15.0	13 3	14 3	9
Ceylon..... ...	13 9	10 1	12 3	5
China..... . ..	10 9	7 3	9 5	6
Java..... . . .	. . .	. ...	14 5	1

In the case of *caper* and *lie* teas, the astringency is often very high, owing to an admixture of *extraneous tannin matters;* but the evidence of the presence of such additions afforded by the estimation of the tannin of tea is, of course, merely inferential. Strong infusions of genuine tea, with the exception of some kinds from India, are generally quite clear, and do not become muddy on cooling. Tea adulterated with catechu gives an infusion which quickly becomes turbid on cooling. More direct proof of the presence of *catechu* may be afforded by the following test devised by Allen, which, however, should always be applied to the suspected tea side by side with a genuine specimen: 1 grm. of the pure tea and an equal weight of the suspected sample are infused in 100 c.c. of boiling water, and the strained liquid precipitated while boiling with a slight excess of neutral lead acetate. 20 c.c. of the filtrate from pure tea (which should be colourless) when treated with a few drops of silver nitrate solution (avoiding excess), and cautiously heated, gives only a very slight greyish cloud or precipitate of reduced silver; but the same tea containing 2% of catechu (purposely added) gives a copious brownish precipitate, the liquid acquiring a distinctly yellowish tinge. With a somewhat larger proportion of catechu, the filtrate from the lead precipitate gives a bright

[1] The writers find that this method gives fairly concordant results on the same sample if the conditions are closely adhered to.
[2] A sample of Indian dust gave 16.6 per cent. of tannin.

green colour on adding one drop of dilute ferric chloride, while the solution of pure tea gives only a slight reddish colour due to the presence of acetate. On allowing this liquid to stand, the adulterated tea gives a precipitate of a greyish or olive-green colour, the pure tea undergoing no change.

These tests, which depend on the properties of catechuic acid, together with the excessive proportion of astringent matters (as shown by the lead process), render the detection of any considerable proportion of catechu tolerably certain; but a means of detecting small additions is still a desideratum.

Extract.—By the term "extract," when used in reference to tea analysis, is understood the sum of the soluble matters extracted from the leaf by boiling water. It includes caffeine, tannin, proteins, gum, dextrin, colouring matter, mineral matter, etc., besides other less important constituents, such as gallic acid, oxalic acid, and quercitin, which substances are present in comparatively small quantity.

According to Allen the extract is best estimated by boiling 2 grm. of the tea in a state of powder with 100 c.c. of water for 1 hour. The liquid is filtered while hot, the residue boiled again with 50 c.c. of water, and the process repeated so long as colouring matter continues to be extracted, the liquid being poured through the filter previously used. After cooling, the decoction is made up to 250 c.c., or other convenient measure, and an aliquot part (1/5) evaporated to dryness for the determination of the extract. The filter and its contents should be dried at 100°, and the insoluble matter detached and weighed. Very constant results are thus obtainable.

The minimum proportion of extract yielded by genuine tea exhausted in a state of powder was fixed by the Society of Public Analysts in 1874 at 30%. Assuming the presence of 7.5% of moisture, this leaves 62.5% for the maximum proportion of insoluble matter. This figure covers almost all legitimate variations in tea, and is considerably in excess of the proportion yielded by green tea, the insoluble matter from which averages 42%, while in black teas the average is only about 50%. In the case of old-leaf Congou teas containing much stalk, which have been stored for some time, the extract may occasionally fall to 30%, corresponding to 62.5% of insoluble matter. In judging a tea by the proportion of extract or insoluble matter, it is very desirable, when possible, to take into account the character of the sample. Thus young leaves (which are to some extent indicated by their size) yield

a notably higher extract than fully grown or old leaves, or specimens containing a considerable proportion of stalk.

G. W. Wigner has recorded the proportions of extract yielded by a sample of tea in powder when boiled with different quantities of water. In each case the tea was boiled with the water under a reflux condenser for 1 hour, the decoction cooled, filtered, and evaporated to dryness.

A.	1 part of tea in 200 parts of water yielded . . .	34.10%	of extract
B	1 part of tea in 100 parts of water yielded . . .	30 55%	of extract
C.	1 part of tea in 50 parts of water yielded .	27 55%	of extract.
D.	1 part of tea in 20 parts of water yielded	22 90	
E.	Exhausted leaves from expt D in 20 parts water	8 17	36.57 of extract.
F	Exhausted leaves from expt E in 20 parts water	3 75	
G.	Exhausted leaves from expt F in 20 parts water .	1.75	

Even after 4 boilings with 20 parts of water, the tea was not completely exhausted. Hence Wigner preferred to estimate the extract by boiling the powdered tea once, for 1 hour, with 100 parts of water, instead of repeatedly exhausting with smaller quantities. Operating in this manner he obtained proportions of extract ranging from 26.15 to 44.85%, the average being 35.70%, containing 4.63 of ash.

Tatlock and Thomson have adversely criticised the methods of estimating the soluble portion of tea on the grounds that the extractions have been insufficient and they suggest that it is better to boil 1 grm. of powdered tea with 400 c.c. of water for 1 hour under a reflux condenser, filter and wash the insoluble matter with 80 c.c. of hot water; dry and weigh the insoluble residue. The results obtained varied from 43.5 to 49.8 for Indian tea, from 41.3 to 48.3 for Ceylon tea and from 38.4 to 46.9 for China tea; figures which are on the whole higher than those given by other observers.

Beythien, Bohrisch and Deiter (*Zeitsch. Nahr. Genussm.*, 1900, **3**, 145) boil 5 grm. of tea with 4 separate quantities of 750 c.c. each of water, and dry and weigh the residue. The results of a large number of teas operated on in this way are given:

	Extract	Total ash	Soluble ash
Maximum.	44 8	6 40	3 99
Minimum.	29 5	5 32	2 08
Average	35 0	5 78	3.13

The method adopted by the A. O. A. C. is that of R. E. Doolittle and F. O. Woodruff (*U. S. Dept. Agr.*, 1907, *Bull.* 105). They recom-

mend boiling 2 grm. of tea with 200 c.c. of water for 1 hour. The extract is filtered through a tared filter and washed with hot water until the filtrate measures 500 c.c. Woodruff and others obtained by this method extracts varying from 42.7 to 53.0% (*U. S. Dept. Agri.*, 1909, *Bull.* 122, 79).

The writers, working by Allen's method on 32 mixed teas, found that the minimum extract was 35% and that it rose as high as 50%.

It should be noted that although different methods will yield varying figures for the same tea, nevertheless for the purpose of detecting adulteration a method such as Allen's (page 621) is quite sufficient in view of the wide variations of teas even of the same class. A point of some interest as regards the detection of excessive stalk is that the stalk of young leaves will yield as much extract as ordinary tea.

The proportion of *extractive matter* yielded necessarily varies with the method used to exhaust the tea, and is, of course, higher when the tea is powdered and the treatment with water long continued and carried to an extreme than when the whole leaves are used and the tea simply infused in boiling water. The latter method commends itself when the object is to study the character of the infusion likely to be yielded in practice, while the former plan gives more information when the object is the detection of adulteration.

An interesting comparison of the results of the two methods has been made by J. F. Geisler, who has published an extensive series of analyses of teas obtained direct from American importers and wholesale houses (*American Grocer*, Oct. 23, 1884; *Analyst*, 1885, **9**, 220). The following table by Geisler shows the proportions of extract, tannin, caffeine, and ash which passed into solution when various representative commercial teas were infused under precisely the same condition by pouring on the leaves 100 times their weight of boiling distilled water, and allowing the liquor to "draw" for 10 minutes. The ratio which the dissolved constituent bore to the total is also given.

A comparison of these figures shows that, as a rule, the finer teas yield to hot water larger proportions of extract, caffeine, and ash than the inferior qualities. On an average, the ash of the extract exceeds by 0.62% the "soluble ash" obtained by treating the ash of the entire tea with water. The proportion of tannin rises and falls with that of the extract, and the ratio which the dissolved extract and tannin bear to the total has a notable relation to the price of the tea.

Kind of tea	Wholesale price per lb in cents	Extract		Tannin[2]		Caffeine	Ash	
		Infusion	Ratio to total	Infusion	Ratio to total	Infusion	Infusion	Ratio to total
Fine Ceylon Pekoe tips[1]...		33 25	76.6	17 19	75.3	2 44	3 44	91.0
Assam.....	23½	29 15	73.5	11 48	60 8	3 30	3.80	70 0
Assam	22½	28 57	72 0	9 50	58 4	2 75	4 40	79.5
Finest Moyune Gunpowder	75	37 32	73.2	16 79	87 8	2 95	4 60	55.8
Common Moyune Gunpowder.	18	28 07	79 4	9 26	77 7	1.67	4 02	66.1
Japan basket-fired		31 75	75 6	11 23	74 5	2 17	4.27	80 8
Japan pan-fired...		34 37	79.6	13 41	94 4	2 07	3.67	63.6
Choicest Formosa Oolong.	65	33.62	75 9	12 91	75 6	2 50	4 00	71.3
Choicest Formosa Oolong	53	33.30	73.7	13.75	68 5	2 42	3 97	66.5
Superior Formosa Oolong	30	29 00	68 6	9 63	59 6	2 12	3 66	62 3
Medium Amoy Oolong	24	27 40	60 9	10 12	56.0	1 92	3.72	68.5
Medium Amoy Oolong	21½	24.50	60.5	7.53	55.6	1 70	3 25	58 9
Choicest Moning Congou	45	24 25	70.6	5 46	41.7	2 87	4 13	73.7
Superior Moning Congou.	27	21 55	57 8	4 44	32 0	2 77	3.70	63 5
Medium Moning Congou.	19½	21 02	68 6	5 55	45 2	2 33	3.22	58 3
Good common Kaisow Congou	17½	23 25	64.1	4 05	38 5	2 35	3 30	59.9
Common Moning Congou[3]	15½	19 50	72 2	4 50	52 9	1 95	2 88	46 8

By the same method of 10 minutes' infusion in boiling-hot water, E. B. Kenrick (*Bulletin* No. 24, Laboratory of Inland Revenue Department, Canada) obtained the following average results from commercial samples of tea:

Description of teas	No of samples	Aqueous extract	Tannin dissolved	Caffeine dissolved	Ratio of aq extract to tannin
Congou.....	10	23 37	5 18	2.65	4 51
Assam	3	38.53	7.49	2.98	3 81
Ceylon	2	27 45	7.85	2.68	3 50
Unclassed black	13	23 76	5.40	2.82	4 40
Japan	18	30 07	9 38	2 45	3 20
Gunpowder.	2	28 55	8.00	2 39	3.57
Young hyson.	5	34.22	10 98	2 52	3 12

From these figures it appears that congou teas yield less extract than green and Japan teas, while Assam and Ceylon teas yield intermediate results. Not only do the Japan and green teas yield more soluble tannin than the black, but the proportion of tannin to the whole extract is greater in the former kinds. On the other hand, the black teas appear to yield more soluble caffeine than the Japan and green teas.

The following figures by Geisler show the influence of the time allowed for infusion upon the proportion of the constituents dissolved, and the difference in the result caused by substituting New York water (Croton River, of 4.96 degrees hardness per 100,000) for

[1] *Jour. Amer Chem Soc*, 1891, 13, 237

[2] The estimations of tannin were made by the Löwenthal method, except in a few instances in which the cupric acetate method was employed

[3] This sample is considered by Geisler to have been adulterated, though its appearance did not indicate any admixture with exhausted leaves.—(Private communication to Allen.)

distilled water. In each case the tea used was the finest Formosa Oolong, and it was infused in 100 parts of boiling water:

	Distilled water				Croton water	
	3 min	5 min	10 min	1 hour	5 min.	10 min
Total extract	25 97	28 37	30 87	33 75	27.47	30.25
Ash	3 72	3 80	4 17	4 33	3 62	4.13
Extract *minus* ash	22 25	24 50	26 70	29 42	23.85	26.12
Tannin	9 75	11 23	13 46	14 94	10.18	10 60
Caffeine	1 95	2 65	2 75	2 85	2 02	2.82
Alkalinity of infusion-ash (= K_2O)	1 03	1 08	1 22	1 28	1 08	1.15

From these results it appears that infusion in distilled water for 3 minutes is insufficient, but in 5 minutes practically as good a result is obtained as in a longer time, without so much astringent matter being extracted. When Croton water is used, 10 minutes gives a materially better result, so far as caffeine and extract are concerned, while the proportion of tannin is not increased in the same proportion. In all these experiments the volatile oil is left out of consideration, though it is to this constituent that the flavor and aroma of the tea is due, and on these characters the commercial value of the tea materially depends. The tannin and extractive matter impart astringency, strength, and body to the infusion. Caffeine, being almost tasteless, is not taken into account by tea-tasters, though physiologically it is the most important constituent of tea.

Tatlock and Thomson publish results of 3 and 5 minutes' infusion of 21 samples of tea, the extract depending to some extent on the mechanical condition of the tea. The general results of these infusions calculated as a percentage on the ingredients extracted are:

	Three minutes' infusion			Five minutes' infusion		
	Max	Min	Average	Max	Min.	Average
Water extract	83	54	66	90	65	79
Caffeine	76	39	62	87	52	77
Tannin	70	39	51	86	50	67

The conclusion arrived at by these observers are (1) that the caffeine in China tea or those containing least tannin is extracted during infusion to a greater extent than the tannin or water extract, and (2) that the tannin extracted is in almost every case lower than the water extract.

In tasting tea, it is usual to infuse the weight of a sixpenny piece (43 grains) of the sample in 3 1/2 fluid ounces of boiling water, and to pour off the infusion after standing from 3 to 5 minutes, according to the practice of the taster. The infusion is not swallowed, and, of course, no sugar or milk is added. In the process of manufacture, the different sized leaves are separated by sifting, and thus broken leaves and dust are obtained, which, though yielding a strong infusion, will be sold at a lower rate. Broken or powdered tea loses its aroma more rapidly than whole-leaf tea. Hence, in judging of the commercial value of a tea, the appearance of the leaf and extent to which it is damaged are taken into account as well as the characters of the infusion. The infusion is judged by its strength or astringency, its flavor, its colour, and its odour. The strength and flavour are dependent on the age, and consequently the size of the leaf, and the time the tea has been kept since its manufacture. A chemical analysis will indicate the strength, but not the flavour of the infusion, and hence is of little use in the valuation of high-priced teas; but as in medium and low-priced teas the strength is of as great or more importance than the flavour, a chemical analysis will, in such cases, go far to indicate the commercial value of the tea. The opinion formed of a tea by a professional taster is sometimes very different from that to which a chemical examination would lead.

In 1874, Allen submitted to two tea-tasters of considerable experience a series of samples which he had specially prepared to test their ability to recognise adulterations of tea by the taste. The following were the opinions expressed:

Nature of sample	A's opinion	B's opinion
No 1 70% of No. 2 and 30% exhausted and redried leaves	Tasted "washed-out," no doubt from presence of exhausted leaves	Very poor; contained many exhausted leaves, ranked *fifth*
No 2 Genuine black tea of fair quality	Genuine	Passed pure; ranked *first*
No. 3 No 2 somewhat crushed.	Mixed with exhausted leaves	Would have been the best, but lacks strength, and is therefore suggestive of exhausted leaves. Ranked *third*
No. 4. 80% of No 2 and 20% of exhausted leaves, to which a little Na_2CO_3 was added while redrying	Genuine; better tea than No 3.	Not pure, but very slightly adulterated with exhausted leaves. Ranked *fourth*.
No. 5. 80% of No 2, 20% of exhausted leaves, and a little catechu	A washed-out tea to which some astringent matter had been added.	Passed pure, and ranked *second*.

It is comparatively unusual for unmixed tea of any kind to be sold retail. Blending of several kinds is very generally practised, and when conducted judiciously materially improves the character of the tea.

Previously infused or exhausted leaves are among the adulterations of tea most difficult to detect, especially when present only in moderate proportion. The sophistication of tea in this manner was formerly extensively practised in England, the exhausted leaves being treated with gum or other matters, and rolled and redried so as to resemble genuine tea.

The treatment of tea with hot water necessarily results in the removal of certain of the ash-constituents, especially the potassium salts of organic acids. Hence the exhausted leaves will contain a smaller proportion of total ash, and especially of ash soluble in water. The extent of the change produced by infusion will, of course, depend on the perfection of the exhaustion. Allen found in a mixture of infused leaves from various teas 4.30% of total ash, of which 0.52% was soluble in water. James Bell (*Foods*, 1, 29) gives the following figures obtained by the analysis of the ash of tea leaves which had been infused in the ordinary way for domestic use, and afterward redried at 100°:

Description of tea	Ash of sample			
	Total, %	Siliceous matters, %	Soluble in water, %	Alkalinity, as K_2O, %
Congou	3 92	0 41	0 54	0 11
Moning	4 53	0 95	0 85	0 28
Orange Pekoe	3 77	0 57	0 68	0 18
Hyson	5 56	1 40	0 76	0 21
Souchong	4 12	0 70	0 81	0 19

The total ash of the foregoing samples averages 4.38%, and the soluble ash 0.73%.

Exhausted tea leaves are also indicated by the deficient extract (and consequently high insoluble matter) and low proportion of tannin. As already stated, the yield of extract depends materially on the condition of the tea, more complete extraction of the soluble matters being effected when the powdered tea is used than when the exhaustion is effected on the leaves in their commercial condition. For the purpose of detecting adulteration, the powdered tea should always be used, or the results will not be fairly comparable.

Essential oil is estimated by distilling a considerable quantity of tea (200 grm.) with 1,500 c.c. of water, and agitating the distillate with ether. On distilling off the ether the tea oil remains. Eder found 0.52% of oil in *gunpowder* and 0.41% in *pekoe bloom* tea by this process. Battershall employed 10 grm. of tea, and saturated the distillate with calcium chloride before agitating ether. A good sample of black tea yielded 0.87% of volatile oil when examined by this method.

The process recommended by the A. O. A. C. is to distil 100 grm. of tea with 800 c.c. of water, extract the distillate several times with light petroleum, evaporate the combined extracts at room temperature, dry in a desiccator and weigh.

Tea oil is a bright yellow liquid, which darkens and resinifies on exposure to the air for a few days, and turns reddish-brown with nitric acid. Even on exposing the aqueous distillate from tea to the air for some time, it loses its aromatic odour, and little or no oil can then be separated from it by ether, and even if the distillate be kept in closed vessels the aroma is soon lost. This explains the fact that tea leaves lose their bouquet by age or exposure.

The nitrogenous substances other than caffeine are calculated from a determination of the total nitrogen by Kjeldahl's method. The nitrogen due to the caffeine found to be present is deducted and the difference, multiplied by 6.25, is taken as proteins. Of the nature of the nitrogenous substances peculiar to tea little is known definitely, and from the point of view of the use of the tea as a beverage, or the detection of adulterants, is of no great value.

Eder found that of the aqueous extract of tea 15 to 16% was precipitated by strong alcohol. A nitrogen estimation in the precipitate gave 12% of proteins and the difference was taken to be gummy matter. According to Eder there is present in tea on an average about 13% of nitrogenous matter insoluble in water. Kozai gave 37 to 39% of crude protein in Japanese teas (calculated on the dry tea) and König's figures show from 18 to 39%.

Kellner (*Landw. Versuchs-Stat.*, 1887, **33**, 370) used a modification of Stutzer's process for the estimation of total proteins. The aqueous decoction of 2 grm. of tea in 100 c.c. of water was treated with 20 c.c. of a 10% solution of cupric sulphate and to this was added standard aqueous sodium hydroxide in such quantity as to leave some copper in solution. The liquid filtered rapidly and was free from proteins The precipitate was washed with hot water and

finally with alcohol. The nitrogen in the dried precipitate was then estimated by ignition with soda lime.

Chlorophyll, resin and wax may be estimated by extraction with ether of the dried residue from the aqueous "extract." It is desirable to extract the ether residue with alcohol, evaporate the alcoholic solution to dryness and extract the resinous mass with benzene. The benzene solution is evaporated to dryness together with the original ethereal extract and treated with hot water to remove any substance which escaped extraction in the first instance.

When either green or black tea is boiled with alcohol or chloroform a solution of a more or less grass-green colour is obtained, owing to the extraction of chlorophyll. E. B. Kenrick states that cheap black teas yield less chlorophyll than the better kinds, and believes that a distinction of practical value might probably be based on a colorimetric estimation.

Gummy matters[1] are estimated by concentrating the aqueous decoction almost to a syrup, treating with excess of alcohol, filtering and washing the resulting precipitate with alcohol. The precipitate is washed off the filter with hot water, and the solution evaporated to dryness at 100°. The organic matter is estimated by the loss on ignition and may be regarded as gum. If more accurate results are required the nitrogen should be determined and the calculated protein deducted from the total organic matter.

Crude fibre may be estimated by the method described in Vol. I., page 70.

Adulterations of Tea.

Before the passing of the Adulteration of Food Act of 1872, tea was subject to adulterations of the grossest kind, most of which were practised prior to importation. By the Sale of Food and Drugs Act of 1875, provision was made for the examination of tea by the Custom House, and the exportation or destruction of very bad parcels. By sections 5 of 11 George I. cap. 30, the adulteration of tea by terra japonica (catechu), leaves other than leaves of tea, or any other ingredients whatever, was punishable by forfeiture and a fine of £100. By section 11 of 4 George II. cap. 14, a penalty of £10 was imposed for

[1] Maurenbrecker and Tollens (*Ber*, 1906, 39, 3581) have examined Java tea for carbohydrates and they obtained arabinose, d-galactose and glucose. They also found 5·6% of pentosans, calculated from the furfural obtained on distillation with hydrochloric acid.

the sale of every pound of tea which was mixed, coloured, stained, or dyed with terra japonica, sugar, molasses, clay, logwood, or with any other ingredients or materials whatsoever. Hence the tea now sold in the United Kingdom is rarely adulterated in the gross manner which was formerly common. This statement does not apply to all countries. In 1888, Wenda and Wiorogorski described various adulterations they had met with in tea sold in Warsaw. Bukowski and Aleksandrow in the same year found as much as 40% of ash in tea, and a considerable proportion of brass-filings in one sample of tea sold in Russia.

The adulterants of tea may be conveniently arranged under the following four heads: 1. Mineral additions used for increasing weight or bulk; such as sand, magnetic iron ore, brass filings. 2. Organic additions used for increasing weight or bulk; such as previously infused leaves, and leaves other than those of the tea plant, as sloe, elder, willow, etc. 3. Adulterants used for imparting fictitious strength, by increasing the astringency or deepening the colour of the infusion; as catechu, sodium carbonate, borax. 4. Facings and colouring materials; as steatite, prussian blue, indigo, turmeric, graphite, etc.

The practice of facing tea, formerly very common, is now confined to certain kinds of green tea, especially gunpowder, and the mineral additions for increasing weight or bulk no longer include (so far as the United Kingdom is concerned) considerable proportions of magnetic iron ore, etc., as was formerly the case.

The mineral adulterants and mineral pigments can be detected in the ash. The tea may be shaken up with a large volume of water and the water separated from the leaves by a sieve, when the insoluble mineral substances used in facing will settle and can be removed by filtration for further examination. Catechu and other soluble substances remain in the filtrate.

Logwood is mentioned by Eder as an adulterant of tea. To detect it, he steeps the tea in cold water. If logwood be present, the resultant solution is changed to a bright green on adding a little sulphuric acid, and to blackish-blue by a solution of neutral potassium chromate.

Facings and colouring materials were formerly almost invariably present in green tea, the object being to impart a hue demanded by custom but not naturally possessed by the leaf. Colouring matters have been extensively employed for transforming black tea of low quality into superior green. The teas consumed by the Chinese and Japanese themselves are not faced. According to Y. Kozai the

maximum proportion of facing in the green tea of Japan is about 0.4%.

If a faced tea be examined under the microscope as an opaque object, the nature of the facing materials may often be recognised. On treating a faced tea with warm water, the colouring matters become detached, and the small portions rising to the surface may be floated on to a glass slide and at once examined under a microscope, while the bulk of the facing is obtained as a sediment when the strained liquid is allowed to stand. This deposit often has a distinctly greenish colour from the presence of prussian blue or indigo. Indigo may be recognised by its behaviour with nitric acid. Prussian blue is best detected by warming the sediment with alkali hydroxide, filtering, strongly acidifying the filtrate with hydrochloric acid, filtering again if necessary, and testing the clear liquid for ferrocyanide with ferric chloride. On treating the sediment with the alkali it is sure to turn brown, but this change must not be regarded as an indication of the presence of prussian blue. The residue left after treatment with the alkali hydroxide should be treated with hydrochloric acid, when the insoluble portion will usually consist of *steatite* or other *magnesian silicate*, the use of which gives the tea a peculiar smooth appearance and slippery feel. *Calcium sulphate* is often employed for facing tea. Caper tea is often glazed with *graphite*. *Turmeric* has been detected by some observers, but in Allen's experience the yellow colouring matter has generally been of a ferruginous nature.

Foreign leaves in tea are legitimately present in small proportion (1 to 3%) to impart bouquet, but larger admixtures can only be regarded as due to adulteration. As a rule, the odoriferous leaves are not allowed to remain in the tea, but having imparted their characteristic fragrance to the tea are removed previously to packing.

For the detection of stems, dust and foreign leaves place 1 grm. of the tea in a 300 c.c. dish and boil with 200 c.c. of water for 15 minutes. This will cause the leaves to unroll and a megascopic examination should reveal the presence or absence of stems and dust, while the leaves will be in a condition for examination as to their form and structure (A.O.A.C.). *Sloe*, *elder*, and *willow* leaves have been formerly met with in England as adulterants of tea. Among the leaves added abroad, and stopped by the Customs, are those of *Chloranthus inconspicuus*, *Camellia sasanqua*, *Eurya Chinensis*, and *sloe*. In 1888 Wenda and Wiorogorski found in the teas sold in Warsaw various foreign leaves,

which they identified by their anatomical characters. Among the leaves recognized were those of *Epilobium angustifolium*, or French willow-herb, which formed the great part of the "tea" sold in certain localities. They also found the leaves of *Epilobium hirsutum* (great willow-herb), *Ulmus campestris* (elm), *Prunus spinosa* (sloe), *Fragaria vesca* (strawberry), *Fraxinus excelsior* (ash), *Sambucus nigra* (elder), *Rosa canina* (dog-rose), and *Ribes nigrum* (black currant) (see plates). The infusion of willow-herb is darker than that of tea, and gives a precipitate of mucilage on treatment with alcohol. An article known in Russia as "Karpar tea" also contains an admixture of the leaves of *Epilobium angustifolium*. Two samples examined by J. Nikitinsky in 1885 yielded 7.87 and 10.43% of ash, 6 representative genuine teas yielding from 5.60 to 6.87%. In the recognition of foreign leaves in tea, chemistry cannot be expected to play a very active part, though it sometimes affords very useful indications. Thus A. Wynter Blyth has pointed out (*Analyst*, 1878, **2**, 39) that a crystalline sublimate (which he believes to be caffeine) is obtainable from a single leaf of tea. For this purpose he boils the leaf for a minute in a watch-glass with a very little water, adds an equal bulk of calcined magnesia, and evaporates the mixture rapidly to a large drop, which is transferred to a microscopic cover-glass and evaporated neary to dryness on a heated iron plate. It is then covered by a ring of glass, and when the moisture is nearly driven off a second slip of glass is added as a cover. At a somewhat higher temperature caffeine volatilises, and on examining the deposit on the cover under the microscope may be recognised by its characteristic appearance. Other leaves than tea may give a crystalline sublimate, but if no sublimate is obtained the leaf cannot be a product of the tea-plant.

P. Kley (*Rec. trav. chim.*, 1901 [*ii*], **20**, 344) used a similar method for the detection of exhausted leaves in tea.

A. W. Blyth has also proposed to utilise the constant presence of manganese in tea leaves as a means of recognition. If a single tea leaf be ignited in platinum, and the ash taken up in a bead of sodium carbonate contained in a loop of platinum wire, on remelting the flux after a minute addition of nitre the green colour of the sodium manganate will be distinctly recognisable. Or a minute quantity of nitre and carbonate of sodium can be at once added to the ash on the platinum foil, when on fusing the mixture a distinct green colour will be obtained if manganese be present.

The following method for the detection of the manganese in the ash of a single leaf has been successfully used by the writers. The ash is treated with a little diluted nitric acid (sp. gr. 1.2) in a test-tube and cooled. A little sodium bismuthate is added to the cold liquid, and the tube is shaken for 30 seconds. The magenta colour of permanganate solution forms at once with genuine tea ash and is best seen by filtering through ignited asbestos.

Manganese is present in the leaves of *Camellia Thea* (tea), *Camellia Japonica*, *Camellia sasanqua*, *Coffea Arabica*, beech, blackberry, and sycamore, but not in hawthorn, ash, raspberry, cherry, plum, and rose; and only faint traces were detected in the leaves of the *Ilex Paraguayensis*, elm, birch, lime, sloe, elder, willow-herb and willow.

For the detection and identification of foreign leaves in tea, the botanical and microscopical characters are best fitted. Some of the samples to be examined should be put into hot water, and when the leaves have unfolded they are spread out on a glass plate and held up to the light, when, with the aid of a lens, the venation, serration, etc., can be readily observed. A valuable aid to the examination consists in treating the leaves with a solution of sodium hypobromite, or, as suggested by A. Wynter Blyth, a strongly alkaline solution of potassium permanganate. In using the reagent, the leaf should be enclosed between two microscopic cover-glasses, a weight being placed on the upper one to keep it in position. On heating the leaf with the reagent, action at once commences, the colouring matter being first attacked and subsequently the cell-membranes. When the action is sufficiently advanced, the leaf is removed, washed, and immersed in hydrochloric acid, which leaves the leaf as a translucent white membrane in which the details of structure can be readily observed. J. Bell removes the skin of the leaf by immersing it in "water containing a few drops of nitric acid," and gradually heating to the b. p., when the skin rises in blisters, and may be readily removed by a camel's-hair brush.

The primary venation of the tea leaf consists of a series of well-defined loops, which are not met with in most leaves likely to be used as adulterants. The serrations are not mere saw-teeth on the margin of the leaf, but actual hooks; they are very strongly marked on mature leaves, but are indistinct or almost wanting in the delicate leaf-buds which constitute "flowery pekoe." The serration stops short abruptly at some distance from the base. The Assam tea leaf is sometimes

biserrate. At the apex of the tea leaf there is a distinct notch, instead of a point. The epidermis of the under-surface is seen under the microscope to consist of distinct sinuous cells, with numerous oval stomata, and a few, long unicellular hairs. On the upper surface the stomata are less numerous. If the under surface of the tea leaf be examined under the microscope after separation of the cuticle, the peculiar and characteristic space between the twin cells of the stomata

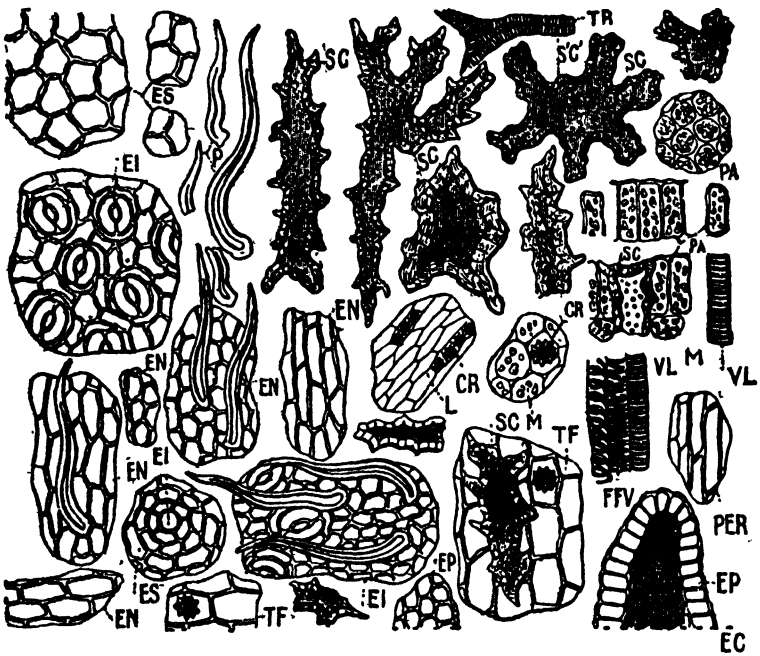


FIG. 3.—Powdered Tea. *cr*, crystals; *ei*, lower epidermis; *en*, neutral epidermis; *ep*, apex of marginal tooth; *es*, upper epidermis; *flv*, débris of fibrovascular bundles; *l*, bast with cluster crystals; *m*, spongy parenchyma; *p*, simple hairs; *pa*, *p'a'*, palisade cells; *per*, pericycle, slightly lignified; *sc*, idioblasts from the mesophyll and cortical tissue; *s'c'*, idioblasts from the pith of the stem; *tf*, tracheids; *vl*, vessel. ×240. (Greenish and Collin.)

may be readily perceived. Tea hairs are conical, pointed, slightly bent toward the base. They have very thick walls, and the central duct usually contains granular matter. Numerous hairs are observable on young tea leaves, but on old leaves they are sometimes wholly wanting.

T. Taylor has pointed out the presence of "stone cells" in the leaves of tea and *Camellia Japonica*, and confirms the observations of Blyth

as to the absence of these formations in the leaves of the willow, sloe, beech, ash, black-currant, raspberry, and *Ilex Paraguayensis*. Taylor prepares the leaves for examination by boiling them in a strong solution of potassium or sodium hydroxide.

It has been pointed out by Tchirch (*Schweiz Wochensch.*, 1905, **43**, 321, and *Pharm. J.*, 1905 [*iv*], **2**, 195) that the idioblasts or "stone cells" are wanting in the leaves of the bud and even in the very youngest leaves they are difficult to find. Tchirch makes a point of the fact that the idioblasts occur more frequently in the oldest leaves and therefore in the least valuable tea.

In the leaf of the blackthorn or sloe (*Prunus communis* or *P. spinosa*) the serratures are direct incisions, numerous, often irregular, and extending to the base. There are no spines. The cells of the epidermis are not sinuous, and are much smaller than those of tea, especially on the under surface. The cells on the upper surface are striated. The stomata of the sloe leaf are smaller and less numerous than those of tea. The hairs are shorter and coarser than those of the tea leaf, are marked in a peculiar manner, and have a club-shaped enlargement at the base. (Plate II, Fig. 26.) A specimen of sloe leaves gathered early in September gave, after drying, the following results (Allen): Moisture, 6.40%; insoluble matter (on whole leaves), 55.90; tannin (by gelatin), 16.00; gum, 8.90; total ash, 8.74; and ash soluble in water, 4.70%.

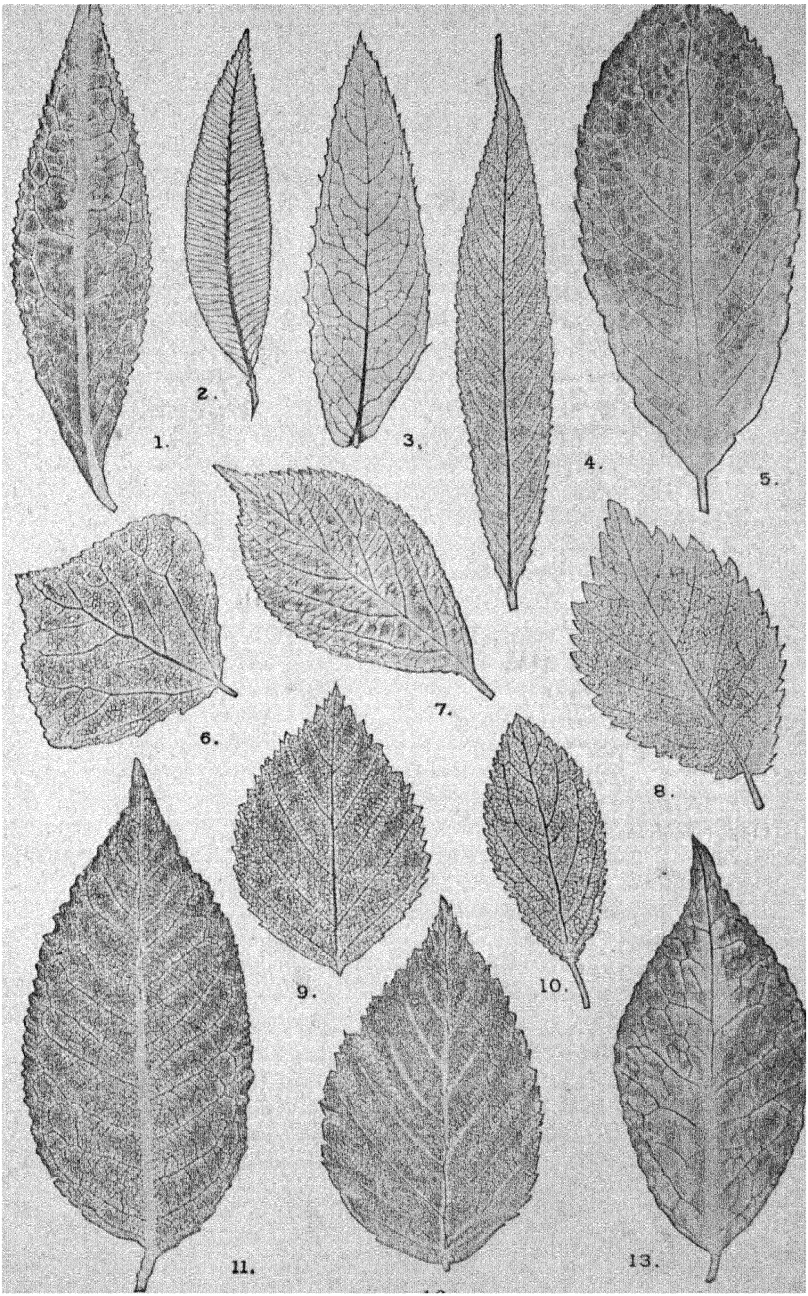
The leaf of the elder (*Sambucus nigra*) is more pointed than that of the tea-plant, and the lobes are unequal at the base. The serrations are direct incisions. The midrib has hairs on it, and on the leaf itself there are two distinct kinds of hairs—one, a short, spinous hair, and the other jointed and club-like. (Plate I, Fig. 7.)

In the leaf of the willow (*Salix alba*) the serrations much resemble those of tea, but the cells of both the upper and under epidermis are much smaller than in tea, and the walls are not sinuous. The hairs, which are very abundant on both sides of the leaf, are long, unicellular and sinuous. The elongated form of the willow-leaf and the character of the venation also distinguish it from tea. (Plate I, Fig. 4.)

The appearance of the leaf of the hawthorn (*Cratægus monogyna* and *C. oxyacantha*) is well known. The cells of the epidermis are mostly quadrilateral, with very sinuous outlines, especially on the under surface. The stomata are oval or nearly round, large, and numerous. (Plate II, Fig. 18.)

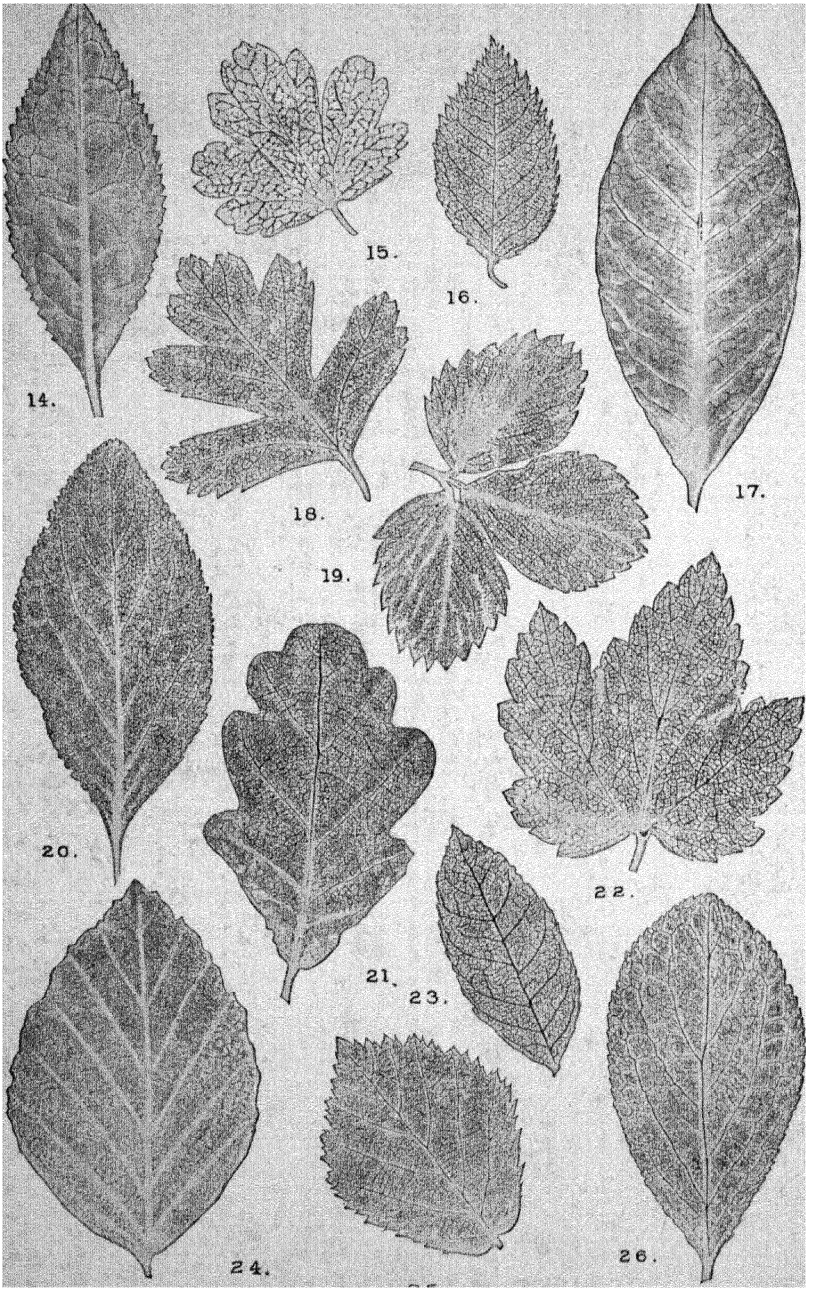
DESCRIPTION OF PLATE I.

1. *Camellia Thea.* Tea.
2. *Marattia Elegans.*
3. *Epilobium Angustifolium.* French Willow or Willow Herb.
4. *Salix Alba.* Willow.
5. *Ilex Paraguayensis.* Paraguay Tea or Brazilian Holly.
6 *Populus Nigra.* Poplar.
7. *Sambucus Nigra.* Elder.
8. *Ulmus Campestris.* Elm.
9. *Betula Alba.* Birch.
10. *Prunus Spinosa.* Sloe or Blackthorn.
11. *Prunus Cerasus.* Cherry.
12. *Rubus Idoeus.* Raspberry.
13. *Camellia Sasanqua.*



12.

PHOTOGRAPHS OF LEAVES.



25.

PHOTOGRAPHS OF LEAVES.

Description of Plate II.

14. *Camellia Thea.* Tea.
15. *Ribes Grossularia.* Gooseberry.
16. *Rosa Canina.* Dog Rose.
17. *Coffea Arabica.* Coffee.
18. *Cratægus Oxyacantha.* Hawthorn.
19. *Fragaria Vesca.* Strawberry.
20. *Pyrus Malus.* Apple.
21. *Quercus Robur.* Oak.
22. *Ribes Nigrum.* Black Currant.
23. *Fraxinus Excelsior.* Ash.
24. *Fagus Sylvatica.* Beech.
25. *Rubus Fructicosus.* Blackberry.
26. *Prunus Communis.* Plum.

The leaves of the beech (*Fagus sylvatica*) are ovate, obscurely dentate, with parallel venations running right to the edge. (Plate II, Fig. 24.)

The leaves of *Chloranthus inconspicuus* are long, oval, serrated, wrinkled, with venations running nearly to the edge, and there by their intersection forming little knots which give the margin of the leaf a very rough feeling. The cells of the epidermis are very large, and the stomata oval and rather numerous.

The leaves of *Camellia sasanqua* are oval, only obscurely serrate if at all, and of a tough leathery texture. The lateral veins are inconspicuous. Both the upper and lower epidermis show a peculiar dotted or reticulated structure, and the lower is studded with numerous small oblong stomata. (Plate I, Fig. 13.)

The leaves of *Lithospermum officinale* (the common gromwell) have been extensively used in Bohemia for adulterating tea. They are lanceolate, with a hairy under-surface, are destitute of alkaloid and essential oil, contain about 9% of fat and 8 of tannin, and leave about 20% of ash on ignition (*J. Chem. Soc.*, 1881, **40**, 131).

The general appearance and venation of tea, and leaves which have been, or may possibly be, employed for its adulteration, are shown in the figures. The illustrations are life-sized reproductions, of photographs of leaves, taken by J. T. Stevenson in Allen's laboratory.

A. Wynter Blyth has pointed out the characteristic appearance of the "skeleton-ash" left on igniting leaves from different sources. The leaf to be examined is placed between two circles of microscopic cover-glass, the upper one weighted with a silver coin, and the whole ignited cautiously in a flat platinum dish, or on platinum foil. Before the carbon is completely consumed the heat is discontinued, and the skeleton-ash examined under the microscope.

Caper Tea.—*Caper* is a name applied to tea which has been made up into small glossy granular masses by the aid of gum or starch. Some years ago the caper tea from the Canton district was invariably adulterated with sandy and magnetic matter, and often with catechu or other extraneous astringents, together with foreign leaves.

Allen was of opinion that caper tea was never genuine and it has been suggested by J. White (*Analyst*, 1899, **24**, 117) that the rounded masses are probably made deliberately so that they may be loaded with mineral adulterants which will remain unobserved by the consumer. This appears to be justified to a certain extent by the fact that the granular masses have sometimes been found to contain a piece of

quartz embedded in the centre; but as caper tea is much in demand among certain classes because of its rough flavour, it must be recognised as a commercial article. The ash of caper tea varies considerably and White found from 6.2 to 7.9%, with not more than 2% insoluble in hydrochloric acid. Some caper teas, which according to White must be considered adulterated, yielded ashes varying from 8.8 to 13.5% and showed from 3.1 to 6.3% insoluble in acid.

Lie tea is the name given to a fraudulent mixture consisting of sweepings and dust of tea and other leaves, mixed with clay, sand, iron ore, etc., and made into irregular masses by means of gum or starch. When put into hot water, lie tea disintegrates and falls to powder. The iodine test for starch may be applied after acidifying the cold liquid with sulphuric acid, and decolourising with permanganate. The ash of lie tea is something as high as 30 to 40%.

The insoluble matter and extract of lie and caper tea are very variable; but the former, exclusive of mineral matter, is usually considerably below the proportion yielded by genuine tea. The gum in caper tea often amounts of 15 or 20%, while the soluble ash is often less than 2%.

The following figures show the results to be expected from the analysis of factitious tea:

	A	B	C
Observer	J Bell	J M Eder	A B Hill
Description	"Mahloo mixture"	Black tea	Green tea
Extract		22 40	37 00
Tannin		19 77 (Catechu detected)	(Catechu detected)
Total ash	9 97	3 07	12 10
Magnetic and sandy matter	4 31		6 00
Soluble ash	1 54	1.12	1 29
Alkalinity, as K_2O	0 17		0 13

The following analyses of samples of spurious tea, received from the U. S. Consuls at Canton and Nagasaki, are by J. P. Battershall (*Food Adulteration*, page 28). No. 1 consisted of partially exhausted and refired leaves known as "*ching suey*" (clear water), a name apparently referring to the character of the infusion. No. 2 was a sample of "lie-tea" made from wampan leaves. No. 3 was a mixture of 10% of green tea with 90% of lie-tea, sometimes sold as "Imperial" or "Gunpowder" tea. No. 4 was a sample of "scented caper," consisting of tea-dust made up into little shot-like pellets by means of "Congou paste" (boiled rice):

	No 1, %	No 2, %	No 3, %	No. 4, %
Insoluble leaf .	70 60	70 55	67 00	60.10
Extract (complete) ..	7 73	14 00	12.76	22 10
Gum . .	10 67	7 30	11 00	11 40
Tannin . .	3 13	8 01	14.50	15.64
Caffeine	0 58	None	0 16	0.12
Ash —Total .	8 62	8 90	7 95	12.58
Soluble in water	0 64	1 86	3 00	3 84
Insoluble in acid	3 92	3 18	1 88	6.60

Certain tea substitutes have been used from time to time mainly on the Continent, but they are all distinguished from genuine tea in being free from caffeine, and are best detected microscopically.

Although the leaves of tea, coffee, and Brazilian holly are almost the only ones known to contain caffeine, a beverage is prepared from the leaves of many other plants in various parts of the world. Thus, *Catha edulis*, a shrub related to the spindle tree, is extensively cultivated in the interior of Arabia, and the leaves, known as Khat, Cafta or Arabian tea, are used both as a beverage and for chewing. Fahum, or orchid tea, is made from the leaves of *Angræcum fragrans*, growing in the Mauritius, and some years since was introduced into Paris as a regular article of commerce. Trillich found 0.2% of coumarin in Fahum tea. Thè Arabe, a substitute for tea which has been sold in Paris, consists of the small leaves of *Paronychia argentea*, a plant growing on the slopes of the Atlas Mountains. Batoum or Trebizond tea is made from the leaves of *Vaccinium arctostaphylos*, a plant closely allied to the cranberry.

Dried leaves which have not been identified have been used in the so-called Hyson and Imperial tea in China. They are destitute of caffeine. A process for the preparation of caffeine-free tea has been patented in Germany.

Commercial tea according to the German standard should fall within the following limits:

Moisture 8 to 12%.

The ash should not exceed 8% of which at least one-half should be soluble in water; the sand (insoluble in hydrochloric acid) should not exceed 1%.

The aqueous extract for green tea must amount to at least 29% and 24% for black tea, both calculated on the dry substance.

Caffeine must not be lower than 1%.

Tannin in green tea, not less than 10% and in black tea not less than 7.5%.

Foreign leaves must be absent.

These limits for caffeine, tannin and aqueous extract are too low, and the upper limit for moisture too high to be considered satisfactory for the tea usually obtained in the British Isles and in America.

Maté, Paraguay Tea.

Maté or Yerba consists of the prepared leaves and twigs of *Ilex Paraguayensis*, or Brazilian holly. Various allied species are recognised, but *Ilex Paraguayensis* appears to be the only one cultivated. It has been grown in Spain, Portugal, and Cape Colony, in addition to its native habitat.

The aroma and quality vary with the time of year when gathered, the leaves possessing most aroma when the fruit is nearly ripe. The twigs are cut from the trees and, after a preliminary drying, are subjected to the action of heat in a torrifier. The leaves are then beaten from the twigs with wooden blades, powdered and packed in cases. A superior product is obtained by drying in large iron pans in the same manner as Chinese tea.

Three different kinds are sold in South America under the following names.

Caá-cuy, the new leaves of the fresh shoots.

Caá-mirim, the leaves freed from the midrib and with twigs and stalks separated.

Caá-guacu or *Yerva de Palos*, the large old leaves with twigs and fragments of wood.

König gives the following as the mean of 15 analyses of maté:

Water,	6.92%
Ash,	5.58%
Nitrogenous substances,	11.20%
Caffeine,	0.89%
Fat (ether soluble),	4.19%
Tannin,	6.89%
Nitrogen-free extractive matter and cellulose,	64.33%
Water extract,	33.90%
Alcohol extract,	33.51%

The caffeine in the foregoing ranged from 0.30 to 1.85%.

Byasson found in caá-guacu, the commonest kind of maté, Caffeine, 1.85%; a substance resembling birdlime, fatty and colouring matters, 3.87; complex glucoside, 2.38; resin, 0.63; mineral matter, 3.92; and an undetermined proportion of malic acid.

Some fresh leaves of *Ilex Paraguayensis*, grown in Cambridge Botanical Gardens, were found in Allen's laboratory to contain 69.1% of water. An analysis of the same leaves after drying at 100° showed: Insoluble matter, 57.94 (=hot-water extract, 42.06); tannin by PbA_2, 15.62; tannin by CuA_2, 15.66; caffeine, 1.13; total ash, 6.14; soluble ash, 3.56; alkalinity of soluble ash (as K_2O), 0.12%.

A. W. Hofmann found in maté 0.3% of caffeine and a variety of tannin identical in every respect with that present in tea. P. N. Arata found the tannin of maté to be analogous to but not identical with that of coffee. On dry distillation he found it to yield resorcinol as well as catechol. Soubeiran and Delondre state that maté contains the same essential constituents as the coffee leaf, and in greater proportion than the coffee seeds. This conclusion is confirmed by Theodore Peckolt in a valuable resumé of the subject (*Pharm. J*, 1883 [*iii*], **14**, 121), including some elaborate proximate analyses of maté.

The aromatic principle of maté has not been isolated, but by dry distillation a volatile oil of phenolic character is obtained.

The *ash* of maté resembles that of tea in containing a notable proportion of manganese.

The leaves of the Yopon (*Ilex cassine*), a shrub or small tree growing on the coast of Virginia and Carolina, have been used as a beverage. F. P. Venable (*Chem. News*, 1885, **52**, 172) found in an air-dried sample: Moisture, 13.19; water extract, 26.55; tannin, 7.39; caffeine, 0.27; and ash, 5.75%. The ash contained manganese.

COFFEE.

Commercial coffee consists of the seeds of *Coffea Arabica* and allied species belonging to the order *Cinchonaceæ*.

Three species of *Coffea*, distinct from each other, are now grown: 1. The *Arabian* or Mocha coffee-plant has short upright branches, with a brittle leaf and seeds usually single in the berries. 2. The *Jamaica* coffee-plant bears longer and more pliable branches than the Arabian, has a tougher leaf, and the seeds are almost always double in

the berries. 3. The *East Indian* or Bengal plant has smaller leaves than the Jamaica coffee, and very small berries. The Liberian coffee-plant (*Coffea Liberiea*) appears to be a distinct species, which is little subject to disease, and has been successfully introduced into the East Indies.

The coffee fruit usually, but not always (see above), contains two twin seeds, which touch each other on the flattened surface. These are contained in a pulp which is removed by water and a process of fermentation; and the membranous pericarp (technically termed "parchment") which incloses each seed is removed by rollers and winnowing. According to Gorter (*Annalen*, 1910, **372**, 237) lactic acid is a product of this fermentation, causing the slimy layer to swell and so to be easily removed.

The *parchment* from coffee-berries is imported to England in considerable quantities, and, when roasted, is said to form an ingredient of the beverage sold in cheap coffee-shops.

An analysis of unroasted "parchment," made by C. M. Caines, showed it to contain: Water, 9.43; essential oil, 0.068; caffeine, 0.27; hot-water extract, 1.61; total ash, 10.41; and soluble ash, 0.19%. A somewhat coffee-like aroma was developed by roasting. The flowers from 20 year-old trees were examined by Graf and found to contain 0.9% caffeine, a reducing sugar, caffetannic acid and phytosterol.

It is stated that the Arabs in the neighbourhood of Jedda discard the kernel of the coffee-berries and make an infusion of the husks (*Pharm. J.*, 1886 [*iii*], **17**, 656).

The coffee-tree is a shrub-like plant cultivated in various tropical countries. The best coffee that reaches England comes form India, Java, and Ceylon. A little "Mocha" coffee comes from Arabia, but the greater part from India. Brazil at the present time furnishes more than one-half of the world's supply of coffee.

The composition of coffee has been given by different observers and a few of the results are shown in the following table:

There has been some diversity of opinion as regards the *sugar* of coffee. Bell believed the sugar to consist of a peculiar species allied to melezitose, but G. L. Spencer definitely proved the presence of sucrose in coffee. Graf showed that the coffee berries contain no dextrose or reducing sugars in the free state; but a methyl-alcoholic extract yielded a large amount of sucrose.

Ewell made an exhaustive study of the sugar in coffee (*Amer. Chem.*

COMPOSITION OF COFFEE.

Observer	Moisture	Nitrogenous matter	Caffeine	Fat	Sugar	Dextrin	Tannin and caffetannic acid	Nitrogen-free substances	Crude fibre	Ash	Aqueous extract
Commaille[1]	6 3 to 15.7	2.6	0 4 to 1.3	12.7	2.6					3 9	
O. Levesie[2]			0.6 to 1 5	14.8 to 21.8			19 5 to 23.7	20 6 to 27.4	29 9 to 36 4	3 8 to 4.9	
J. Bell:[3]											
Mocha raw	9 0	9 9	1 1	12 6	9.6	0.9	8.5		38 0	3 7	
Mocha roasted	0 6	11 2	0.8	13 6	0 4	1.2	4 7		48 6	4 6	
East Indian raw	9 6	11 2	1 1	11 8	8 9	0 8	9 6		38 6	4 0	
East Indian roasted	1.1	13 1	1 1	13 4	0 4	1 4	4 5		47 4	4 9	
König[4]											
Raw	10 7	12 6	1 1	11.8	7 6	0 9	9 0	20.3	24 0	3.0	30.8
Roasted	2 4	14.1	1.2	13.9	1 3	1 3	4 6	39 9	18 1	4 7	28 7
Tatlock and Thomson.[5]											
Costa raw	8 2		1.2	14 3						3 8	30 8
Costa roasted	5 5		1.2	12.5						3 8	30.3
Costa roasted	2.2		1 4	13 0						4.2	30.7
Mysore raw	10 9		1 2	11.9						3.9	31 0
Mysore roasted	6 7		1 3	12.0						4.1	29.1
East Indian roasted	3 1		1 5	12 9						4.3	29 1
Mocha	5 7		1 2	14 0						4.0	30 8
Caffeine-freed coffee	1 2		0 1	13 1						4.3	27 4

[1] *Mon. Scient.*, 1876 [*iii*], 6, 779 Mean of 24 Mysore raw coffees. Nitrogenous substances given as legumen-casein and protein, sugar given as glucose.

[2] *Arch Pharm.*, 1876 [*iii*], 8, 294. Mean of 7 raw coffees. The non-nitrogenous matter is taken as gummy matter and cellulose is reckoned as crude fibre.

[3] *Foods*, 1, 43. Bell also gives an alcoholic extract of 6 9 and 4 3 for the raw and 14.1 and 12 7 for the roasted coffee.

[4] 30 raw and roasted coffees by various analysts.

[5] *J. Soc. Chem. Ind.*, 1910, 29, 138 All figures calculated on the dry coffee Tannin was found in raw Costa to the extent of 2.8%; sugar calculated to dextrose 0 6% in roasted coffee.

J., 1892, **14**, 473); he found 6% of sucrose in the fat-free coffee extractable by 70% alcohol; the insoluble matter after acid hydrolysis gave galactose. Distillation with hydrochloric acid yielded furfural equivalent to 9% of pentose. Ewell also obtained a gummy substance which on hydrolysis gave rise to a reducing sugar, furfural, and mucic acid resulted on oxidation. From this Ewell concluded that the gummy substance was a compound of pentose and galactose.

Schultze and Maxwell state that raw coffee contains galactan, mannan and pentosans, the latter present to the extent of 5% in raw and 3% in roasted coffee. Hehner and Skertchley (*Analyst*, 1899, **23**, 178) have utilised the production of furfural on distillation with hydrochloric acid to estimate the pentosans in coffee.

Baker (*Analyst*, 1902, **26**, 116) found that manno-arabinose or manno-xylose formed one of the important constituents of coffee berry substance and yielded mannose on hydrolysis. He pointed out that the latter substance could readily be detected by the formation of the hydrazone (Baker and Pope, *Trans.*, 1900, **77**, 704).

Caffetannic acid, called by Payen chlorogenic acid, exists in coffee-berries in the proportion of 3 to 5%, as a double caffetannate of potassium and caffeine (Payen). It is prepared by diluting an alcoholic infusion of coffee with water, filtering from precipitated fatty matter, and precipitating the boiling filtrate with lead acetate. On decomposing the washed precipitate with hydrogen sulphide free caffetannic acid is obtained. It forms a yellowish-white powder, or groups of colourless mammillated crystals. It is very soluble in water, less soluble in alcohol, and only very sparingly in ether. Caffetannic acid has an astringent taste, and the solution reddens litmus. It gives a dark green colouration with ferric chloride, and precipitates the sulphates of quinine and cinchonine. It reduces silver nitrate on heating, forming a metallic mirror. The salts turn green in the air.

Tatlock and Thompson (*J. Soc. Chem. Ind.*, 1910, **29**, 138) are of opinion that roasted coffee contains no tannin, and that the lead precipitate contains colouring matter. They found 4.5% of tannin (precipitable by gelatin or alkaloids) in raw coffee.

The tannic acid of coffee was formerly supposed to be a glucoside which on prolonged boiling with alkali hydroxides yielded a reducing sugar and caffeic acid. Graf (*Zeitsch. angew. Chem.*, 1901, **14**, 1077) failed to obtain an osazone of this sugar after either acid or alkali hydrolysis, and showed that the reducing substance was precipitated

by lead acetate; from these facts Graf concluded that caffetannic acid was not a glucoside.

The recent work of K. Gorter (*Annalen*, 1908, **358**, 327; **359**, 217; 1910, **372**, 237; 1911, **379**, 110) shows that caffetannic acid is a mixture of chlorogenic acid with caffalic acid and other substances. *Chlorogenic acid*, $C_{32}H_{38}O_{19}$, can be purified through its calcium salt and then crystallises in needles m. p. 208°. It acts as a dibasic acid and on hydrolysis with alkali hydroxides yields caffeic (3–4 dehydroxycinnamic) and quinic acids. Chlorogenic acid gives a characteristic colour reaction and has by this means been detected in other seeds. Charaux (*J. Pharm. Chim.*, 1910, [*vii*], **2**, 292) has determined the chlorogenic acid in coffee and various other plants by hydrolysis and separation of the caffeic acid obtained. *Coffalic acid*, $C_{34}H_{54}O_{15}$ is obtained in the form of prisms m. p. 255°, having a sweet taste. It yields isovaleric acid on boiling with alkali hydroxides.

The caffeine is stated to exist in Liberian coffee in combination with chlorogenic acid as potassium caffeine chlorogenate, $C_{32}H_{36}O_{19}K_2$-$(C_8H_{10}O_2N_4)_2,2H_2O$, which Gorter isolated in colourless prisms. The compound is soluble in water but the caffeine cannot be extracted from the dry crystals with anhydrous solvents whereas when moist the whole of the caffeine can be completely removed. Gorter states that the fact that caffeine cannot be completely removed from coffee with anhydrous organic solvents is due to this combination of the caffeine with chlorogenic acid. Lendrich and Nottbohm (*Zeit. Nahr. Genusm.*, 1909, **17**, 241) on the other hand, maintain that the retention of caffeine is due to adsorption by the tissue of the coffee bean.

On dissolving caffetannic acid in alkali hydroxide or ammonia, and exposing the solution to the air, the liquid acquires a bluish-green colour owing to the formation of the oxidation-product, *viridic acid*, or *viridinic acid*, which is an amorphous brown substance, very soluble in water to form a solution which is turned green by alkalies. It gives a bluish-green precipitate with barium hydroxide sulution and a blue with lead acetate. Viridic acid dissolves in concentrated sulphuric acid to form a crimson solution, which on dilution with water gives a flocculent blue precipitate.

A. Nestler (*Zeit. Nahr. Genussm.*, 1903, **6**, 1032) has made use of the production of the green colour by alkalies to decide whether coffee is present in mixtures or in extracts.

The principal *alkaloid* of coffee is caffeine which occurs to the

extent of 1.0 to 1.3% and is frequently nearer the latter value. It has been shown by Gorter to exist in coffee as potassium caffeine chlorogenate. Paladino (*Gazzetta*, 1895 [*i*], **25**, 104) isolated another base from coffee extract, by boiling with milk of lime, and after-treatment with lead acetate extracting the caffeine by chloroform. The aqueous solution was acidified with sulphuric acid, concentrated, and treated with a solution of potassium-bismuth-iodide, when crystals of the base "*coffearine*" were obtained as a double salt of bismuth and coffearine iodide. Coffearine, $C_{14}H_{16}O_4N_2$, occurs in colourless deliquescent needles melting at 140° and giving a faint alkaline reaction; it forms a *hydrochloride*, $C_{14}H_{16}O_4N_2HCl,H_2O$, melting at 180°. Some doubt was thrown on the occurrence of this alkaloid and it was attributed in part to the action of the lime on the caffeine. Graf repeated Paladino's experiment and obtained the alkaloid even when lime was not used. Coffearine is present in very small amount and is not extracted from the aqueous decoction by chloroform. Forster and Riechelmann also observed a second alkaloid in coffee, in their process of determining caffeine (*Zeitsch. offent. Chem.*, 1897, **3**, 129 and *Chem. Centr.*, 1897, **1**, 1259).

The *fat* of coffee has been studied by Rochleder, who stated that it contains glycerides of palmitic acid and of an acid, $C_{12}H_{24}O_2$. Tretzel found glycerides of palmitic, stearic and oleic acids, and free oleic acid. The fat of roasted coffee contains some dihydroxystearic acid. The following values for the fat of coffee are due to Hilger and Juckenack. (*Forschungsber. Lebens*, 1895, **2**, 223.)

	Acidity (as oleic acid)	Saponification value	Ether number	Iodine number		Reichert-Meissl value	Mol. wt of fatty acid	Refractive index	Glycerol	Unsaponifiable
				Fat	Fatty acid					
Raw...	2 05	157.2	153.2	82.4	89 4	0	282.0	1.4695	9.48	6.87
Roasted	2 79	162 7	157 2	84 0	85 0	0 34	285 1	1.4715	9.39	6.08

Tatlock and Thomson give the following values:

	Raw	Roasted
Sp gr.		.9354
Iodine value	99.0	99.0
Saponification value	179.5	179.5
Unsaponifiable.	5.26	5 51

Meyer and Eckert (*Monatsh.*, 1910, **31**, 1227) have separated a fatty oil and a wax from raw coffee. The oil has the saponification value 160 to 182 and iodine value 90.1 to 91.2. The fatty acids are mainly linoleic, palmitic and carnaubic with small quantities of oleic, daturic and caproic acids. The wax contains carnaubic acid combined with a resin alcohol.

The unsaponifiable matter of coffee fat includes Phytosterol. According to De Negri and Fabris the sp. gr. of the fat at 15° varies from 0.9510 to 0.9525, and the saponification value from 165.1 to 173.4. The R. M. value is given by Spaeth as 1.65 to 1.7.

The nitrogenous constituents of coffee other than caffeine have not been studied to any great extent, but the proteins have been stated to consist of legumin or vegetable casein.

Polstorff (*loc. cit.*) obtained about 0.25% of trigonelline. Gorter confirms the presence of this substance and is of opinion that Paladino's coffeurine is really trigonelline.

Roasting of Coffee.—The roasting of coffee is best carried out at a temperature of from 200 to 250°, and during the process considerable changes occur. Jaeckle (*Zeitsch. Nahr. Genussm.*, 1898, **1**, 457) has shown that caffeine is volatilised to a slight extent and certain other products are produced, such as acetone, furfural, ammonia, trimethylamine, formic and acetic acids. Moriari and Scoccianti (*Gazzetta*, 1895 [*i*], **25**, 115) had, however, stated that when heated to 260° no trimethylamine was formed, but pyridine and its homologues occurred in appreciable quantities. The fat is decomposed to some extent and the free fatty acid is thereby increased. The sugar becomes caramelised and the caffetannic acids lose about half their weight. Hilger and Juckenack state that the losses on roasting are much higher when the coffee is glazed with sugar (*Forschungber. Lebens*, 1897, **4**, 119) on account of the higher temperature required. According to these observers the losses for ordinary coffee are 19.3% total, 9.7% of the original fat and 21.1% of the original caffeine; for "sugar glazed" coffee the total loss is 15.3%, 18.3% of the original fat and 44.3% of the original caffeine.

Herfeldt and Stutzer (*Zeit. angew. Chem.*, 1895, **8**, 469) have published figures showing a large increase in the fat of Santos coffee on roasting; it is probable that the fat of the original raw coffee was incompletely extracted.

During the process of roasting, the aroma of coffee is developed and

the toughness of the beans destroyed, so that subsequent grinding is facilitated. If the roasting be insufficient, the rawness is not destroyed and the flavour not fully developed; while if over-roasted, the product has a nauseous empyreumatic flavour.

When roasted to a yellowish-brown, coffee loses, according to Cadet, about 12.5% of its weight, and in this state is difficult to grind. When roasted to a chestnut-brown is loses 18%, and when is becomes entirely black, though not all carbonised, it has lost 23%. In practice, the loss of weight in roasting coffee is between 12 and 20% (of which about 8% represents water removable at 100°), and if the latter figure is reached, the product is injured. According to Watson Will, the usual yield of roasted coffee is about 98 lb. from 1 cwt. of raw berries. This corresponds to a loss of 12.5%.

König found that on roasting coffee-berries to a light brown the total loss of weight was 17.77%, of which 8.66 was water and 9.11% organic matter. The original coffee contained 11.19% of moisture, and after roasting, still retained 3.19%.

According to Paul and Cownley (*Pharm. J.*, 1886 [*iii*], **17**, 655, 821) there is no appreciable loss of caffeine by volatilisation during the roasting of coffee, unless the process is carried to excess. But Paul admits that the water condensed in the place leading from the roasting often contains some caffeine, which he considers has been carried over mechanically. Watson Will (*ibid.*, page 684) states that he has never failed to find caffeine in the sublimate obtained in coffee-roasting.

The chemistry of the roasting of coffee has also been studied by O. Bernheimer (*Monatsh.*, 1880, **1**, 456), who roasted coffee till it had lost about 25% of its weight. 50 kilogrm. of coffee yielded 5 litres of aqueous distillate and 680 grm. of solid matter floating thereon. On agitating this with ether, fatty acids, quinol and caffeol were extracted, while caffeine, acetic acid, methylamine and trimethylamine remained in the aqueous liquid. On evaporating the ethereal solution, and fractionally distilling the residual dark, coffee-smelling oil, a few drops of an acetone-like liquid passed over, followed by a little acetic acid and water. Between 200° and 300° *caffeol* distilled, and above that temperature palmitic and other solid fatty acids. On neutralising these and the 200–300° fraction with sodium carbonate, a viscid dark oil was thrown down The uncondensable vapours consisted chiefly of carbon dioxide, and by passing them through dilute hydrochloric

acid a resinous substance having the appearance of pyrrol-red was deposited.

It should be pointed out, however, that Jaeckle was unable to detect Bernheimer's caffeol.

Caffeol, $C_8H_{10}O_2$, according to Bernheimer, is an oily liquid smelling very strongly of coffee. It boils at 196°, and is not solidified by a freezing mixture. It is not sensibly soluble in cold water, to which, however, it imparts its characteristic odour. It is slightly soluble in hot water, very slightly in aqueous potassium hydroxide, and with great facility in alcohol and ether. The alcoholic solution gives with ferric chloride a red colouration, said not to be destroyed on adding sodium carbonate. By fusion with potassium hydroxide, caffeol yields salicylic acid, and, according to Botsch (*Monatsh.*, 1880, 1, 621) is isomeric with methyl-salicyl alcohol, the two compounds having the following constitution:

$C_6H_4(O.CH_3).CH_2OH$ — Methyl-salicyl alcohol.

$C_6H_4(OH).CH_2.OCH$ — Caffeol.

A valuable contribution to the knowledge of the volatile oil of coffee was made by Erdmann (*Ber.*, 1902, **35**, 1846). Roasted coffee was heated with superheated steam, whereby Erdmann obtained 0.06% of a yellowish-brown oil having a powerful odour of roasted coffee. The oil possessed the sp. gr. 1.0844 at 16° and contained nitrogen. By treatment with sodium hydroxide solution it was separated into 42% of acid substances and 58% of non-acid material. The acid portion contained acids of the nature of methylbutyric acid and phenolic compounds were also found; furfuryl alcohol occurred to a large extent in the non-acid portion. The characteristic odouriferous substance of the oil boiled at 93° at 13 mm. and contained 9.7% of nitrogen. The yield of this last was only 0.89 grm. from 65 kilos of coffee and it possessed the odour of roasted coffee to a marked degree. Erdmann further found that the aroma of coffee was produced when caffeine, sucrose, and caffetannic acids are heated together and not if any of the three are absent.

The dietetic value of coffee is possibly dependent as much upon the presence of caffeol as on that of caffeine. According to M. Fargas the effect of caffeol on the heart's action is the opposite to that of caffeine, and increases the strength and rapidity of the pulsations.

Katz states that 85–95% of the caffeine is extracted by boiling water and a cup holding 150 c.c. contains about 1.5 grains of caffeine.

According to Couty, Guimaraes, and Niobey (*Compt. Rend.*, 1884, **99**, 85) coffee diminishes the activity of the simple combustions which produce carbon dioxide, but increases the formation and excretion of urea, and the assimilation of meat and other nitrogenous foods. It is a complex aliment which renders the organism capable of consuming and destroying larger quantities of nitrogenous substances, and hence may be regarded as an indirect source of available energy.

Coffee-berries vary considerably in *size* and character according to their origin. West Indian coffee-berries are regular in size, pale yellowish, firm and heavy, with a fine aroma, and they lose comparatively little on roasting. Brazilian coffee is larger, less solid, greenish or white, and usually classed as "low" or "low middling." Javanese coffee-berries are smaller, slightly elongated, light, and deficient in aroma and essential oil. When new, Java coffee is pale yellow, and of less value than when old and brown. The deeper colour is due to curing as well as age. It has been artificially coloured. Ceylon produces all descriptions of coffee, but the ordinary plantation coffees are even-coloured, slightly canoe-shaped, strong in aroma and flavour, heavy, and more susceptible of adulteration than the other kinds. Genuine Mocha coffee is small and dark yellow in colour, and considered of the highest quality. The following table shows the number of seeds required to fill a 50 c.c. measure (Thorpe's *Dict. Applied Chem.*, 1, 578):

Fine brown Java	187	Good ordinary Java	223
Fine Mysore	198	Fine Ceylon plantation.	225
Fine Neilgherry. .	203	Good average Rio . .	236
Costa Rica . .	203	Medium Ceylon plantation .	238
Good ordinary Guatemala	207	Manilla	248
Good La Guayro	210	Ordinary Mocha . .	270
Good average Santos	213	West African	313
Fine long-berry Mocha . .	217		

Analysis of Coffee and Coffee Mixtures.

The analysis of raw coffee beans is not usually required, as any factitious beans would be detected readily and it is doubtful whether such coffee is on the market. The following details for the analysis therefore apply to roasted coffee only.

Whole Beans.—Examine megascopically in order to detect foreign substances. Artificial coffee beans are apparent from their exact regularity of form. Coffee pellets made from roasted wheat mash are of brown colour, possessing a very characteristic ellipsoidal form (A. O. A. C.).

Detection of Sugar Glazing.—None of the proposed methods gives sugar alone; but two of them are fairly satisfactory. Stutzer recommends shaking 10 grm. of coffee with 250 c.c. of cold water for 5 minutes, diluting to 500 c.c. and decanting at once. The aqueous extract is filtered and the solids determined by evaporation of an aliquot portion and drying at 100°. (A.O.A.C.)

Hilger's process (*Zeitsch. anal. Chem.*, 1897, **36**, 226) is more satisfactory, according to Fresenius and Grünhut, and is, shortly, as follows: 10 grm. of coffee beans are digested 3 times for half an hour with quantities of 100 c.c. of cold alcohol (1 volume 90% alcohol to 1 water). The alcohol is decanted each time, made up to 500 c.c. and filtered. An aliquot portion is evaporated to dryness, weighed and the ash deducted. To allow for the action of the solvent on the beans, deduct 0.83 for each 100 parts of dry coffee. The difference may be taken as due to caramel.

The detection of glycerol in coffee berries is not altogether satisfactory; but may be found in the solutions obtained in the test for caramel. A portion of the solution is evaporated to about 10 c.c., treated with 5 grm. of sand and 3 c.c. of milk of lime (15% CaO) and evaporated almost to dryness. The moist residue is then treated with 50 c.c. of 90% alcohol (by volume) worked into a smooth paste, heated on the water-bath to incipient boiling, decanted through a filter and repeatedly washed by decantation with small quantities of hot 90% alcohol until the filtrate amounts to 150 c.c. The filtrate is evaporated to a syrup at a temperature below 90°, washed into a stoppered cylinder with 20 c.c. absolute alcohol and 3 successive quantities of 10 c.c. of ether added, shaking after each addition. When clear the alcohol-ether solution is filtered off, the cylinder and filter washed with a mixture of absolute alcohol and ether (2 to 3), the filtrate evaporated, and dried for 1 hour at 100°. The weight, after deducting the ash, is taken as glycerol. This process will include any other organic matter which is soluble in the solvents. Grünhut (*Zeit. anal. Chem.*, 1899, **38**, 37) has pointed out that although the alcohol-ether residue may yield oxalic acid on oxidation, yet on distillation with potassium hydrogen sulphate no acrylic aldehyde resulted, indicating that some substance other than glycerol was present.

Facing materials such as *fat*, *paraffin*, *vaseline* and *waxes* may be detected by the method of Späth (*Forschungsber. Lebens*, 1895, **2**, 223). Extract 100 to 200 grm. of the beans with light petroleum for 10 min-

utes. Decant the petroleum spirit and repeat the process a few times. Evaporate the petroleum extract to dryness; digest with warm water; extract the fatty matter with light petroleum, filter and evaporate to dryness. Examine the residual fatty matter by the usual methods for fats and waxes. For *shellac* and *resin*, shake the beans with absolute alcohol for about 10 minutes; evaporate to dryness in a dish and ignite the residue; the characteristic resinous odour on burning indicates the adulterant. It should be noticed that the Liebermann-Storch reaction for resin cannot be applied directly as coffee-berry oil itself answers to the reaction. The resin should be separated from the ethereal solution of the oil by means of sodium hydroxide solution. *Dextrin* is obtained after applying the test for resin by testing the beans with hot water. The aqueous extract yields tests for sugar after hydrolysis with mineral acid if dextrin is present. *Artificial colouring* matters can be detected by shaking the beans in a thick stoppered bottle and examining the powder obtained. Graphite, however, adheres to the beans closely and is not removed by shaking, but is readily detached by warming with alcohol containing a little potassium hydroxide.

Ground Coffee.—For analysis the sample should be powdered so as to pass through a sieve with 0.5 mm. mesh. The *moisture* and *ash* of coffee are estimated as for tea, and valuable information is sometimes obtained from the estimation of the soluble ash, chlorine and phosphoric anhydride. In the experience of the writers, the moisture rarely exceeds 3% although much higher proportions are occasionally found depending upon the degree of roasting.

The ash of pure coffee is generally between 3.5 and 4.5%, rarely, if ever, exceeding 5%, and even when a considerable proportion of chicory is present it seldom rises beyond 6%. Any notably higher proportion will indicate the presence of a *mineral adulterant*. The ash should be white, or nearly so, a marked red tint indicating an added compound of iron.

The composition of the ash of coffee presents considerable differences from that of chicory, as is apparent from the following results or analyses by H. Ludwig (*Arch. Pharm.*, 1872 [*iii*], 1, 482) and James Bell (*Foods*, 2, 46, 57).

	Coffee-beans H Ludwig		Coffee-beans, eight samples J. Bell	Chicory root, Eight samples J. Bell	
	Gneiss soil	Limestone soil		Deducting SiO_2 and sand	Including SiO_2 and sand
K_2O .	14.13	44 03	53.20 to 55 80	27 85 to 46 27	24.88 to 33.88
Na_2O .	5 84	5.85	Not detected	3.17 to 16 90	2.04 to 15.10
CaO .	8 64	4.89	4 10 to 6.16	7.65 to 10 81	5.00 to 9.60
MgO ..	8.14	8 01	8 20 to 8 87	5 33 to 8 08	3.42 to 7.22
FeO_3 .	16.54	1.96	0 44 to 0.98	3 50 to 8 29	3.13 to 5.32
P_2O_5 .	18.65	10.54	10 15 to 11 60	9 59 to 12 61	6 65 to 11.27
SO_3 ..	15.28	1.64	3 09 to 5 26	8 38 to 11 78	5 38 to 10.53
Cl..	Trace	0 98	0 26 to 1.11	5 03 to 6 08	3.23 to 4.93
CO_2 ..	8.34	21 24	14 92 to 18.13	2 04 to 4 60	1 78 to 3.19
SiO_2	1 65	0 37	0.00 to 0.45	.	2 61 to 12 75
Sand .	None	None	None	...	8.08 to 23.10

Ludwig found in each case a notable amount of soda, a result which disagrees with Bell's statement that this base is absent from coffee ash. Ludwig's figures also show an enormous variation in the proportions of K_2O, Fe_2O_3, SO_3, and CO_2, according to the nature of the soil on which the coffee-plant is grown. The sample of coffee from a gneiss soil must be regarded as highly abnormal. In Allen's experience the ash from genuine coffee was never observed to have a red colour, as would be the case with the ash of a specimen containing a considerable proportion of iron.

C. Kornauth showed the ash to contain 54.43% K_2O, 0.29% Na_2O, 12.52% P_2O_5, 4.11% SO_3 and 0.45% Cl; he stated that the ash should be free from silica and that the sodium oxide should not exceed 0.5%. If the chlorine exceeds 0.6% the coffee has probably been adulterated or damaged by sea water. If the Na_2O in chicory-ash be calculated into its equivalent of K_2O, and the figure thus found added to the actual K_2O, the percentage is not greatly different from the proportion of potash found by Bell in coffee-ash. The proportion of ferric oxide is notably greater in chicory than in coffee. Hence chicory-ash always has a red tinge which is absent from the ash of genuine coffee. (*Cf.* Petermann, page 653.) A notable difference is observable in the proportions of CO_2 and Cl, and a very wide distinction in the figures for sand and silica. In only one of the eight samples of coffee did the silica even approach 0.5%, and in another portion of the same coffee, which was properly screened before roasting, the silica of the ash fell to *nil.*

Defert found that in coffee from São Paula the percentage of K_2O

increased in the plant with distance from the roots and was highest in the leaves, while CaO was highest in the root and least in the leaves.

In consequence of the large proportion of potassium carbonate in coffee-ash, the percentage of the total ash soluble in water is much greater than in the case of chicory-ash, and attempts have been made to utilise this fact for ascertaining the proportion of chicory present in mixtures of the two. Thus Allen found from 60 to 85% of the total ash of coffee to be soluble in water, whereas on an average only 34% of the total ash of chicory was soluble in water. But this proportion is gravely affected by the proportion of actual sand which may be present. This varies in commercial chicory from a trace up to 4.5%, which difference is quite sufficient to invalidate deductions based on the ratio of the total to the soluble ash. By comparing the soluble ash with the total ash *minus* sand and silica, somewhat more reliable results are obtained, but at best the method is only capable of affording a rough indication of the proportion of chicory present. It may, however, serve to point to the presence of a foreign ingredient, which can then be identified and determined by other means. The following ash-analyses, by James Bell, are interesting in this connection.

	Lupins	Acorns	Maize	Parsnips	Dandelion root
K_2O	33 54	54 93	30 74	56 54	17 95
Na_2O	17 75	0 63	Not found	Not found	30 95
CaO	7 75	6 01	3 06	6 85	11 43
MgO	6 18	4 32	14 72	6 49	1 31
Fe_2O_3		0 54	0 84	0 53	1.27
P_2O_5	25 53	11 15	44 50	13 84	11 21
SO_3	6 80	4 79	4 13	4 07	2.37
Cl	2 11	2 51	0 50	2 09	3 84
CO_2	0 56	13 69		11 44	6.21
SiO_2, etc	0 87	1 01	1 78	0 57	11 26
	101 09	99 58	100 27	102 42	97 80

The following centesimal figures by Way and Ogston refer to the ash of other roots:

	Turnip	Beet	Carrot
Fe_2O_3	0.14 to 0 66	0.52 to 3 74	0 59 to 1 66
Cl	3 to 5	25 to 30	3 to 4.6
CO_2	9.5 to 15	15 to 21.6	15 to 19

Estimation of Fat.—Dry 2 grm. of coffee at 100° and extract with light petroleum for 16 hours; evaporate the solvent, dry residue at 100° and weigh (A. O. A. C.). The writers find that more complete extraction is obtained by macerating the coffee with a flat-topped glass rod in a deep flat platinum dish with light petroleum and allowing to settle for a few minutes; the petroleum is filtered into a tared flask and the operation is repeated about 16 times. Although this method is much more tedious than other extraction methods, higher results are generally obtained.

The fat of coffee is tolerably constant in amount, and hence the proportion serves as a useful indication of the amount of certain admixtures. Thomas Macfarlane, stated that the petroleum-ether extract from previously dried coffee ranges from 10 to 12%. Only 1 sample out or nearly 50 examined showed less than 10, and no sample gave as much as 13%, although 12.5% was reached in a few instances. By the maceration method described above the fat is usually near 13% calculated on the dry coffee and is frequently higher. Chicory yields about 1% when similarly treated,* and 3 samples of roasted barley gave from 1.31 to 1.54%, but Tatlock and Thomson found 2.73% of fat in a sample of chicory, and the writers have recently found 2.42%.

Caffeine.—The various methods for estimating caffeine have been described under tea, and are suitable for coffee but larger quantities must be taken (from 6 to 12 grm.); extraction by means of chloroform, after moistening with ammonia, is especially suitable. The residue contains fat, however, and should be boiled with water, filtered through a cotton-wool plug, and evaporated to dryness or extracted with chloroform. If desirable the nitrogen may be determined. Gomberg's periodide method has been recommended as convenient for use with the filtrate from the caffetannic acid determinations (*U. S. Dept. of Agric.*, 1909, *Bull.* 122, 78).

The caffeine of coffee is remarkably constant and is generally from 1.0 to 1.3%, and, although much lower figures have been published, these results are probably due to defective methods.

Paul and Cownley (*Pharm. J.*, 1887 [*iii*], **17**, 565, 648, 821, 921) recommended the adoption of 1.30% of caffeine as a constant for all genuine coffee and used this figure to estimate the proportion of coffee in a mixture. Allen considered 1.20% a safer number to adopt. As genuine coffees may contain appreciably less caffeine than 1.3%, the deficiency in caffeine should be used in conjunction with other data, or

results wide of the truth may result. In any case it is necessary to ensure that the whole of the caffeine has been extracted by an examination of the residues obtained in any particular process.

These statements apply to ordinary coffee, but certain varieties of coffee have been described containing no caffeine but a bitter principle, *cafamarine*. Bertrand (*Compt. Rend.*, 1901, **132**, 162, and 1905, **141**, 209) describes 4 new species, C. *Humblotiana*, C. *Gallienii*, C. *Bonnieri*, C. *Mogeneti*, grown in Madagascar and Grand Comoro. 1 kilogrm. of coffee-berries from C. *Humblotiana* gave no caffeine and the plant was considered a distinct species by Bertrand and not merely C. *Arabica* grown on different soil, since the latter when grown on the same soil, under similar conditions, gave the normal proportion of caffeine. The following analyses of C. *Humblotiana* and C. *Arabica* grown on the same soil are published by Bertrand:

	Water	Ethereal extract	Alcoholic extract	Reducing sugar	Non-reducing sugar	Total nitrogen	Ash	Caffeine
C. Humblotiana	11.6	10.7	8.4	0 8	4 2	1 5	1 8	0.0%
C. Arabica	9.7	5.8	12.1	0 3	4.9	1 9	3 7	1.3%

Hanausek describes a wild coffee (probably C. *bourbonica*) free from caffeine. The beans are egg-shaped and more slender than those from C. *Arabica*.

Besides the varieties of naturally occurring caffeine-free coffees, certain coffees have recently been placed on the market which purport to have been freed from the greater part of the caffeine and are sold as "caffeine freed." These coffees are stated to be prepared by submitting the coffee-berries to the action of superheated steam, then ammonia, sulphur dioxide or hydrogen chloride gas is introduced and the caffeine is extracted by a suitable solvent such as alcohol or benzene; after removal of the solvent the action of the steam is continued It is probable that the essential oil of coffee is returned after extraction of the caffeine.[1]

Estimation of Caffetannic Acid.—Although the tannic acids of coffee have been shown to consist of a mixture of tannin (Tatlock and Thomson), coffalic, chlorogenic, and other acids (Gorter), nevertheless,

[1] Lendrich and Murdfeld (*Zeit. Nahr. Genussm.*, 1908, 15, 705) give 0.2% caffeine as the mean of 14 caffeine-free coffees and the unusually high proportion of 17.1% of fat.

by working under definite conditions, more especially as regards the quantity of lead acetate, results of value may be obtained. Krug's method as modified by Woodman and Taylor (*U. S. Dept. of Agric.*, 1909, *Bull.* 122, 83) gives concordant results. To 2 grm. of ground coffee add 10 c.c. of water and shake continuously for at least 1 hour; add 25 c.c. of 90% alcohol and continue the shaking for half an hour; filter and wash with 90% alcohol, until filtrate is about 50 c.c. in volume. Heat the filtrate to boiling (in a vessel suitable for use in a centrifugal machine) and add drop by drop 6 c.c. of a saturated solution of lead acetate. Separate the precipitated lead salt by means of a centrifugal machine and decant the supernatant liquid through a tared filter; repeat this twice with 90% alcohol. Transfer the precipatate to the filter, wash free from lead with alcohol and finally with ether; dry at 100° and weigh. The weight of the precipitate multiplied by 0.51597 gives the weight of caffetannic acid. The percentage of lead in the precipitate as obtained varies between 48.3 and 48.7%, the amount required by $Pb_8(C_{16}H_{16}O_8)_2$ is 49%. Trillich and Göckel (*Zeitsch. Nahr. Genussm.*, 1898, 1, 101) use 3 grm. of coffee and boil 4 times for half an hour with water; and the whole is made up to 1,000 c.c. To 400 c.c. of the clear filtered liquid 1. c.c. of basic lead acetate solution is added and the precipitate is allowed to settle all night. The precipitate is filtered off, washed, removed from the paper, suspended in water and decomposed with sulphuretted hydrogen; after filtration from the lead sulphide, the liquid is evaporated to dryness and weighed.

Trillich and Göckel give the following values for raw New Granada coffee: Krug's original method 11.3, Bell's method 5.3, Trillich and Göckel's method 11.37%. According to O. Woodruff the results by Trillich and Göckel's method are generally higher than those obtained by Krug's method.

The aqueous extract of coffee is remarkably constant in amount, and is very little affected by variations in the roasting. Instead of weighing the actual extract, Graham, Stenhouse and Campbell (*J. Chem. Soc.*, 1857, 9, 33) determined the sp. gr. of the aqueous infusions of coffee and various roasted vegetable matters. Their me hod was to treat the roasted substance with ten times its weight of cold water, raise the liquid to the b. p., and observe the sp. gr. of the filtered liquid after cooling to 60° F. (= 15.5° C.). The following is a classified arrangement of their results:

Substance	Sp. gr of 10% infusion	Substance	Sp gr. of 10% infusion
Coffee:		**Roots:**	
Mocha	1008 0	Chicory, Yorkshire	1019 1
Neilgherry	1008 4	Chicory, English.	1021.7
Plantation Ceylon	1008 7	Chicory, Foreign	1022.6
Java	1008 7	Chicory, Guernsey	1023.3
Jamaica	1008 8	Average	1021.05
Native Ceylon	1009 0	Parsnips	1014.3
Costa Rica	1009 0	Carrots	1017.1
Costa Rica	1009.05	Turnips	1021.4
Average	1008.7	Dandelion	1021 9
		Red beet	1022.1
Leguminous Seeds:		Mangold wurzel	1023.5
Lupins	1005 7		
Peas	1007 3	**Cereal Products:**	
Beans	1008 4	Brown malt	1010.9
		Black malt	1021.2
Miscellaneous:		Rye meal	1021 6
Spent tan	1002 1	Maize	1025 3
Acorns	1007.3	Bread raspings	1026 3

These results show a marked distinction between coffee, leguminous seeds, and acorns on the one hand, and cereal products and chicory and other roots on the other. Unfortunately, with the exception of chicory and coffee, they apply merely to single specimens of each kind of substance.

Experiments made by Allen gave a mean sp. gr. for coffee-infusions precisely identical with that obtained by Graham, Stenhouse and Campbell (1008.7). Hence in a series of experiments made in Allen's laboratory, the sample of coffee was well boiled with 10 parts of water, the solution filtered, and the residue washed with hot water till the filtrate measured 10 c.c. for every 1 grm. of the substance employed. Operating in this manner, the infusions from 14 specimens of ordinary commercial roasted coffee (ground in the laboratory) were found to have a sp. gr. ranging from 1006.8 to 1008.5, with an average of 1007.9 (*Analyst*, 1880, **5**, 1). O. Hehner has met with a genuine coffee giving an infusion-density of 1010.2.

J. Skalweit has shown that the sp. gr. of the aqueous infusion is not sensibly affected by the extent to which the coffee has been roasted.

By the exhaustion-process, Allen obtained the following results from samples of commercial chicory (undried):

	Sp. gr of 10% infusion
Yorkshire Chicory, under-roasted	1025.9
Yorkshire Chicory (same sample), highly roasted	1019.0
Chicory of unknown origin	1021.1
Chicory of unknown origin	1020 0
Chicory of unknown origin	1023 4
Mean	1021 9

It is evident that the density of chicory infusions varies much more than that of coffee, a fact which prevents the method from furnishing more than an approximate determination of the proportion of coffee and chicory in a mixture of the two. A sharper result may be obtained by previously drying the sample at 100°, and hence eliminating the somewhat serious error due to varying proportions of moisture. Adopting 1,024 as the normal sp. gr. of the infusion of dried chicory and 1,009 as that of dried coffee, the percentage of real coffee in a mixture of the two will be found by the following equation, where d is the sp. gr. of the 10% infusion and C the percentage of coffee in the sample:

$$C = \frac{(1024 - d)\,100}{15}$$

A. McGill (*Trans. Royal Soc. Canada*, 1887) finds that the sp. gr. of the infusion of coffee and chicory is materially affected by the fineness of the powder, the time occupied in heating the decoction to boiling, and the time during which the boiling with water is continued.

McGill's method is best carried out as follows:

Weigh into a tared flask the equivalent of 10 grm. of dried coffee; add water until the contents weigh 110 grm., connect to a reflux condenser and heat so that boiling commences in 10 to 15 minutes. Boil for 1 hour, cool for 15 minutes, weigh again and make up any loss by the addition of water, filter through a dry filter and take the sp. gr. of the filtrate at 15°. The average sp. gr. of a 10% extract of pure coffee is 1.00986 at 15°, and of chicory under the same conditions (for 3 samples) sp. gr. 1.02821. The approximate percentage of chicory may be calculated by the following formula:

$$\%\ \text{chicory} = 100 - \frac{(1.02821 - \text{sp. gr.})}{0.01835}$$

The *refractive index* of the above solutions by Zeiss' refractometer is $N_D^{20} = 1.3377$ for 10% coffee extract and $N_D^{20} = 1.3448$ for 10% chicory extract.

Thos. Macfarlane obtained the following results by the application of McGill's method for ascertaining the infusion-density and actual determination of the soluble extract. This last estimation was made by thoroughly extracting the dried sample with petroleum spirit, and then treating the redried substance with boiling water. Instead of evaporating the solution, the insoluble matter was redried and weighed, the loss showing the "water extract:"

	Water extract	Infusion sp. gr at 62° F.
Santos coffee	22 44	.. . .
Mocha coffee	21 92	1009 78
Java coffee	20 42	1009 73
Java coffee, with 10% Chicory	25 90	1011 58
Java coffee, with 20% Chicory	30 75	1013.44
Java coffee, with 30% Chicory	37.40	1015 28
Java coffee, with 40% Chicory	43.36	1017 08
Java coffee, with 50% Chicory	49 84	1018.66
Java coffee, with 60% Chicory	53 82	1020.48
Java coffee, with 70% Chicory	60 34	1022.70
Java coffee, with 80% Chicory	65 93	1024.15
Java coffee, with 90% Chicory	71 41	1026.42
Chicory	77 73	1028.32

Tatlock and Thomson (*J. Soc. Chem. Ind.*, 1910, **29**, 138) give the sp. gr. of 10% infusion as from 1.0099 to 1.0102. McGill (*Canadian Bull.*, 1909, **172**) gives for the sp. gr. of a 10% extract of 55 samples of genuine coffee figures varying from 1.0083 to 1.0104 with a mean value 1.0093.

It is evident that the sp. gr. of the aqueous infusion is really a function of the solid matter dissolved by the water, and a close approximation to the percentage of the latter can be obtained by dividing the difference between the sp. gr. and 1,000 by the number 0.375 or multiplying it by 2.67. This factor is deduced from the known solution-densities of caramel and the carbohydrates. J. Skalweit (*Rep. Anal. Chem.*, 1882, **2**, 227), as the result of direct experiment, gives the following data: At 17.5°, 1.001 sg. gr. of 20% infusion represents 0.36 extract per 100 c.c.; 1.115 sp. gr. of 20% infusion represents 27.24 extract per 100 c.c ; 1.235 sp. gr. of 20% infusion represents 48.25 extract per 100 c.c. Thus if a coffee-infusion have a sp. gr. of 1009.0, the proportion of matter soluble in water will be

$$\frac{1009.0 - 1000.0}{0.375} = 24.0\%.$$

The figures for soluble extract obtained by T. Macfarlane (Ottawa) by the analysis of 54 samples of commercial coffee ranged from 21.5 to 26.5%, with an average of about 24%. The samples were dried at 100°, deprived of fat by treatment with petroleum spirit, re-weighed, and then exhausted with water. Instead of evaporating the infusion and weighing the soluble extract, the insoluble residue was dried and weighed, and the loss gave the soluble extract. A. Smetham has also proposed to wash, dry, and weigh the insoluble matter left on the filter.

Alfred E. Johnson states the soluble extract from previously dried (roasted) coffee to be very constant at 24%, and the extract from dried chicory to average 70%, and on these figures bases the following process for the analysis of coffee mixtures.

The ground coffee is dried at 100° and 5 grm. of the moisture-free sample boiled for 15 minutes with 200 c.c. of water. After settling for a few minutes, the liquid is poured off through copper wire-gauze or coarse muslin into a 250 c.c. flask. The grounds are boiled with 50 c.c. of water for 5 minutes and the liquid strained as before. The contents of the flask are cooled, made up to 250 c.c., agitated, and poured on to a dry filter. 50 c.c. of the filtrate, rejecting the first portion (equal to 1 grm. of the dry sample), is then evaporated in a flat dish over boiling water, and the residue (representing the extract from 1 grm.) dried in the water-oven and weighed. Then:

$$\frac{100(70 - \%\text{ of extract found})}{46} = \text{percentage of coffee in sample.}$$

The results thus yielded by coffee and its principal adulterants are given on pages 658, 659.

Winton estimates the *soluble solids* in ground coffee by 8 hours' maceration of 4 grm. of the sample in a 200 c.c. graduated flask filled to the mark with water. The flask is shaken frequently and then allowed to stand for a further 16 hours, filtered, 50 c.c. of the filtrate evaporated in a flat dish, and dried at 100° and weighed (A. O. A. C.).

Reducing sugar may be estimated (A. O. A. C.) in this solution by the ordinary methods, after clearing with lead acetate. The results are calculated to dextrose (Vol. 1, page 320).

Sucrose may be estimated polarimetrically (A. O. A. C.) by taking half the standard quantity, extracting and clearing with basic lead acetate (Vol. 1, page 309).

Starch, if present, may be estimated (A. O. A. C.) by the diastatic method (Vol. 1, page 422).

Proteins are estimated from total nitrogen by Kjeldahl's method, after deducting the nitrogen due to caffeine. The difference multiplied by 6.25 is taken as protein, but the results have very limited application. The *crude fibre* is determined as usual but is rarely required.

For the detection of *adulteration* in roasted coffee it is usually sufficient to estimate moisture, ash (soluble and insoluble), caffeine, fat and the 10% extract. Additional information in doubtful cases may be optained from the alkalinity of the soluble ash, the proportion of nitrogen and from the amount of chlorine which in adulterants is frequently higher than in coffee.

Adulterations of Coffee.—Commercial coffee is subject to a variety of sophistications, both in the berry and after grinding.

Imitation coffee-berries were formerly manufactured of fire-clay. These were mixed with genuine berries and roasted with them, when they absorbed some of the colouring matter and oil, and so remained a close imitation. On breaking such spurious berries the colour would be seen to be principally on the exterior. The determination of the total ash and silica would at once lead to the detection of such a fraud.

In 1850, Messrs. Duckworth of Liverpool took out a patent for moulding chicory into the form of coffee-berries, and several kinds of factitious coffee-berries have been described.

A factory for the manufacture of imitation coffee-berries on the scale of 40 to 50 kilogrm. daily was seized at Lille by the French Government. It appeared in evidence that the composition of the product was: Chicory, 15 kilogrm.; flour, 35 kilogrm.; ferrous sulphate, 1/2 kilogrm.

Factitious coffee-beans seized in Roumania consisted of coffee-grounds, chicory, and peas.

In America there are several firms which extensively manufacture imitation coffee-beans and "coffee-pellets." These preparations usually consist of wheat-flour, chicory, bran, and occasionally coffee. Samples purchased and examined by the chemists of the United States Department of Agriculture gave the following results:

Appearance	Sp. gr.	Composition
Roasted beans	1.195	Wheat-flour.
Roasted beans	1.198	Wheat-flour, coffee, and chicory.
Roasted beans	1.111	"Kunst Kaffee." Wheat-flour, coffee, and chicory.
Roasted pellets	1 119	Wheat-flour, bran, and probably rye.
Roasted pellets	1.183	
Roasted pellets	1.193	
Raw beans	...	Wheat-flour and coffee.
Roasted beans	1 211	Wheat-flour
Light-coloured beans	1 174	Wheat-flour and probably sawdust.
Dark-coloured beans	1.134	
Roasted beans	1.118	Wheat-flour
Roasted granules	.....	Hulls of peas, with molasses
Roasted lumps	.....	Bran and molasses
Roasted granules	.	Pea hulls and bran

A. W. Rehnstrom (*Eng. Pat.*, 14,970, 1889) described a substitute for coffee prepared by boiling down whey or milk in a vacuum to a pasty consistency, forming the product into cakes, drying it below 100°, cutting it into pieces the size of coffee-beans, and roasting.

L. Jaunnes, in 1891, examined a factitious coffee consisting of acorns and cereals.[1]

An imitation coffee examined by J. König (*Zeitsch. angew. Chem.*, 1888, **1**, 630) closely resembled real coffee in appearance, but all the berries were precisely the same shape. Under the microscope, wheat-starch was detected, and Konig concluded that the article consisted of roasted wheat dough of low quality. E. Fricke (*Zeitsch. angew. Chem.*, 1889, **2**, 310) has described a factitious coffee containing caffeine, and apparently made from lupine-seeds. K. Portèle (*Chem. Zentr.*, 1890, **61**, 135) has described factitious coffee-beans sold under the name of "Kunst Kaffee." The following were the compositions of the samples referred to above:

	Portèle	König	Fricke
Moisture	1 46%	5 14%	(Analysed after drying)
Proteids	13 93%	10 75%	17 90%
Fat	3.86%	2.19%	2.03%
Starch, sugar, gum, etc.	64 01%	76.76%	.....
Cellulose	15 83%	3.96%	10.83%
Caffeine	0.07%	...	0.94%
Ash	2 53%	1 20%	2 27%
	101 63%	100 00%	100 00%
Matter soluble in water	21.53%	29.28%	24.85%

[1] The writers have found a sample of factitious coffee to consist of roasted maize, polished and glazed.

R. Wolffenstein (*Zeitsch. angew. Chemie*, 1890, **3**, 84) has described two varieties of factitious coffee, respectively known in Germany as *Domkaffee* and *Allerweltkaffee*. Both preparations were entirely destitute of caffeine. One consisted practically of chicory, while the other contained large quantities of lupines. From the latter spec men Wolffenstein isolated a brown colouring matter having the spectroscopic and chemical characters of *Cassella-brown*. It was soluble in alkalies and in water, but was completely precipitated from its solutions by hydrochloric acid. 14 grm. of the sample extracted with water and precipitated with acid yielded 1.67 grm of the colouring matter.

Factitious coffee-beans are, with very rare exceptions, heavier than water, while genuine roasted beans are invariably lighter, unless much over-roasted. In taking the sp. gr., 20 beans should be immersed in brine, which is then diluted with water till 10 of the beans float and the remainder sink. The result shows the average density; but individual factitious beans often vary considerably from the mean.

In genuine coffee-beans a portion of the fine membrane or "parchment" with which the berries were invested will almost always be found adhering in the cleft. The microscopic structure of the bean, as seen in a thin section, or of the powder affords a certain means of recognising its nature. Most factitious beans contain starch, which is entirely absent from genuine coffee. Chicory and other roots are readily recognisable by the microscope. The methods used for the examination of ground coffee may also be applied.

G. Würtz (*Zeitsch. Nahr. Genussm.*, 1898, **1**, 248) has called attention to the practice of washing coffee beans, colouring and drying in a centrifugal machine with fine sawdust. By this means the furrows are filled with wood powder, making them a fine white and enhancing the value of the beans.

Dangway beans, the seeds of *Cassia tora* or *C. occidentalis*, abundant in British Burmah, have been prepared and patented as a substitute for coffee (*Eng. Pat.*, 15,564, 1888). In Germany, the ground and roasted seeds have been sold under the name of "Mogdad coffee," and it is said that a smaller proportion than 20% in coffee cannot be detected either by the taste or the appearance of the sample. Dangway beans leave about 10% of ash on ignition, and have a characteristic microscopic appearance which has been described and illustrated by A. Wynter Blyth (*Food; Composition and Analysis*). They sink

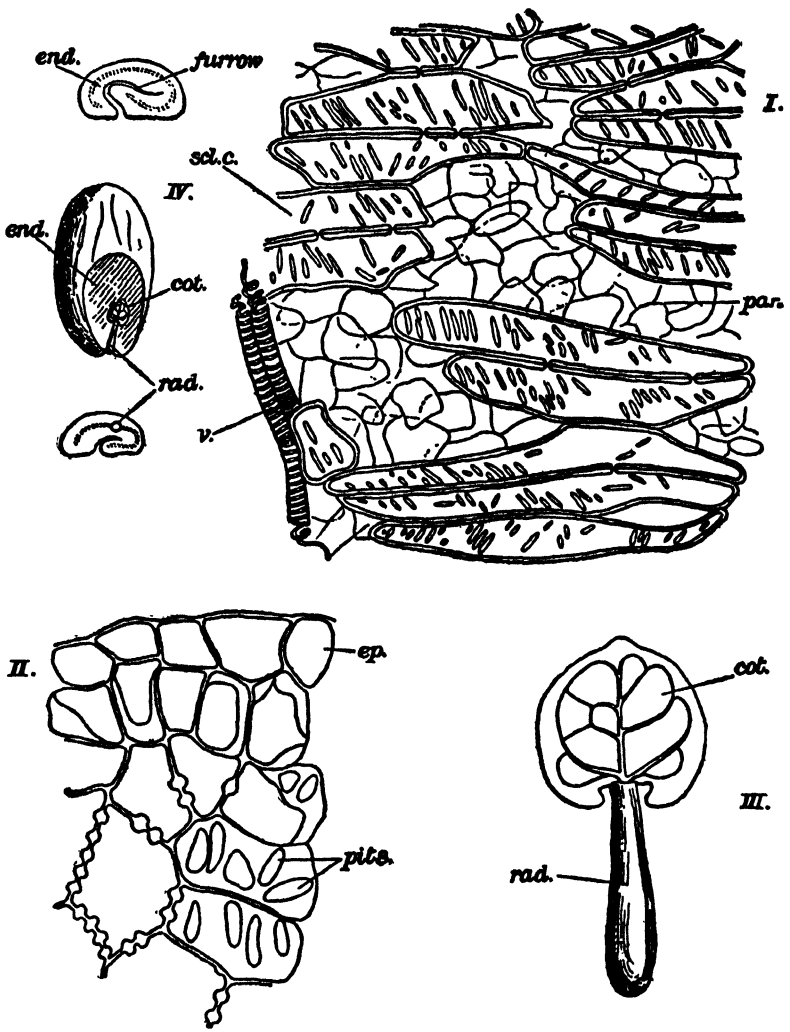


FIG. 4.—Coffee. I., portion of the seed coats, surface view; *par.*, collapsed parenchymatous tissue; *scl. c.*, sclerenchymatous cells; *v.*, vessel, ×220. II., transverse section of outer part of endosperm. *ep.*, epidermis, ×220. III., embryo of seed; *cot.*, cotyledon; *rad.*, radicle. IV., sections of seed; *cot.*, cotyledon; *end.*, endosperm; *rad.*, radicle. (Greenish.)

very rapidly in water and colour brine more intensely than do coffee beans. Dangway beans contain a tannin distinct from caffetannic acid. They are destitute of caffeine, but O. Hehner has detected a minute quantity of some other alkaloid.

The use of *Mussaenda Borbonica* seeds, to be mixed and roasted

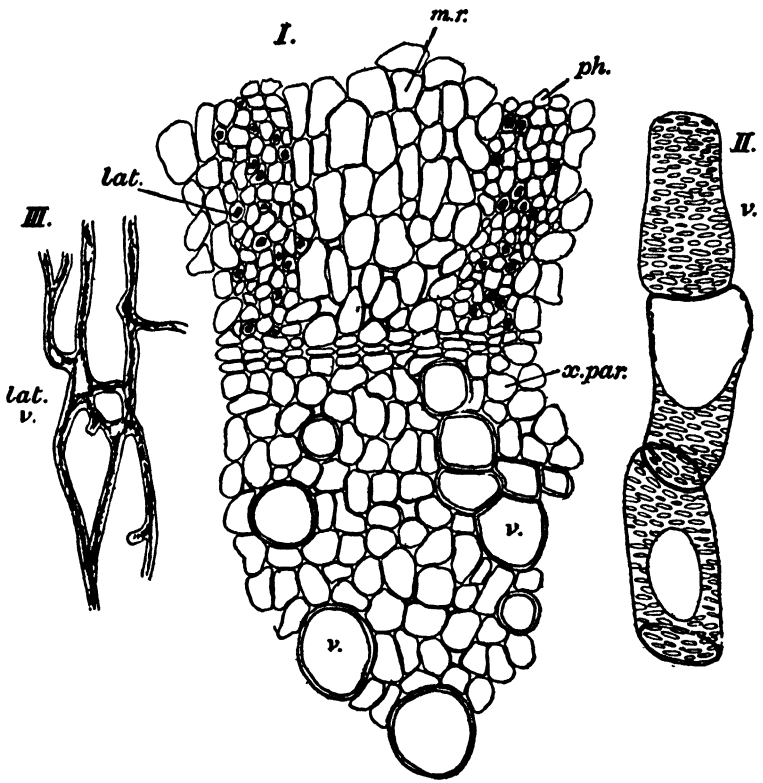


FIG. 5.—Chicory Root. I, transverse section; *lat.*, laticiferous vessel; *m.r.*, medullary ray; *ph.*, bast; *v.*, vessel; *x.par.*, wood parenchyma. II., radial section of vessels. III., laticiferous vessel isolated by maceration with potash. ×220., (Greenish.)

with coffee-beans or entirely substituted for them, has also been patented (*Eng. Pat.*, 14,945, 1888). Investigations at Kew Gardens show the supposed *Mussaenda* seeds to be really those of *Gærtnera vaginata*. They contain no caffeine.

The beans of a species of *Phaseolus* are reported by E. Fricke to be roasted, ground, and sold as "Congo coffee." The berries are very large—214 filling a 100 c.c. measure—and of shining black colour. The infusion is very astringent and contains no caffeine or other crystallisable alkaloid.

To distinguish lupine-seeds from coffee-beans, Hager treats 3 grm. of the powdered sample with 20 c.c. of water and filters after half an hour. The filtrate from genuine coffee will be feebly yellow and not taste in the least degree bitter, while in the presence of lupine-seeds a marked bitter taste will be observed.

Bertarelli (*Zeitsch. Nahr. Genussm.*, 1900, 3, 681) pointed out that

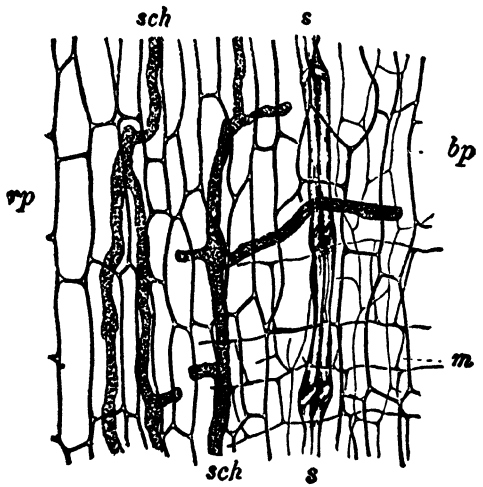


FIG. 6.—Chicory Root, radial section of cortex. *bp*, bast parenchyma; *m*, medullary ray; *rp*, cortical parenchyma; *sch*, laticiferous vessels. ×160. (Moeller.)

roasted coffee has been soaked in a 5% solution of borax by which means some of the moisture lost during roasting is absorbed The borax introduced in this way is too small to increase the ash notably and it is therefore desirable to test for borax when a roasted coffee is found to contain a high proportion of moisture. Morpurgo has called attention to the artificial colouration of coffee berries. He found coffees coloured by mixture of graphite, bone black, soot, ultramarine, Prussian blue, lead chromate, tannate or iron, yellow ochre,

chalk, talc and anline colours. Some samples were found to be polished with wax, resin and sandarac. The colour and appearance of the berries when examined with a lens will frequently indicate such sophistication.

According to L. Padé (*Bull. Soc. Chim.*, 1887, **47**, 501), raw coffee which has been damaged by sea-water is sometimes washed, decolourised with lime-water, again washed, dried rapidly, and coloured either by slight roasting or by dyeing with azo-oranges. By such manipulations, green Santos coffees are said to be increased 25% in value, and made to pass for Java growths. E. Waller states that South American coffees are often exposed to a high moist heat, which changes their colour from green to brown, in imitation of Java coffee. He found coffee-berries coloured with Scheele's green, yellow ochre, chrome-yellow, burnt umber, Venetian red, etc. When possible, such facings should be detached by agitating the berries with cold water and examining the sediment. Organic colouring matters can be detected by soaking the berries in alcohol, which is not coloured by genuine coffee. On evaporating the alcoholic solution to dryness, and taking up the residue in water, a solution will be obtained which will give the characteristic reactions of the coal-tar dyes.

The sp. gr. of 24 samples of genuine raw coffee-berries was found by Padé to range from 1.368 to 1.041, while the density of the same samples, after roasting in the ordinary manner, varied from 0.635 to 0.500. Raw coffee which is lighter than water may be suspected of having been damaged by sea-water or other means, and subsequently washed and improved in colour by partial roasting.

The sp. gr. of coffee-berries is ascertained by Padé by a special apparatus described in his paper. In the case of unroasted coffee, the gravity can be readily observed by immersing a few of the berries in saturated brine, which is then diluted with water till the coffee remains suspended in the liquid, the sp. gr. of which is then taken. With roasted coffee, the brine must be replaced by the very lightest gasoline, the density of which can be increased if necessary by the gradual addition of ordinary kerosene. Another plan of ascertaining the sp. gr. of coffee-berries is to introduce as many as possible into a tared 50 c.c. flask or other vessel of known capacity. The weight is then ascertained, and the flask filled to the mark with mercury. The weight is again observed, when the increase will be the weight of mercury required to fill the interstices between the berries:

According to J. König (*Zeitsch. angew. Chem.*, 1888, **1**, 630) coffee is often roasted with an addition of glucose-syrup, which makes the decoction look stronger and causes the berries to hold an additional 7% of water. L. Padé states that roasted coffee-beans can be made to take up nearly 20% of water by steaming them and coating them with glycerin, palm-oil, or vaseline to prevent evaporation. The sp. gr. of the berries is thereby raised to 0.650–0.770, and hence is sensibly above 0.635, which is the maximum figure for genuine roasted berries.

Van Hamel Roos (*Revue Intern. des Fals.*, 1890–1, **4**, 166) has called attention to an ingenious method of sophisticating coffee-berries. A sample examined by him had the microscopic structure of genuine coffee, but showed an almost entire absence of fat globules, and gave an ether-extract of less than 1% (instead of 12 to 14). Roos suggests that the berries had been used for preparing coffee-extract, and then re-roasted with addition of a little sugar.

As a coating for coffee, T. W. Moore patented (*Eng. Pat.*, 5033, 1889) a mixture of milk or condensed milk, ground or powdered glue, "liquid glycerin," and refined lard; with the addition in some cases of bicarbonate of soda, fine salt, and vinegar!

Ground Coffee.—Besides the foregoing sophistications and substitutions of the coffee-bean, ground coffee is liable to various adulterations. Wallace (*Analyst*, 1884, **9**, 42), names the following articles as used for adulterating coffee: Chicory, caramel, dried and roasted figs, dried dates, date-stones, decayed ship biscuits, beans, peas, acorns, malt, dandelion root, turnips, carrots, parsnips, and mangold-wurzel. Some of these can be tolerated when practised in moderation, provided that the fact and proportion of admixture are duly acknowledged; but it must be remembered that all these additions, including *chicory*, the least objectionable and by far the most widely used, are destitute of the volatile oil and peculiar alkaloid which give to coffee its most valued properties. The diminished consumption of coffee in England is doubtless largely due to the frequency and extent of its sophistications in the past.

The chief adulterants likely to be met with in ground coffee are: 1. Mineral matters; 2. roots, such as chicory, dandelion, turnip; 3. seeds and seed-products, such as beans, acorns, and cereals; and 4. saccharine matters, such as caramel and roasted dates and figs.

In *Bulletin* No. 29 of the Laboratory of the Inland Revenue Depart-

ment, Canada, T Macfarlane, states that: "There are, moreover, large quantities of a substance imported under the name of essence of coffee, for adulterating purposes, which is a species of burnt sugar, and, from its containing dextrin, is probably made from some of the bye-products of the glucose factories. It costs in New York and Philadelphia from 3 to 5 cents per lb. As it possesses no organic structure it is apt to be overlooked in the microscopical examination. It contains about 75% of matter soluble in water, which has great colouring power, and a little of it is capable of imparting a strong brown coffee colour to water."

Caramel, when added as such, may often be distinguished under a low microscopic power by the jet-black colour of the particles. These dissolve easily in water with intense brown colour, and the solution has a bitter taste.

A factitious caramel has been manufactured by adding to glucose about 1/8 of its weight of a brown coal-tar dye, *Naphthol Brown*.

A useful preliminary test for ground coffee consists in gently strewing some of the powder on the surface of cold water. The oil contained in coffee prevents the particles from being readily wetted by the water, thus causing them to float. Chicory and the majority of coffee adulterants contain no oil, and their caramel is very quickly extracted by the water, with production of a brown colour, while the particles themselves rapidly sink to the bottom of the water. On stirring the liquid, coffee becomes tolerably uniformly diffused without sensibly colouring the water, while chicory and other sweet roots quickly give a dark brown, turbid infusion. Roasted cereals do not give so distinct a colour.

According to A. Franz (*Arch. Pharm.*, 1876 [*iii*], **8**, 298), if 2 c.c. of a 10% infusion of coffee in boiling water be treated with 0.3 c.c. of a 2.5% solution of cupric acetate, and the liquid filtered, a greenish-yellow filtrate is obtained. If chicory be similarly treated, a dark red-brown filtrate results, the colour of which changes on standing. 10% of the adulterant can thus be detected.

The colour of an infusion of chicory is said to remain unaltered on addition of a solution of ferric chloride or sulphate, while the brown colouring matter of coffee infusion turns green, and is partially precipitated as bluish-green flakes. In an infusion of mixed chicory and coffee, the reagent forms a precipitate, and leaves the liquid more or less brownish-yellow. The deposition of the precipitate is facilitated

by rendering the liquid slightly alkaline by ammonia (*Dingler's polyt J.*, 1874, 211, 78).

Albert Smith (*Pharm. J.*, 1880 [*iii*], 11, 568) recommends, for the detection of chicory in coffee, that 10 grm. of the sample should be boiled with 250 c.c. of water, and the liquid strained and precipitated with a slight excess of basic lead acetate. On allowing the precipitate to settle, the supernatant liquid will be colourless if pure coffee has been under treatment, but in presence of chicory will be coloured to a greater or less degree according to the proportion present, which can be estimated from the depth of tint by a process similar to that of nesslerising water.

The three foregoing tests are occasionally of service for the examination of infusion of coffee when the solid article is not available, but they cannot be regarded as so satisfactory as the actual recognition of the adulterant by the microscope.

Tatlock and Thomson (*J. Soc. Chem. Ind.*, 1910, 23, 138) give an analysis of chicory for comparison with coffee. Their results are as follows: 75.8% water extract, 1.9% of soluble ash, 2.0 of insoluble ash and 2.73% of oil, and 4.8% moisture. The cupric reducing power of a 10% extract was 24.2 as compared with 0.6 for roasted coffee. The great majority of seeds likely to be met with in coffee contain a notable quantity of *starch*. This is true of beans, peas, acorns, and all cereals and products therefrom. Hence if starch be absent, the freedom of the coffee from all this class of adulterants is certain. If present, the nature of the admixture can usually be ascertained by a microscopic examination of the prepared sample. For this purpose the coffee should first be exhausted with ether to remove fat, and then treated with alcohol to dissolve the colouring matter. In the residue, the starch and other structures will be readily perceptible.

For the detection of starch, Allen boils the coffee for a few minutes with about 10 parts of water. When the liquid has become perfectly cold, some dilute sulphuric acid is added, and then a strong solution of potassium permanganate dropped in cautiously, with agitation, till the colouring matter is nearly destroyed, when the liquid is strained or decanted from the insoluble matter. On now adding a solution of iodine to the solution, a blue colouration will be produced if any starch be present. As little as 1% can be readily detected in this manner.

Some operators employ animal charcoal for decolourising the coffee

infusion before testing for starch. The addition of starch-holding adulterants to coffee is rare in the United Kingdom, but in the United States and Canada is very common, the adulterants there found including wheat-flour and bran, buckwheat, barley, maize, peas, pea-hulls, etc. In 1875 a large seizure was made in the East of London of a mixture of 10% of coffee with 90% of roasted acorns. Roasted acorns were first placed before the English public as "Pelotas coffee," and subsequently as "coffee surrogate," but the manufacture of both these preparations was stopped by the Excise Authorities.

The insoluble matter remaining after treating the coffee with water and decolourising with permanganate can be advantageously examined under the microscope for chicory and other non-starchy additions, the structure of which is more readily observed after the removal of the colouring matter.

F. M. Rimmington (*Pharm. J.*, 1880 [*iii*], 11, 529) recommends, for the removal of colouring matter, that the sample of coffee should be boiled for a short time with water containing a little sodium carbonate. After subsidence, the liquid is poured off, the residue washed with water, and then treated with a weak solution of bleaching powder until decolourisation is effected, which usually occurs in 2 to 3 hours. The real coffee will then form a dark stratum at the bottom of the beaker, and the chicory a light and almost white stratum floating above it, showing a clear and sharp line of separation.

Under the microscope, chicory is readily recognised by the peculiar dotted appearance of the vessels, often occurring in bundles, and by the characteristic appearance of the large cells. Dandelion, turnips, and other sweet roots present a close similarity to chicory, and can only be safely distinguished therefrom by careful microscopic comparison of the sample with the actual roots in question.

The microscopic appearance affords the only certain means of identifying chicory and other roots in coffee, and the same statement applies to saccharine fruits, such as roasted figs, dates, raisins, etc. The adulterants of coffee are best examined as transparent objects under a moderate power, and, except where starch is to be identified, by unpolarised light.

The nature of an adulterant of coffee having been ascertained by the aid of the microscope or other means, an attempt may be made to deduce the proportion present from the chemical composition of the sample. When only one adulterant is present, this may sometimes be

effected with a fair approximation to accuracy; but even in the case of chicory it is not always possible to ascertain the proportion within a somewhat wide limit.

Commercial chicory is prepared from the root of *Cichorium intybus*, which is cut into slices, kiln-dried, and then roasted in the same manner as coffee, usually with the addition of a small proportion of fat of some kind. The preparation and use of roasted chicory appears to have originated in Holland about 1750. A. Mayer (*Bied. Central.*, 1885, 828) gives the following as the composition of 3 samples of Dutch chicory root: Water, 72.0 to 77.3%; albuminoids, 1.1; fat, 0.2; inulin and other non-nitrogenous matters insoluble in alcohol, 12.0 to 17.3; crude fibre, 1.4 to 1.8; sugar, etc., 5.0 to 6.0; bitter extract, 0.05 to 0.15; and ash, 1.4 to 1.9%. Mayer found the bitter substances extracted by chloroform to be soluble in water and alcohol, insoluble in ether, and absorbed by bone-charcoal. They were decomposed by boiling with dilute sulphuric acid, but did not by such treatment yield any substance capable of reducing Fehling's solution.

A. Petermann (*Bied. Central.*, 1883, 843) gives the following results of analyses of 2 samples of roasted chicory, one of which was coarsely and the other finely ground. The ash was somewhat higher than usual, but was perfectly white. The *fat* shown was probably not all natural to the chicory, as the proportion recorded is largely in excess of that found by other observers. The water also is much above the usual proportion in recently roasted chicory (5 to 7%, and the albuminoids below the usual range (8.75 to 11.50.—O. Hehner).

	Coarse grains	Fine powder	
Water (lost at 100–105°)	16 28	16 96	
Glucose	26 12	23 76	Soluble in hot water = 57.96.
Dextrin, inulin	9.63	9 31	
Albuminoids	3 23	3 66	
Colouring matter and bitter extractive	16 40	17.59	
Ash in soluble portion	2.58	2 55	
Ash in insoluble portion	4.58	5.89	Insoluble in hot water = 26 14.
Albuminoids	.15	2.98	
Fat	5.71	3.92	
Cellulose	12.32	13.37	

Bernard Dyer (*Analyst*, 1898, **23**, 226) gives the following analyses of chicory:

	Total matter insoluble in water	Ethereal extract	Nitrogen	Total ash	Ash soluble in water	Sand
Chicory "nibs," described as "medium roast"	22 40	2.57	1.53	4.63	2.50	0.70%
Chicory "nibs," described as "dark roast."	50.30	2.43	1.67	4.70	2.99	0.30%
Ground chicory.....	22 27	2 17	1.33	5 53	2 43	1.43%
Ground chicory	21 50	1.90	1 34	5.23	2.07	1 43%
Ground chicory.....	35.50	3.43	1 50	5.13	2 57	0 77%
Ground chicory.....	37.80	3 87	1.52	8 23	1 60	3 97%
Ground chicory..	22 77	3 17	1.25	5 13	3 30	1 60%
Ground chicory.....	22 50	3 67	1.23	5.73	3 23	1.63%
Ground chicory.....	23 50	2 60	1 29	5 63	2 97	1.47%
Ground chicory.....	22 50	2 60	1.29	5.33	3 20	1.47%
Ground chicory.....	22 63	2.57	1.29	5.70	2 60	1.47%

The above results are calculated on the dry substance, the percentage of moisture in the sample ranging from 1 to 4%.

J. Wolff (*Analyst*, 1899, **4**, 157, 187) shows a mean of 6.1% of inulin in 6 roasted chicorys.

The following analyses by C. Krauth (*Ber.*, 1878, **11**, 277) give some comparative figures for coffee and its more probable adulterants. Except in the case of the last column, the results apply to the substances previously dried at 100°:

	Ash	Fat	Sugar		Soluble in water	Insol. in water	Moisture in undried substance
			Before hydrolysis	After hydrolysis			
Coffee, roasted, 5 samples.	4.19 to 6 38	11 76 to 15 6	0 2	24 29	22 47 to 25 21	74.79 to 77.53	1.47 to 4 37
Chicory, roasted	10 83	1.15	23 40	22 14	65 42	34.58	4 30
Chicory, unroasted ..	5 35	0.43	23 84	Not determined	78.71	21.28	6.89
Rye, roasted....	2.43	1.68		75 37	31.92	68.07	0.28
Wheat, roasted.....	1.80	2 75		.. ...	52.65	47.35	.....
Coffee, with 10% rye	4 31	14 16	0.19	29.65	25.98	74.46	2.15
Coffee, with 10 % wheat	5.10	12.55	2.30	23.15	30.63	69.36	2.30

The following analyses by König show the composition of certain adulterants of coffee:

	Chicory	Figs	Acorns	Rye
Water	12.16	18.98	12.85	15.22
Nitrogenous matters	6.09	4.25	6.13	11.84
Fat	2.05	2.83	4 61	3.46
Sugar	15.87	34 19	8 05	3 92
Other non-nitrogenous matters	46.71	29.15	62.00	55.37
Cellulose	11.00	7.16	4 98	5 35
Ash	6.12	3.44	2.12	4.81
Matters soluble in water	63 05	73 81	.. .	45 11 ?

The following table shows the published results of analyses of coffee substitutes said to be manufactured respectively from acorns, rye, and barley:

	"Acorn coffee"	"Rye coffee substitute"	"Barley coffee"	"Barley coffee"
Water	12 85	2 22	3 45	6 41
Nitrogenous matters	6 13	11 87	9 38	10 56
Fat	4 01	3 91	3.25	1.04
Sugar	8.01	.... ..		
Starch	62.00	8 34	70 13	68.38
Dextrin		49.51		
Other non-nitrogenous matters		9 83		
Cellulose	4 98	9.78	4.25	10.56
Ash	2 02	4 54	3 36	3 04
Matters soluble in water	..	61 33	31 20	34 37
Glucose formed by boiling with dilute sulphuric acid.	. . . .	. ...	69 28	67 19

The "acorn coffee" was analysed by König, who found from 20 to 30% of starch and 6 to 8% of a variety of tannic acid. The "rye coffee substitute" was prepared by Behr Bros. The analyses of "barley coffee" are by C. Kornauth.

Moscheles and Stelzer have published complete analyses of several coffee substitutes (*Chem. Zeit.*, 1892, **16**, 281). One of these contained lupines (which they consider a very reprehensible addition), and another was destitute of coffee, but contained 0.31% of caffeine, due to the presence of powdered kola-nut.

The tinctorial power of the infusion was suggested by Graham, Stenhouse and Campbell (*J. Chem. Soc.*, 1857, **9**, 36) as a means of determining adulterants in coffee. They found that the depth of colour of the liquid obtained by infusing coffee and its adulterants in 2,000 times their weight of boiling water varied remarkably, caramel giving about seven times and chicory about 3 times as deep a colour as

coffee.[1] But their experiments showed that 4 different samples of pure coffee varied in tinctoral power between 143 and 183, as compared with caramel as 1,000, and no doubt samples of chicory would be found to present at least as great difference in colouring power, according as they happened to be lightly or strongly roasted. Nevertheless, Allen found (*Chem. News*, 1874, **29**, 140) that the tinctorial power of an infusion of mixed samples of chicory was almost exactly 3 times that of an infusion of average or mixed coffee, and that different samples of chicory did not very more than from 2.8 to 3.2 in colouring power when compared with the same sample of coffee. In order to estimate the proportion of chicory in a sample of coffee mixture, a standard mixture should be prepared by mixing together several representative samples of genuine ground coffee with an equal weight of mixed chicory.[2] 1 grm. of this standard coffee mixture (containing 50% of coffee), and the same weight of the sample to be tested, are boiled for a few minutes with 20 c.c. of water. The liquids are cooled and passed through a double filter, the insoluble portions being repeatedly boiled with fresh quantities of water till no more colour is extracted. The solution of the standard mixture is then made up with water to 200 c.c., and the solution of the sample to 100 c.c. 10 c.c. of this latter liquid is poured into a narrow graduated tube, and some of the standard solution into another tube of exactly equal bore. If the sample consists of pure coffee, the 2 liquids will now be of exactly similar tint; but if chicory be present, the solution of the sample will be the darker, in which case water is gradually added till the tints are precisely equal. When this point is attained, the volume of the sample solution is observed. Every 1 c.c. of water added represents 5% of chicory in the sample. Thus if the liquid measure 17 c.c., the sample contains 35% of chicory.

[1] The following are the relative amounts of various roasted substances found by Graham, Stenhouse, and Campell to impart an equal depth of colour to the infusion:

Caramel.	1.00	Parsnips . .	2 50	Coffee.	5.46 to 6 95
Mangold wurzel. . .	1.66	Maize and rye.	2.86	White lupin-seed. .	10 00
Black malt.	1 82	Dandelion root. . . .	3 33	Beans and peas. .	13.33
White turnips.	2 00	Red beet	3 33	Spent tan .	33 00
Carrots.	2 00	Bread raspings. . . .	3.64	Brown malt.	40.00
Chicory, darkest Yorks	2.22	Acorns.	5 00		

[2] If the standard coffee mixture be kept, it undergoes a change which modifies, even in a dry state, the colour of the infusion. A permanent standard of the right tint can be made by mixing solutions of ferric, cobalt, and copper sulphates in proper proportions. The yellowish-brown glass employed in Lovibond's tintometer for the colorimetric determination of carbon in steel can also be employed as a standard, if its value be previously ascertained. The tints are best observed by placing a piece of wet filter-paper behind the tubes while they are held up to the light.

J. R. Leebody (*Chem. News*, 1874, **30**, 243) has described a similar method, but, instead of observing the colour of the solutions transversely, he dilutes the solution from 1 grm. of the coffee to 700 c.c., and observes the colour from above, as in nesslerising water.

The observation of the infusion-colour is occasionally very useful as an indication of the presence of caramel added as such, since in that case the colour will be greatly in excess of the proportion of chicory or other adulterant as deduced by other methods.

In view, however, of the wide variations in tinctorial power of coffee and its adulterants, the colorimetric methods are of very limited application; but may be occasionally of value as a confirmatory test when adulteration has been otherwise detected.

Coffee extracts are prepared with limited success by subjecting roasted coffee to treatment with boiling water or steam and adding the volatile products to the aqueous extract. Generally, the product is deficient in caffeine, and does not contain all the extractive matter of the coffee; nor, when diluted with the appropriate amount of water, is the colour the same as that of the freshly-prepared liquid. To remedy this defect caramel is often added, together with strong alcohol as a preservative. In one patent, addition of chicory and sugar is prescribed. The following results were obtained by A. Domergue (*J. Pharm. Chim.*, 1892, **25**, 243) by the examination of 6 samples of coffee extract:

	Water	Extract dried at 100°, %	Caffeine, %	Ash, %
A	86 3	13 7	0.106	0.61
B	82 4	17 6	0.105	0.79
C	58 99	41 01	0.060	4.30
D	72.8	27 2	0 040	3.10
E	69 9	30.1	0.050	1.40
F	80.74	19.26	0.096	1.83

Samples A and B were prepared in the laboratory. C, D, and E were coloured with caramel. Domergue regarded the proportion of caffeine as the best indication of the value of a coffee extract.

The *Lancet* (1894 [*ii*], 43) gives the following results of analyses of coffee and coffee and chicory extracts and essences:

Sample	Dried extract	Caffeine	Mineral matter	Description
Standard infusion	2.10	0.26	0.11	10% infusion of best mocha.
1	60.30	1.17	2 50	Pure extract.
3	82.20	0 58	2 20	Pure extract.
4	19.00	0 87	2 20	Pure essence.
6	67 40	3.55	10 00	Pure extract.
8	67.60	1 00	3.30	Pure extract.
10	31 62	0 46	1 20	Pure essence.
12	38 60	0 44	1 36	Pure essence.
14	40.70	1.35	3 92	Pure essence
2	82.40	0 30	1 50	Coffee and chicory.
5	73.00	0.23	1 85	Coffee and chicory.
11	36 10	0 31	0 80	Coffee and chicory.
9	64.10	0.47	2.15	Dandelion coffee essence.

At a dilution of 2 teaspoonfuls to a cup of 10 ounces (which dilution is somewhat less than that generally recommended by the makers), 1 only of the extracts yielded a liquid closely resembling in appearance, flavour and analytical data a genuine coffee infusion. It is noteworthy, moreover, that this extract (No. 6 above) was described as a very concentrated and expensive product, prepared especially for confectioners.

Moor and Priest (*Analyst*, 1899, **24**, 281) in 10 samples obtained figures in fair agreement with those given above. In their opinion the low caffeine contents are due to unavoidable loss during manufacture, owing to the caffeine being precipitated as an insoluble tannate, which, they state, may sometimes be seen at the bottom of the bottle.

Tatlock and Thomson (*loc. cit.*) give the following figures for coffee and chicory essences, mixed with sugar:

	Coffee and chicory essences		Essence of coffee (French)
	1	2	
Caffeine	0 2	0 3	0 7
Crystallisable sugar	38.4	39 7	}
Uncrystallisable sugar	12.9	10 1	} 40 0
Other organic matter	16 3	15 9	}
Ash	1 5	1 6	3.3
Water	30.7	32 4	56 0

Utilising their observation that, whatever the degree of exhaustion of the coffee, the caffeine and mineral matter bear the same relation to the total extract, Tatlock and Thomson conclude that these 3 extracts are equivalent to 17, 27 and 57% respectively of dry coffee.

According to Allen, notable proportions of tin and copper have been detected in coffee extracts and essences.

It may be interesting to note that Clayton (*Analyst*, 1897, **22**, 172)

has estimated the caffeine in 11 samples of coffee infusion supplied in London "Coffee-palaces." He found proportions ranging from 0.014 to 0.039%, with a mean of 0.026%. A 10% infusion of coffee yielded 0.12%. Hence the strength of the infusions examined ranged from 1 to 3 parts of coffee per 100 parts of water.

Kola.

The Gourou or Kola-nut, from a tree belonging to the family *Sterculiaceæ*, is chewed and used for preparing a beverage in Western Africa, by the negro inhabitants of the West Indies, Brazil, etc.

Kola-nuts are oblong, 3 forming a ball fully 2 in. in diameter, and resembling a very large horse-chestnut. Those imported from Trinidad and the West Indies are often not much more than half this size. The individual nuts have a rugged, dark brown surface. Inside they are light brown, becoming rusty on exposure, and tough as wood. When fresh the taste is first sweet, then astringent, and finally bitter. After drying the bitterness diminishes.

Various other African plants yield seeds closely resembling the true Kola. Of those used to adulterate Kola, only two, according to the analyses of Heckel and Schlagdenhauffen, contain caffeine, viz., *Cola Ballay* nut, 1.05%, and *Cola Gaborensis*, 0.26%.

From the nut of *Sterculia* or *Cola acuminata*, the female or true Kola, Heckel and Schlagdenhauffen (*Pharm. J.*, 1883 [*iii*], **14**, 584) obtained the following products:

Extracted by Chloroform:	Caffeine,	2.348%
	Theobromine,	0.023%
	Fats,	0.585%
	Tannin,	0.027%
Extracted by Alcohol:	Tannin,	1.591%
	Kola red,	1.291%
	Glucose,	2.875%
	Salts,	0.070%
Undissolved:	Starch,	33.754%
	Gum,	3.040%
	Colouring matters,	2.561%
	Proteins,	6.761%
	Cellulose,	29.830%
	Ash,	3.325%
	Water,	11.919%

Uffelmann and Bömer (*Zeit. angew., Chem.*, 1894, **71**, 710) give the following mean composition of 10 samples, ranging in price from 4/- to 20/- per lb.

Water,	13.35%
Total nitrogen,	1.53%
Protein,	5.91%
Caffeine and theobromine,	2.08%
Ether extract,	1.35%
Starch,	45.44%
Tannin,	3.79%
Cellulose,	7.01%
Other nitrogen-free extractive matter,	18.21%
Mineral matter,	2.90%

Knox and Prescott (*J. Amer. Chem. Soc.*, 1897, **19**, 63) have estimated the moisture and the total alkaloids, free and combined, in 1 sample of dried and in 4 samples of fresh kola seeds. They consider that discrepancies in analyses published hitherto are to be attributed to incomplete liberation of the caffeine from its state of combination and to faults in the method of estimating the alkaloid. They employed Gomberg's volumetric method for estimating the caffeine (page 612).

The following table shows their results:

Sample	Fresh Kola				Dried Kola		
	Moisture %	Free alkaloids %	Combined alkaloids %	Total	Free alkaloids %	Combined alkaloids %	Total %
No. 1. Dried Kola, mixed, I.	6.16	.....	.....	.....	1.859	1.783	3.642
Dried Kola, mixed, II.	.....	.....	.....	.....	1.828	1.836	3.664
Average	.	. . .	.	. .	1.843	1.809	3.653
No. 2. Fresh Kola, red and white seeds. Average.	53.9	0.534	0 884	1.418	1.158	1.922	3.080
No. 3 Fresh Kola, red and white seeds, very mouldy. Average.	53.9	0.569	0 854	1 423	1.235	1.854	3.089
No. 4. Fresh Kola, white seeds. Average.	51.2	0.578	1.018	1.596	1.186	2.085	3.271
No. 5. Fresh Kola, red seeds. Average.	57.3	0.478	0.693	1.171	1.120	1.625	2.745

According to E. Knebel (*Apoth. Zeit.*, 1892, 112), Kola-nuts contain a glucoside, ***Kolanin***, which on boiling with water, or by treatment with dilute acids, splits up into caffeine, glucose, and Kola-red, $C_{14}H_{12}(OH)_8$. This last product is an extremely unstable substance, taking up oxygen during the drying of the nuts, with separation of water and formation of gallotannic acid, $C_{14}H_{10}O_9$.

Knox and Prescott (*loc. cit.*), however, are of opinion that kolanin is really a mixture of the tannates of caffeine and theobromine. An artificial tannate of caffeine prepared by them closely corresponded in ultimate composition and in physical and chemical characteristics with kolanin from natural sources. Moreover, sterilisation, although inhibiting the formation of Kola-red, did not interfere with the liberation of the alkaloids. Hence they conclude that there is no evidence that Kola-red and caffeine are joint products of the hydrolysis of a glucoside. Perrot and Goris (*Pharm. J.*, 1908 [*iv*], **26**, 31) regard kolanin as a mixture of kola red and caffeine. They state that it is not glucosidal and cannot be regarded as a definite constituent of Kola.

Goris and Chevalier (*Pharm. J.*, 1908 [*iv*], **26**, 31) have isolated a phenolic substance, ***kolatëin***, $C_8H_8O_4$, from fresh Kola seeds, as small colourless crystals m. p. 148°, slightly soluble in water and readily soluble in alcohol, acetone and ethyl acetate. The yield obtained was 0.75% of the fresh seeds. Under suitable conditions of oxidation it yielded Kola-red. Kolatin is stated to be to a certain extent antagonistic physiologically to caffeine and hence the seeds, sterilised before drying, have an action different from that of the dried seeds in which the kolatin no longer exists, it having been converted into Kola-red.

Tschirch and others have pointed out that the formation of Kola-red is due to the presence of a ferment in the fresh seeds and that sterilization by heating at 80° for 30 minutes prevents the development of the coloured substance.

Goris (*Bull. Soc. Pharm.*, 1911, 138) has also detected a second phenolic substance, ***kolatëin*** (m. p. 257–8°) resembling phloroglucinol in some of its properties.

Theobromine is present in kola seeds in small amount. Knox and Prescott found it averaged about 1.5% of the mixed alkaloids. According to Dekker (*Schweiz Wochenschr.*, 1902, **40**, 569) the proportion is much higher in Kola *leaves*. From young leaves he obtained 0.15% of alkaloids which proved to be a mixture of caffeine and theobromine

in the ratio of 1 of the former to 2 of the latter. Polstorff found 0.25% of betaine in kola nuts (*Chem. Zentr.*, 1909, **2**, 2014).

Monaron and Perrone state that powder and extract of Kola-nuts have a far greater power of diminishing the elimination of phosphates and nitrogen than caffeine alone has. Kola-red has a diminishing influence, but both it and caffeine act better in their natural combination than separately. Caffeine has a diuretic action, whereas Kola is anuretic. The drug prevents waste of brain as well as of muscular tissue.

False Kola, Male Kola, or Kola Bitter, is the seed of *Garcinea kola*, a plant of the family of the *Guttiferæ* growing in Liberia and Central Africa. On extracting the seeds with chloroform, ether, and alcohol, no caffeine is obtained, but only resins. One of these gives a violet colouration with ferric salts, while the other is dextrorotatory and precipitated by tartar emetic and basic lead acetate. The physiological action of the extract of kola bitter is attributable to these resins.

Guarana.

This product occurs in the form of cylindrical masses about 30 mm. thick and 10 to 30 cm. long. It is an indefinite mixture of various materials, of which the seeds of *Paullinia sorbilis* appear to be the only constant and characteristic ingredient. It is prepared by the Guaranis, a tribe of half-savage Indians on the upper Amazon. Its only interest is as a source of caffeine, of which is contains a notable proportion. Stenhouse obtained 5.04%, and F. V. Green 5.05%. E. R. Squibb found 4.83% (*Ephemeris*, **2**, 615). J. H. Feemster (*Pharm. J.*, 1882 [*iii*], **13**, 363) obtained from 3.9 to 5.0% of caffeine from 5 samples of guarana. The alkaloid is readily isolated in a state of purity by boiling the substance with water and litharge for some hours, or until the liquid is colourless and the deposit settles readily, concentrating the filtered liquid, and agitating with chloroform. M. Nierenstein (*Ann. Trop. Med. Parasit.*, 1910, **4**, 115) described a new alkaloid, *β-guaranine*, obtained from the seeds of *P. trigonia*, a variety of guarana used as a remedy against diarrhea in Brazil. The formula ascribed to β-guaranine is $C_{49}H_{47}O_{21}N_4$ and Nierenstein was unable to detect caffeine in the guarana examined by him.

A valuable historical and descriptive account of guarana is given by P. H. Marsden (*Ann. Trop. Med. Parasit.*, 1910, 4, 105).

Guarana is official in the United States and many of the Continental pharmacopœias. The *United States Pharmacopœia* requires that it shall yield, when assayed by the official process, not less than 3.5% of its alkaloidal principles.

COCOA AND CHOCOLATE.

By R. WHYMPER.

The cacao tree, *Theobroma cacao*, flourishes in warm moist climates. It is indigenous to tropical America, and grows freely there under cultivation.

The chief centres of cocoa-growing are Mexico, the Isthmus of Panama, Trinidad, St. Lucia, Cuba, Hayti, Jamaica, San Domingo and Guadeloupe; in South America, Venezuela, Columbia, Ecquador, Peru and the northern part of Brazil, especially Para, are of importance.

The cacao tree has been successfully naturalised in the Philippine Islands, Java, Celebes, Amboyna, in Ceylon and parts of Africa and Australia.

Although the cocao seeds from different districts vary considerably in appearance and flavour, they do not present any sharp distinction in chemical composition. It is of importance to note, however, that the various processes practised in the different districts between the time of plucking and shipping are entirely responsible for the development of the aroma, the freedom of the seeds from earth, clay, etc., the moisture content, and other properties which are of economic importance to the buyer, and which cause the beans from each district to have a different market value.

The fruit of the cacao tree contains from 25 to 40 almond-shaped seeds closely packed in a mucilaginous pulp.

The seeds or beans are separated from the pulp and subjected to a period of fermentation, either in the pulp itself, which starts fermenting in 24 hours, or else the seeds are packed into tubs, where, with their natural moisture and the small quantity of pulp adhering to them, they are allowed to ferment for 60 hours. The temperature generated during the normal progress of fermentation is, of course, a variable one in different districts, but in Jamaica it is considered a good fermentation when a gradual rise of temperature from 30° to 43°, extending over 3 days, is maintained.

The results of fermentation seem to be a little doubtful, though the practical benefits to be attained are, the conversion of the harsh red colour of the bean to a rich chocolate hue, the hardening of the shells, and the modification and improvement of the odour and flavour. Examination and analysis show that during the alcoholic fermentation of the sugars of the pulp, sufficient heat is generated to bring about hydrolysis of some of the bitter and astringent matter of the bean; at the same time a certain amount of mineral matter, chiefly potash and phosphoric acid, is removed in the liquid diffusing from the fermenting bean (J. H. Hart, "Cacao," 1900, 106, and A. Preyer, "Der Tropenpflanzer," 1901, 5).

The fermented beans are then dried in the sun or by artificial heat, when they are ready for exportation.

Some cacaos, after or during drying, are coloured with red earth or clay to improve the appearance. This treatment is obviously accompanied by increase in weight.

The process of roasting to which the beans are next subjected is one of great importance and takes place between 130 and 140°, a temperature below that used for roasting coffee.

During roasting, the aroma is developed, water and acetic acid are expelled, the astringent properties are modified and the husk of the bean, which represents 8 to 14% of the entire seed, is rendered more friable, and in a condition to be readily removed.

N. P. Booth and others (*Analyst*, 1909, **34**, 137) give the following tables illustrating the changes which take place in the bean during roasting.

TABLE I.

Constituents	Grenada bean (with shell)		Trinidad bean (without shell)	
	Raw, %	Roast, %	Raw, %	Roast, %
Moisture	6.32	3.10	6.67	4.45
Fat	46.50	46.96	54.60	55.70
Nitrogen	1 96	1 86	2 28	2.32
Fibre	3.60	3.90	2.45	2 48
Total ash	2.86	3.12	2.87	2.73
Siliceous matter	0.10	0.12	0.03	0.08
Soluble ash	1.26	1.44	0.94	0.95
Alkalinity as K_2O	0 68	0.75	0.42	0.43
Cold-water extract	13.50	12.90	12.73	12.00

The figures bear out the author's experience, though occasionally a diminution in the fat content is found, if the beans have been fiercely or too long roasted.

The roasted bean is cooled as rapidly as possible, being constantly stirred to prevent the self-contained heat from spoiling the roast. When thoroughly cool the beans are passed through a "nibbing" or "kibbling" machine, which cracks the shell and frees the kernel, which by a process of winnowing and sieving is separated from the husk, and falls into receptacles according to its size. Crushing, mixing with sugar, flavours, etc., refining, blocking off and other details of chocolate manufacture follow this process but cannot be dealt with here. (Dr. Paul Zipperer, "The Manufacture of Chocolate," 1902, also Auguste Jacoutot, "Chocolate and Confectionery Manufacture." Whymper, "Cocoa and Chocolate," 1912.)

An extremely large number of analyses of raw and commercial cocoas have been made, and, as it is essential that the source from which the cocoa came should be known, all analyses of unnamed specimens have been excluded. The following analyses of commercial raw cocoa, after removal of the husk, are by Eastes and Terry (*Pharm. J.*, 1885 [*iii*], **15**, 764).

Kind of cocoa	Moisture %	Fat %	Theobromine, %	Ash %	H_3PO_4 %
Caracas	4 75	53 65	1 08	2 76	1 36
Carupano	5 04	47 38	0 87	3 69	1 39
Grenada	5 59	47 12	1 42	2 81	0.91
Guayaquil	3 68	52 97	1 74	3.28	0 85
Para	4 39	57 07	1.00	3 09	1 30
Surinam	2 55	53 70	1 42	2.44	0.85
Trinidad (common)	5 62	45 71	1 05	2 79	0 89
Trinidad (St Antonio)	4 72	53 57	1 94	2 70	1.15

Ridenour (*Amer. J. Pharm.*, 1895, **67**, 207) has published a series of analyses, of raw and roasted cacao beans, which do not agree with observations made by other chemists. Although the values for fat, albumen, starch and ash differ so essentially from results hitherto obtained that they cannot be taken as representing the analyses of normal beans, they must not be discredited as inaccurate.

Some of the most recently published analyses of nibs (roasted and shelled cocao) from known sources are those made by N P. Booth

(*Analyst*, 1909, **34**, 143), and are thoroughly typical of cocaos from which the husk has been removed as far as possible by mechanical means. The second table shows the analyses of the husk separated from several of the varieties of the nibs.

ANALYSES OF NIBS (BOOTH).

Constituents	African %	Grenada %	Guayaquil %	Trinidad %	Caracas %	Bahia %	Ceylon %
Total mineral matter...	2.52	2.60	3.16	2.73	3.24	2.68	3.81
Soluble mineral matter.	0.98	1.04	1.32	0.95	1.58	1.22	1.66
Siliceous matter.....	0.05	0.03	0.04	0.08	0.08	0.05	0.03
Alkalinity of mineral matter as K_2O.	0.38	0.55	0.53	0.44	0.74	0.51	0.67
Cold water extract.....	11.80	9 80	11.40	12.00	.....	9.50	11.90
Nitrogen.....	1.84	2.26	.....	2.32	.....	1.98	2.44
Fat	50.20	50.80	.....	55.70	.....	44.40	50.20
Fibre.....	.....	2.94	.....	2.48	.....	.....	2.36

ANALYSES OF HUSK (BOOTH).

Constituents	African, %	Guayaquil %,	Ceylon, %
Total mineral matter.....	5.63	8.19	6.61
Soluble mineral matter..	3 53	5.25	4 78
Siliceous matter.....	1 79	1.45	1 00
Alkalinity as K_2O..	2 63	3.36	2.54
Cold water extract.....	20.40	24.60	20 70
Nitrogen..	2 91	2.13	2.40
Fat.....	3 50	5.90	3 10
Fibre.....	15 70	12.85	12.80

These tables show the content of such constituents as will enable the chemist to detect the presence of husk among the nibs, and include the analyses of nibs and husk as far as will be found usually necessary for practical purposes. It may, however, be required to analyse the bean more completely, when the following table will be found useful for reference:

ANALYSES OF COCOA NIBS.

Constituents	Raw,[1] %	Raw,[2] %	8 varieties Raw,[3] %	8 varieties Roasted,[4] %	8 varieties Raw,[5] %	8 varieties Roasted,[5] %	Average of 12[6] varieties Raw, %
Moisture	7.6	5.0	2.5–5.6	3 7–4 4	6 2–8.3	6.3–8.5	
Fat	49.9	51.0	45.7–57 1	45 3–54 4	50 4–53.7	46.9–52.1	43 0
Theobromine	3.3	1.5	0 9–1 9		0 3–0.8	0.3–0.5	1.0
Ash	4.0	3.6	2.7–3.7	2 4–3.9	2.7–4.1	2.9–4 8	3 7
H_3PO_4			0 9–1 4	0 9–1.9			
Protein matter	10.9	17.0		7.4–13.0			10.5
Cellulose	10.6 (rol.)	8.0			14.5–24 1	18.2–24 4	14 4
Lignin							5 9
Starch	2.4			26 3–39 4 (Total carbohydrates)	5 8–11.1	8 7–12 6	4.7
Glucose							1.5
Cane-sugar					7 9–13 7	7 2–8 6	0 9
Astringent principles	0.2						
Cocoa-red		3 0					
Tartaric acid	3.4						
Gum	2 4	10 9, etc					

[1] Boussingault (*Ann. Chim Phys.*, 1883 [v], 28, 433).
[2] Church (*Foods*, 200)
[3] Eastes and Terry (*Pharm. J.* [iii], 15, 764).
[4] Heisch (*Analyst*, 1876, 1, 142).
[5] Zipperer ("Investigations of Cacao and its Preparations," 56, 57; also "The Manufacture of Chocolate," 33, 34).
[6] Ridenour (*Amer. J. Pharm.*, 1895, 67, 207).

Cocoa-essence, -extract, -powder, etc.

The definition of cocoa-powder as adopted by the Union of German Chocolate Manufacturers is "The product obtained after pressing out a portion of the cacao butter from roasted and shelled cacao beans. Soluble cacao is prepared by treatment with alkalies, steam pressure, water, or by some other suitable process"

The following table shows the analyses of several well-known brands of cocoa powders, made by different investigators

The points of importance for the analyst to observe in investigating the purity of cocoa powders are the presence of husk, the alkalinity of the ash (due to the treatment of the bean with alkalies in the preparation of so-called "soluble" cocoas), and the presence of added starch and sugar.

The relatively large amount of cocoa fat in the cocoa bean renders the latter too rich and indigestible for consumption by persons suffering from gastric affections This circumstance has caused a demand for preparations of cocoa containing less fat—an end which is obtained by expressing a quantity of the fat from the nibs by heat and pressure, the resulting powder being known as cocoa-powder, -essence or -extract Cocoa-powder particularly lends itself for adulteration to the unscrupulous manufacturer who is able to add powdered husk—a bye-product of little nutriment and value—starch and sugar, and to sell the mixture as pure cocoa-powder Such adulterations can, of course, be readily detected by analysis, the methods for which are described later

There are, however, many nourishing cocoa-powders on the market which contain selected arrowroot and other starches, with or without the addition of sugar, though they cannot be said to be adulterated but rather improved by the addition. Such as these, provided that they are not sold as pure cocoa-powder, and that the presence of added starch, sugar, etc , is clearly notified on the package, are bought by the public with a full knowledge of what they contain [1]

So-called "soluble" cocoa-powders are now in considerable demand and are the result of cocoa so treated with alkalies that they are readily miscible, in the form of an emulsion, with water or milk. (J M. Albahary, *Ann Falsif*, 1910, **3**, 159–165)

[1] Epps' cocoa contains 40% cocoa, 16% West Indian arrowroot, 44% sugar (Evidence given in case of "Gibson v Leaper")

ANALYSES OF COCOA POWDERS.

Constituents	Fry's Cocoa Extract		Van Houten's Cocoa		Rowntree's Extract of Cocoa		Epps' Prepared Cocoa	Chocolat Menier	Bensdorp's Pure Royal Dutch Cocoa	Cadbury s Cocoa Essence
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(1)	(2)	(2)
Moisture		4 33		4 53		4 05			4 59	4 00
Ash { Total	4 24	4 28	8 64	8 19	8 48	7 70	3 15	1 40	6 69	4 70
Ash { Acid equivalent*	5 80		16 05		16 60		2 60	2 05		
Fat	30 95	31 16	29 80	29 78	27 56	30 82	25 94	21 31	33 06	27 58
Extractive soluble in water		5 26		9 88		7 48			8 52	6 48
Theobromine		1 36		0 69		1 08			0 88	0 70
Starch		16 07		21 26					11 33	21 05
Protein matter as albumen		12 78		17 03		15 22			11 41	13 58
Fibre	3 89		4 38		4 42		1 51	1 10		
Cane-sugar							26 0	58 0		
Added starch	Nil		Nil		Nil		Much arrow root	Nil		

* Acid equivalent = No of c c of $N/10$ acid required to neutralise ash from 2 grm of sample
E E Ewell (*U S Dept of Agriculture, Division of Chemistry*, Bull No 13, Pt VII, 933)
2. Florence Yaple (*Chem Zeit*, 1895, Rep 21, 240)

To give cocoa this character, it is subjected to treatment either in the raw or roasted state, with or without the partial removal of fat:

a. By means of water and heat, with or without pressure.

b. With alkalies such as potassium and sodium carbonates, magnesium carbonate (Dutch method), solution of ammonia or ammonium carbonate (German method), or weak solutions of potassium or sodium hydroxides.

The treatment of the bean with the fixed alkalies[1] can readily be detected by the increase in the quantity and alkalinity of the ash. Treatment by the German method is more difficult to detect.

The following are the results of the analyses of 4 cocoa powders examined by Stutzer (*Zeitsch. angew. Chem.*, 1891, 368) for the purpose of determining the effect of the process of manufacture on the chemical constituents. A. was composed of 40% Ariba, 40% Machala, and 20% Bahia cocao, and was manufactured by Wittekop & Company without the use of chemicals. B. is a sample of a well-known cocoa manufactured in Holland with the addition of potassium hydroxide.[2] C. and D. are German cocoas, which, in Stutzer's opinion, were prepared by the use of ammonia:

	A. %	B. %	C. %	D. %
Water	4 30	3 83	6 56	5.41
Fibre	3 36	} 37 48	39 99	36 06
Nitrogen-free extract	38 62			
Total nitrogenous substances[1]	20 84	19 88	20 93	19 25
Fat	27 83	30.51	27.34	33.85
Ash[2]	5 05	8 30	5 18	5.43
	100 00	100 00	100 00	100 00
[1] *Nitrogenous substances*				
Containing total nitrogen	3 68	3 30	3 95	3 57
Composed of:				
Theobromine	1 92	1 73	1 98	1 80
Ammonia	0 06	0 03	0.46	0 33
Amino-compounds	1 43	1 25	0 31	1 31
Digestible albumen	10 25	7.68	10 50	7 81
Indigestible nitrogenous substances	7 18	9 19	7 68	8 00
Containing nitrogen	1 15	1 47	1 23	1 28
Proportion of total nitrogen indigestible	31 20	44.50	31.20	35 80
[2] *Ash*				
Containing: Total P_2O_5	1 85	2 52	2.14	2.05
P_2O_5 soluble in water	1.43	0 50	0 74	0.77
Ratio of *total* P_2O_5 to *soluble*	100 77	100 19	100:34	100 37
Ash soluble in water	3 76	4 76	2.82	2.76
Ratio of *total* ash to *soluble*	100 74	100 57	100:54	100:49

[1] (Am Ed) In the United States the addition of alkali is permitted as long as there is no excess in the finished product It would only seem possible to determine this by the use of indicators as deductions based upon the alkalinity of the soluble ash would have to take into account, first, the alkalinity of the natural ash, which varies; second, the added alkalinity due to alkali used to neutralise resins, etc. Litmus paper probably is the best indicator to use

[2] An analysis of the ash of Van Houten's cocoa by König (in 1880) showed: Total ash 7.84; K_2O, 3 52; CaO, 0 27; MgO, 0.81; P_2O_5, 1.84.

E. G. Clayton (*Chem. News*, 1902, **86**, 51) has made analyses of different samples of cocoa essences, which consist of partially defatted and otherwise treated cocoas. A very complete investigation into the component parts of the ash obtained from them has been made.

It is often desirable to ascertain the proportion of real cocoa in a mixture, but owing to the fact that a large quantity of the natural fat has been removed this becomes a matter of considerable difficulty.

J. Bell ("Analysis and Adulteration of Foods") has adopted the plan of stating the fat and non-fatty constituents separately, and the following analyses made by him represent the composition of certain commercial preparations of cocoa:

Constituents	Finest Trinidad nibs	Cocoa extract	Flake cocoa	Cocoa-tina	Prepared cocoa	Chocolat de Santé
Moisture	2.60	5 76	5 49	3.52	4 95	1 44
Fat ...	51 77	29 50	28 24	23 98	24.94	22 08
Added sugar	Nil	Nil	Nil	Nil	23.03	61.21
Added starch	Nil	Nil	Nil	Nil	19.19	2.00
Non-fatty cocoa (by difference)	45.63	64 74	66 27	72.50	27 89	13.27
Nitrogen.....	2 95		3 06	4.07	2 24	.. ....

"Chocolate-surrogate" or "suppen powder" will occasionally be met with, consisting of a small quantity of cocoa with cocoa waste, sugar, meal, spices and colouring matter in varying quantities.

"Cocoa tea" of Germany and "miserables" of Ireland are independent articles of commerce and consist of cocoa husks only, the infusion of which in boiling water is drunk after the manner of tea.

Chocolate, Milk Chocolate and Preparations of Chocolate.

In its simplest form, commercial cocoa consists of the roasted and husked seeds ("nibs") ground to a paste or semi-fluid, and run into moulds in the form of cakes. Flake chocolate is met with and is made by passing the decorticated seeds through a particular form of roller sets. Such cocoas are frequently termed "bitter chocolates" or "base."

The terms "cocoa" and "cocoa-powder" have been applied to mixtures of real cocoa with sugar. Such a mixture, in order to avoid misconception and confusion, should be termed "chocolate."

"Chocolate" is defined by the Union of German Chocolate manufacturers as a "mixture of roasted and shelled cacao with sugar (cane

or beet-root) and an addition of cacao butter, vanilla, vanillin, cinnamon, cloves and other spices or their essential oils"

The process of converting "bitter" chocolate into what is known as "chocolate" is of little interest to the analyst, as it does not involve any change in chemical constituents of the "base," the only changes being those of addition, while the actual process is purely mechanical.[1]

The quantities of materials added to bitter chocolate or "base" depend upon the quality and nature of the resulting chocolate required.

The following analyses by N. P. Booth (*Analyst*, 1909, **34**, 145) show a few of the variations that may occur:

Constituents	I %	II %	III %	IV %	V %	VI %
Fat	34 60	22 10	28.10	25.90	23.00	30.70
Sugar	46 00	54 00	52 20	48.00	56 00	44 00
Nitrogen	0.86	0.92	1.14			
Mineral matter	1.45	1.22	1 50	1.04	1.01	1.96
Cold water extract	50.00	58.10	58.20			
Moisture				1.80	0 80	1.20
Foreign starch	Nil	Nil	Nil	15.00	8 00	20 00
Character of starch				Arrowroot	Wheat	Maize
Fat-free cocoa matter.				10 00	12 00	8 30
Fibre (calculated on fat-free cocoa matter).				8.70	10 00	16.60

The first 3 columns are analyses of chocolates free from foreign fats and starches; the latter 3 are of chocolates containing foreign starches; while VI also shows a high ash due to the presence of added red ochre.

Among other starches, wheat, barley, maize (cornflour), rice, sago, and arrowroot are frequently to be found added to chocolate: there are also many cacao-butter substitutes now on the market which from their cheapness or hardening qualities are much in demand. Methods of detection and analyses of these fats are fully described later.

Besides sugar, saccharin is used as a sweetening agent, though only for diabetic chocolates, as it is expensive and does not give weight in proportion to its sweetening qualities. Chestnut, bean and acorn meal are variously added on occasions for flavour, and their presence can be detected under the microscope.

Vanilla, vanillin, cloves, cinnamon, nutmeg and mace, cardamons and various essential oils are principally used for flavouring purposes.

Salep, an amylaceous powder from the tubers of various kinds of orchids, is but little used now for its flavour.

[1] A. Jacoutot ("Chocolate and Confectionery Manufacture"). P. Zipperer ("Manufacture of Chocolate," 2d Ed., 1902). Whymper ("Cocoa and Chocolate," 1912).

Milk Chocolate, Nut Chocolate.

The addition of milk in the powdered or condensed form to ordinary sweet chocolate has become extremely popular with every chocolate manufacturer, and is in much demand by the chocolate-eating public for its smoothness and softness to the palate. Such an addition, however, considerably complicates the analysis by increasing the nitrogen, and introducing fresh varieties of sugar and fat.

As no other reducing sugar is likely to be used in chocolate-making, the presence of milk may be inferred from the reducing power of the cold water extract, and after allowance has been made for the reducing power of the cold water extract of genuine cocoa can be estimated by this means.

The analyses of milk chocolate given here are by N. P. Booth (*Analyst*, 1909, **34**, 145):

Constituents	I, %	II, %	III, %	IV, %	V, %	VI, %
Cocoa fat	27 40	31.60	25.70	23.40	27.70	22.50
Milk fat	8.30	3.00	7 60	8.10	5 70	8.40
Milk-sugar	11.10	5 00	10 40	9 00	7.50	5.50
Cane or beet sugar	39 10	54 30	38 40	42.70	42 90	50 20
Foreign starch	Nil	Nil	Nil	Nil	Nil	Nil
Cocoa shell	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	1.10	0.76	1.68	1.28	1 30	1.10

The first 3 are analyses of English, the latter 3 of Swiss and German milk chocolates.

A further series of analyses of milk chocolate have been made by O. Laxa (*Zeitsch. Nahr. Genussm.*, 1904, **7**, 471), a few of which are given below:

Constituents	Peter's Gala, %	Peter's Croquettes, %	Suchard's Milka, %	Cailler's Croquettes, %
Moisture	0.77	1.79	1.22	2.26
Total protein	9 66	9.15	8.13	10.94
Fat	31 47	31 91	32.33	31.12
Milk-sugar	7 32	7 42	8.70	7.84
Cane or beet sugar	27.51	39 42	35.93	33.68
Ash	1.87	1.96	1.82	2.28

In nut chocolate, ground hazel nuts or almonds, usually roasted, take the place of the milk solids in milk chocolate, and, beyond the necessary amount of sugar, the balance should be supplied by the product of the cocoa nib.

Component Parts of Cacao Beans.

Husk (12–18% of Whole Bean).—The shells or husks from the cacao bean are used for making cheap chocolates either *per se* or by addition to cocoa nibs. They have also found a use as cattle food.[1] For this latter purpose many analyses or valuations have been made, of which the few following are selected from those most recently published:

Constituents	I,[2] %	II,[3] %	III,[4] %	IV,[5] %	V,[6] %	VI,[7] %
Water	12.57	5.12	...	...	9 30	12.51
Fat	3 30	12.92	3.6	...	3.83	4.23
Ash	7 35	6 92	5.7	6 46	8 26	10.20
Proteid	14 69	16.44	12 8	...	18 81	13.69
Fibre	16.33	13 17	...	13 05	13.85	16.71

Fibre (12–18% of Husk).—The quantity of cellulose present in a cocoa is a guide to the extent to which husk has been included. Fincke (*Zeitsch. Nahr. Genussm.*, 1907, **13**, 265) has published the following information:

Constituents	Cocoa husks, %	Cocoa powder, %
Crude fibre	20 21	9.28
Cellulose	9 88	3 57
Lignin	9 92	0.24

These figures are, however, in excess of those usually found.

The analyses made by Ludwig (*Zeitsch. Nahr. Genussm.*, 1906, **12**, 153) of known mixtures of husk and cocoa show to what extent the fibre (estimated by a modified König's method) varies with the quantity of husk present.

Six samples of cocoa powder gave 4.98 to 5.96% (average 5.60%) of crude fibre, calculated on fat-free material; the quantity of fat present in the samples was 25.05 to 27.92%, giving an average of 25.78%.

[1] (Am. Ed.) In the United States experiments have been made to utilise the shells but with results that are not very encouraging. To the writer's personal knowledge they have been tried for cattle feeding, but only small proportions are tolerated in the rations. They make an excellent dressing for the soil but they would not bring much for this purpose in addition to hauling, etc. Shells are of value for extracting "Dutch IIa" cacao butter, however.

[2] G. Paris (*Zeitsch. Nahr. Genussm.*, 1898, 6, 389).

[3] F. T. Schutt (*Annual Rep. on Experimental Farms* (Canada), 1898, 151).

[4] J. Dekker (*Chem. Zentr.*, 1902, 2, 1217).

[5] H. Luhrig (*Zeitsch. Nahr. Genussm.*, 1905, 9, 263).

[6] A. Smetham (*J. Royal Lancs. Agric. Soc.*, 1909).

[7] Zipperer (*Untersuch. über Cacao und dessen Präparate*, 55).

The sample of cocoa husk contained 3.08% fat and 14.47% crude fibre.

A mixture of equal parts of the above-mentioned 6 cocoas was then made, to which varying proportions of husk were added. The following results were obtained:

	Fat	Crude fibre found	Crude fibre in fat-free powder calculated
Cocoa	25 78	5 50	5 60
Cocoa + 5% husk	24 37	6 03	6.16
Cocoa + 10% husk	24 00	6 39	6.71
Cocoa + 20% husk	21 44	8.06	7.79
Cocoa + 40% husk	16 62	10 18	9.74
Cocoa + 60% husk	12.04	11 72	11.48
Cocoa + 80% husk	8 26	13 28	13.04

The fibre, which is present in the cacao husk and other fibrous tissues in intimate association with sugar compounds, such as pentosans, is capable of being estimated by determination of the quantity of the latter.

Many chemists have given attention to the estimation of pentosans in cocoa during recent years and the following table compiled by R. Adam (7th *Internat. Cong. App. Chem.*, VIII, C, 194) is a fair sample of the results obtained:

	Moisture	Fat	Starch	Ash	Cellulose	Pentosans			
						Nibs		Shells	
						Initial substance	Powder with 30 % fat	Dry substance	Substance with 10 % moisture
Ariba	8 27	45 15	5.83	3 88	4 48	1 71	2.79		
Ariba, roasted	8 52	50 07	9 10	3 89	3 70	1 29	2 19		
Ariba, shells								9 97	9.11
Port au Prince	7 77	46 35	5 97	4 15	5 19	1 59	2 60		
Port au Prince, roasted	4 73	51 87	8 40	3 49	4 31	1 27	1 99		
Port au Prince, shells								7.57	6 92
San Thomé	8 08	46 61	5 69	4 28	4 43	1 43	2 34		
San Thomé, roasted	5.71	50 20	13.27	3 89	4 33	1 45	2 57		
San Thomé, shells								8 49	7 92
Caracas	7 77	45.54	5 48	4 91	6 18	1 56	2 71		
Caracas, roasted	7.48	49 24	9 85	3 92	4 24	1 19	1 86		
Caracas, shells								7.78	7 11
Bahia	5.96	42 10	7 53	3 63	7 86	2 19	3 14		
Bahia, roasted	3 71	50.19	9 61	3 24	3 93	1 77	2.73		
Bahia, shells								9 45	8 70
Soconusco	2.95	48 38	8.33	3.21	3 34	1 59	2 60		
Soconusco, roasted	5 00	50.22	9.58	3.76	3 78	1 21	2.06		
Soconusco, shells								10 53	9 51
Average	6 43	44 4	8 22	4 00	4 78	1 53	2.47	9 96	8.21

Ash (7–16% of Husk).—The ash obtained by burning the husk has been the subject of much analysis, and, though it depends largely upon the source of origin of the bean and the treatment to which it was there subjected, the following analysis of ash from a Trinidad sample gives a fair idea of the constituents (R. Bensemann, *Repert. der analyt. Chemie*, 1885, **5**, 178): potassium oxide (K_2O) 25.87; sodium oxide (Na_2O) 2.73; calcium oxide (CaO) 5.10; magnesium oxide (MgO) 5.21; ferric oxide (Fe_2O_3) 0.34; aluminium oxide (Al_2O_3) 0.71; silica (SiO_2) 2.42; phosphoric anhydride (P_2O_5) 4.70; sulphuric anhydride (SO_3) 3.40; chlorine (Cl) 1.02; carbonic anhydride (CO_2) 16.29; water 2.26; oxygen (O equivalent to chlorine) 0.29.

Duclaux (*Bull. Soc. Chim.*, 1872, 33) found small quantities of copper in the ash after burning cocoa. Galippe (*J. Pharm., Chim.*, 1883 [*v*], **7**, 506) confirmed this later, finding quantities of this metal varying from 0.01 to 0.03 grm. per kilo of cocoa. The greater part of the copper existed in the husk, and in some inferior chocolates as large a proportion as 0.12 grm. copper per kilo of cocoa was present.

The other constituents of cocoa husk, namely, fat, cqcoa-red, alkaloids, tannin and other astringents, are either of no importance to the analyst or will be considered under cocoa nibs, which contain them in greater quantity.

Cocoa Nibs (82–88% of Whole Bean).—Cocoa nibs contain, in varying quantities, water, starch, protein matter, cocoa-red, theobromine, fat, grape and cane-sugar in small quantities, fibre, and mineral matter.

Starch (4–13% of Nibs).—Cacao starch is one of the smallest kinds that appear in the vegetable kindgom. It usually consists of minute globular granules, generally separate but sometimes in aggregations of 2 or 3 granules. In common with other starches it is gelatinised by boiling water and shows the blue colouration with iodine solution.

Protein Matter (10–17% of Nibs).—The protein-matter is present in the bean mainly as globulin and is found in larger quantity in the unfermented than in the fermented bean.

Forster's (*Hygienische Rundschau*, 1900, **10**, 305) experiments on the digestibility of cocoa powder in the human stomach showed that 80% of the nitrogenous substance was readily assimilated.

Leffman and Beam (*Food Analysis*, 1901, 275–282) state that the nitrogenous constituents are of 3 kinds:

1. Non-proteins not precipitated by hydrated copper oxide (theobromine, caffeine and amino-compounds).

2. Digestible albumens, insoluble in water in presence of hydrated copper oxide, but soluble when treated with acid gastric juice and alkaline pancreatic extract.

3. Insoluble and indigestible nitrogenous matter.

	I, %	II, %	III, %
Nitrogen as soluble compounds (alkaloidal) . .	31.43	26 95	29 79
Nitrogen as digestible albumen	33.34	40 61	22 62
Nitrogen as indigestible matter .. .	33.33	32 44	47 83

Cocoa-red (3–5% of the Whole Bean).—Many investigators have assigned the peculiar aroma and flavour of the cacao bean to this constituent.

The pigment is formed as the fresh colourless bean ages and dries (similar to oak and kola nut red), and occurs during the decomposition of a glucoside *cacaonin*, when theobromine, caffeine and dextrose are also produced. (C. Schweitzer, *Pharm. Zeit.*, 1898, 389).

Hilger (*Apoth. Zeit.*, 1892, 469) has closely followed the production of cocoa-red during fermentation of the bean, and concludes that the cocoa-red isolated in the ordinary way is a mixture of pure non-nitrogenous cocoa-red and some glucoside. It can be isolated by treating the roasted beans with petroleum ether, to remove the fat and part of the free theobromine, then with water to extract the remainder of the theobromine, caffeine, sugar and salts, and finally with alcohol to extract the cocoa-red. After further purification, a substance termed "true cocoa-red" is obtained, having a formula $C_{17}H_{12}(OH)_{10}$. In this form it is closely akin to tannin, which it resembles by yielding formic acid, acetic acid and catechol by treatment with potassium or sodium hydroxide.

A. W. Blyth ("Foods, Their Composition and Analysis," 1909, 363–375) states that fat-free cocoa is only partially deprived of its cocoa-red by solvents, unless a mineral acid has been previously added. By adding a few c.c. of hydrochloric acid and gently warming for a few seconds, the red colouring matter is dissolved with ease by amyl and ethyl alcohols.

The cocoa-red is insoluble in ether or petroleum ether, but slightly soluble in carbon disulphide. With the aid of sodium or potassium

hydroxide it is easily dissolved, from which solutions it can be precipitated by means of mineral acids.

Cocoa-red is a sensitive reagent to acids and alkalies; mineral acids turn it red with violet fluorescence, alkalies usually strike a dirty green.

The aqueous alkaline solution is bitter and astringent and forms a precipitate with salts of iron, copper and silver.

Theobromine (0.9–3.0% of Nibs; 0.4–2.0% of Husk) **and Caffeine** (0.05–0.36% of Whole Bean).—Both alkaloids are found in the unfermented and fermented beans, and the former is prepared commercially from the husks.

Theobromine is a pure white powder, subliming at 220°.

Theobromine and caffeine both give the murexide reaction when treated with chlorine water, forming amalic acid. This, rapidly dried down on a watch glass with the addition of a drop of ammonia, at the end of the operation produces a violet colouration which readily distinguishes theobromine and caffeine from other plant alkaloids which do not belong to the xanthine group.

The following analyses by Wolfram (*Zeitsch. anal. Chem.*, **18**, 346) show the percentage of theobromine in nib and husk of various beans:

Bean	Theobromine	
	Bean, %	Husk, %
Caracas	1 63	1.11
Bahia	1 64	0.71
Domingo	1 66	0 56
Guayaquil	1 63	0 97
Puerto Cabello	1 46	0.81

P. Traganowski (*Arch. Pharm.*, 1877 [*iii*], **10**, 32) found from 1.2 to 4.6% of theobromine in cocoa and concluded that the proportion of alkaloid does not always bear a relation to the quality and value of the cocoa.

The recorded values of theobromine are very variable and untrustworthy, arising from the lack of any very accurate and reliable means of estimation. Leffman and Beam (*Food Analysis*, 1901) give Weigmann's figures as follows:

	Bean.	*Husk.*
Theobromine,	1.26	0.50
Caffeine,	0.17	0.15

Gum (2% of Nibs).—The gum from cocoa is precipitated by ethyl

alcohol from an aqueous extract of the fat-free bean. It resembles gum arabic but is dextrorotatory.

Cocoa Fat or Butter (45–55% of Nib, 4–5% of Husk) (*Oleum Theobromatis, British Pharmacopœia*) (see also Vol. 2, pages 71, 176). —Cocoa fat is a mixture of glycerides of fatty acids and contains in addition to stearin, palmitin and laurin, the glyceride of arachidic acid. It is solely extracted from cocoa beans and must not be confused with coconut fat from *Cocos nucifera*. To prevent this misunderstanding it is usually termed "cacao butter."

It is a yellowish fat of firm consistency, of a strong cocoa flavour and aroma; its fracture is partly granular.

The degree of fermentation and roasting to which the beans have been subjected have important bearing on the proportion of fat that the bean will finally yield to solvents. The percentage of fat is independent of the locality whence the bean originated (Davies and McLellan, *J. Soc. Chem. Ind.*, 1904, **23**, 480).

The following figures bearing on this subject are of importance:

	Unfermented	Mildly fermented	Fully fermented	Highly fermented	Very high roast	Very low roast
Fat, %. .	54 68	56 73	57.35	58.23	54.0	52.7

The specific gravity of the fat is variously given. Hager gives the normal sp. gr. at 15° as 0.95 for fresh, and 0.945 for old butter, though later values for fresh butter are given as 0.96–0.98.

Butter extracted by solvents shows a higher sp. gr. than expressed butter; thus Rammsberger gives 0.85 for expressed butter and 0.99 for extracted butter. At 100° Allen gives a value of 0.86.

The melting-point is usually given at 33°, but figures varying from 28° to 36° are given by various investigators as the m. p. of cacao butter from different sources and extracted by different means. Old butter has undoubtedly a higher m. p. than fresh.

White and Braithwaite (*Brit. and Col. Druggist*, 1897, 21) give the following m. p.

Bean	M. P.
Guayaquil	33.6–33.9°
Grenada	33.0–33.3°
Trinidad	31.5–32.5°
Caracas	33.0–33.6°
Ceylon	33.9–34.2°

Generally it may be taken that the m. p. of genuine cacao butter should not fall below 32°, nor over 35°.

Adulteration in cacao butter cannot be detected by the alteration in m. p.

The iodine absorption of a large number of samples of cacao butter from different sources has been determined by Filsinger (*Zeitsch. anal. Chem.*, 1896, **35**, 517) and found to range from 33.4 to 37.5; this is the average range for genuine cacao butter.

Strohl (*Zeitsch. anal. Chem.*, 1896, **35**, 166) obtained a fat from Bahia beans giving an iodine value 41.7, but this may have been due to the beans having been over-roasted, or to the fat having been extracted by petroleum of high b. p.

Shell butter has a higher iodine value of 39–40 (Filsinger).

The saponification value, or Koettstorfer's number, amounts to 192–202 (Filsinger) and usually to 194–195. Its determination may be useful in detecting an admixture of coconut fat. Many other determinations of this figure show similar values (Lewkowitsch, Weigmann, Wright, etc.).

The Reichert-Meissl value expressing the potassium hydroxide value of the volatile fatty acids present is of value. It amounts to 1.6 c.c. *N* potassium hydroxide, if the fat is extracted from pure cocoa; while fat from a milk chocolate requires 2.5 c.c. (Welmans).

The refractive index taken on a Zeiss butyro-refractometer at 40° C. is 46–48°, and corresponds to a refractive index of 1.4565–1.4580 (Strohl).

The following table shows normal figures, of cacao butter compared with fats which are likely to be used as adulterants, estimated by the writer:

Fat	M. P.	M. P. of fatty acids	Iodine value	Saponification value	Refractive index on Zeiss' butyro-refractometer at 40°
Cacao butter	30–34.5°	48–52°	34–37.5	192–202	46–48
Earth nut oil		27–31°	92–101	190–197	55.0
Cotton-seed oil		38–40°	106–111	191–197	58–60
Oleomargarine	32–33°	42–43°	43–49	195–197	45–48
Beef tallow	43–49°	43–46°	35–37	193–198	49
Coconut fat	22–28°	24–25°	6–10	250–270	35.5
Paraffin	50–80°	3–4°			

Lewkowitsch (*J. Soc. Chem. Ind.*, 1899, 556) gives the following table of constants for cacao butter:

Sample	M. P.	M. P. of fatty acids	Iodine value	Saponification value	Reichert-Meissl value
1. Sample to be tested.	26.6–28.0°	47.2–49 2°	34.27–36.99	191.8–194.5	0.38–0.94
2. Cacao butter prepared in lab. from nibs	28.4°	.....	35.65	192.9	0.50
3. Genuine cacao butter kept 10 yrs. in a sealed bottle.	27.0°	48.0–48.27°	36.92	192.0	0.20
4. Genuine English cocoa butter (fresh)	28–33°	48.55–49.1°	35.37	193 5	0.38
5. English cocoa butter (another sample).	28°	48 85–49.2°	34.86	193.1	0.33
6 Dutch sample cocoa butter (fresh)	27.2–33°	. . . . .	34 55	192.8	0 83

Sugar and Plant Acids [Cane sugar about 1.0% / Dextrose about 1.5% / Tartaric acid about 3.0%] of Nibs

The presence of sugar in raw cacao beans was first pointed out by Schweitzer (*Pharm. Zeit.*, 1898, 390). The sugar is formed by the action of the cocoa ferment on the glucoside cacaonin during the operation of fermenting and drying the beans.

Malic and tartaric acids have been observed, but neither is of special interest to the technical chemist.

Fibre[1] (2–6% of Nibs).—Cellulose, of which the greater part of the fibre consists, forms the chief constituent of the cell walls and vascular tissues. It is found in the bean with many impurities, such as cocoa-red, gum, mucilage, etc.

Cocoa nibs, cocoa powders and chocolate should contain but little fibre, and where found in quantities exceeding 6% its presence indicates either intentional adulteration of the cocoa with husk or carelessness in the nibbing process, which should almost completely separate nib from husk.

Mineral Matter or Ash (3–4% of Nibs).—A high ash content indicates adulteration with husk, or treatment of the bean with alkali

[1] The allowance by the U S. Dept. of Agric. in its published "Standard" is 3 5%. The amount found in sweetened chocolate must be calculated to a fat- and sugar-free basis and thence to the nibs.

in the process of preparing so-called "soluble" cocoas. It may also indicate addition of mineral colouring earths, such as red ochre.

R. Bensemann (*Rep. f. analyt. Chemie*, 1885, **5**, 178) has given a detailed analysis of the ash from burnt cocoa nibs; the figures following show the mean values obtained from 5 different varieties of beans:

Potassium oxide (K_2O) 32.25; sodium oxide (Na_2O) 2.17; calcium oxide (CaO) 4.0; magnesium oxide (MgO) 16.14; ferric oxide (Fe_2O_3) 0.36; aluminium oxide (Al_2O_3) 0.33; silica (SiO_2) 0.19; phosphoric anhydride (P_2O_5) 31.56; sulphuric anhydride (SO_3) 3.07; chlorine (Cl) 0.29; carbonic anhydride (CO_2) 6.80; water 1.94.

Copper has also been found in the ash obtained from the nibs (see under Husk Ash).

Matthes and Rohdlich (*Zeitsch. öffentl. Chem.*, 1908, **14**, 166) found the soluble silica in cacao beans to vary from 0.02–0.88%. The alkalinity of the ash will detect the presence of added alkali; thus Bordas (*Ann. Falsif.*, 1910, **3**, 61) finds an alkalinity of 2.40–3.05% K_2O calculated on pure dry fat-free cocoa, while samples of soluble cocoa gave values equivalent to 4.82–6.41% K_2O.

The United States standards for cocoa, chocolate and preparations of the same are given in "Food Inspection Decision 136," United States Dept. of Agric., as follows:

After consideration of the evidence submitted in regard to the meaning of the terms "chocolate" and "cocoa," the Board of Food and Drug Inspection has reached the conclusion that the definitions laid down in the "Standards of Purity for Food Products," adopted by the Committee on Food Standards, Association of Official Agricultural Chemists, and printed in Circular No. 19, Office of the Secretary of Agriculture, are substantially correct. By these definitions the names "chocolate," "plain chocolate," "bitter chocolate," "chocolate liquor" and "bitter chocolate coating" are applied to the solid or plastic mass obtained by grinding cocoa nibs without the removal of fat or other constituents except the germ, containing not more than 3% of ash insoluble in water, 3.50% of crude fibre, and 9% of starch, and not less than 45% of cocoa fat.

Sweet cocoa and sweetened cocoa are terms applied to cocoa mixed with sugar (sucrose) and contain not more than 60% of sugar (sucrose), and in the sugar and fat-free residue no higher percentage of either ash, crude fibre, or starch than is found in the sugar and fat-free residue of chocolate.

Cocoa nibs, and cracked cocoa, are the roasted broken seeds of the ocoa tree freed from shell or husk.

Milk chocolate and milk cocoa, in the opinion of the Board, should ontain not less than 12% of milk solids, and the so-called nut chocolate hould contain substantial quantities of nuts.

If sugar is added, for example, to milk chocolate, it should be labeled 'sweet milk chocolate," "sweet nut chocolate," etc.

When cocoa is treated with an alkali or an alkaline salt, as in the o-called Dutch process, and the finished cocoa contains increased ineral matter as the result of this treatment, but no alkali as such is resent, the label should bear a statement to the effect that the cocoa ontains added mineral ingredients, stating the amounts.

Cocoa and chocolate containing an appreciable amount of free lkali are adulterated. In the opinion of the Board, cocoa not treated vith alkali is not soluble in the ordinary acceptance of the term. Cocoa before and after treatment with alkali shows essentially the ame lack of solubility. To designate the alkali-treated cocoa as 'soluble" cocoa is misleading and deceptive.

Analysis.

The object of the analysis of cocoas and chocolates from the point of iew of the public analyst is the detection of adulteration, which may e practised by the addition of excess of husk, cacao butter substitutes, tarch and foreign colouring matters.

Microscopic Examination.—A preliminary examination of the ample under the microscope will reveal the presence of any added tarch, when both the nature and origin of the starch[1] and its approxinate proportion may be determined.

The microscope will further aid the detection of excess of husk, vhich can be recognized by the presence of characteristic spiral vessels nd numerous hyaline masses of mucilage which are tinged pink when reated with ruthenium red and 10% lead acetate solution.

The direct estimation of moisture, mineral matter, soluble ash, ilica, fat, fibre, cold water extract, total nitrogen, added starch (if ound under the microscope), and added sugar will be required to be nade in samples of cocoa, chocolate, and their preparations.

[1] (Am. Ed.) With the use of a polarising attachment to a microscope adulteration vith foreign starches such as wheat or corn shows very vividly. As the analyser is rotated hese foreign starches show luminosity and play of colours while the cacao starch does not pparently seem affected.

The methods of conducting these analyses are simple and require little detailed explanation. It is essential, however, for the analyst to have availiable a number of reliable analyses made by other observers for purposes of comparison, and it is for this reason that so many examples have been included.

Methods of Analysis.—The methods of analysis given here are capable of being applied, where necessary, to the raw and roasted bean, husk, nibs, cocoa-powders, -extract, etc., chocolate and preparations.

Moisture.—The presence of much moisture is objectionable in bean or cocoa-powders, and is impossible in chocolate properly manufactured.

2 grm. of the material are weighed out and maintained at 100° in a water oven till no further loss of weight occurs. The difference represents moisture. (See also Vol. 1, page 64.)

Ash.—The deliberate addition of mineral matter to cocoa and its preparations is rarely practised. Occasionally red ochre is met with in chocolate of poor quality, and the preparation of "soluble" cocoa by the Dutch method causes a higher ash figure, due to the presence of fixed alkalies.

In the absence of adulterants, the ash figure is of value as an indication of the amount of cocoa matter present; this has already been discussed.

The 2 grm. of material from the estimation of moisture are charred at the lowest possible temperature and burnt until free of carbon; if a carbon-free ash cannot be obtained in this way it is advisable to exhaust the charred mass with water, filter, burn the filter-paper and contents strongly till a white ash is obtained, add the filtrate to the ash and evaporate to dryness; the whole is then heated to a dull redness and weighed. (See also Vol. 1, page 72.)

Soluble Ash.—The ratio of ash soluble in water to the total ash is of importance as confirming the presence of red ochre or other insoluble mineral matter. If the weight of soluble ash is more than half that of the total ash, the addition of husk or alkali may be suspected (K. Farnsteiner, *Zeitsch. Nahr. Genussm.*, 1908, **16**, 625).

The total ash is boiled with water, the solution filtered through an ash-free filter-paper, and the deposit washed with hot water until the filtrate equals 60 c.c. The filter-paper and contents, and the filtrate, are separately placed in platinum dishes; the former after drying, the latter after evaporation, are then ignited and weighed.

Siliceous Matter or Ash Insoluble in Acid.—As this figure calculated as percentage of the ash is considerably higher in the husk than the nib of cocoa, the estimation may often be valuable.

The ash obtained from 2 grm. of material is boiled with 25 c.c. of 10% hydrochloric acid for 5 minutes; the insoluble matter is collected in a Gooch crucible, washed well with hot water, ignited and weighed.

Alkalinity of Ash.—K. Farnsteiner (*Zeitsch. Nahr. Genussm.*, 1908, **16**, 625) has stated that a cocoa, having an alkaline reaction and yielding an ash from 20 grm., requiring more than 15 c.c. of normal acid for its neutralisation, must be considered as having been treated with either potassium or sodium carbonate. An analysis of the ash will show which salt is present, as ordinary cocoa ash contains but little sodium salt.

If the alkalinity of the total ash exceeds 15 c.c. of normal acid and the insoluble ash is more than 60% of the total ash, magnesium carbonate has been added.

The simplest method of estimating alkalinity is to treat the entire ash with excess of $N/10$ hydrochloric acid and titrate the excess of hydrochloric acid with $N/10$ sodium hydroxide solution, using methyl orange as indicator. The alkalinity is best expressed as the number of c.c. of $N/10$ acid required to neutralise the ash from 1 grm. of sample.

Cold Water Extract.—This figure is remarkably constant in the case of the cocoa nib (about 24% of fat-free cocoa), and usually ranges from 40% to 70% in chocolate. In the latter case the cold water extract represents not only the cold water extract of the cocoa, but also the sugar.

2 grm. of the material are placed in a 100 c.c. flask and shaken with 50 or 60 c.c. of water, made up to 100 c.c., and left standing overnight. 25 c.c. are then filtered off, evaporated, dried and weighed.

It has been found more rapid and accurate to work on fat-free material.

Total Nitrogen.—Estimations of total nitrogen by the ordinary Kjeldahl method or by combustion with soda lime, give satisfactory results. In the cocoa nib it varies from 1.5 to 2.5%, and in unadulterated samples of chocolate the cocoa is the only nitrogen-containing constituent. (See also Vol. 1, p. 59.)

Fibre.—The estimation of fibre is of considerable value as a means of determining the proportion of husk. In cocoa free from husk it will usually amount to 2 or 3%, but will exceed this limit in propor-

tion to the amount of husk present. König's method and its modifications have been found to be most satisfactory, and the following from the "*Official and Provisional Methods of Analysis, Association of Official Agricultural Chemists*,"[1] gives good results:

2 grm. of dry material are treated with ether (or the residue from the estimation of the fat by ether extraction may be used).

The residue is placed in a 500 c.c. flask, to which 200 c.c. of boiling, 1.25% sulphuric acid, is added; the flask is converted into a reflux condenser and the boiling continued for 30 minutes. Air should be bubbled through the flask if frothing occurs.

The liquid is filtered and the residue, after thoroughly washing free from acid, is rinsed back into the clean flask and boiled for 30 minutes with 200 c.c. of 1.25% sodium hydroxide solution. The residue is filtered off and washed successively with hot water, alcohol and ether. It is then dried and weighed.

The dry residue is ignited and weighed, and the loss regarded as crude fibre.

Another method depending upon the estimation of furfural, a decomposition product of pentose and other sugars, with which the fibre is intimately connected, has been used with success by Hehner and Sketchley,[2] Adan[3] and others. It was suggested by Tollens in conjunction with Gunther and de Chalmont, and has been shown to have an efficiency of from 99.1% to 101.1% in a number of experiments carried out by Adan.

3 to 4 grm. of the sample are placed in a Wurtz flask of 250 c.c. capacity, with 100 c.c. of dilute hydrochloric acid of 1.06 sp. gr. (equivalent to 12% HCl). The contents are then distilled until 30 c.c. have passed over, when 30 c.c. of dilute acid are let into the flask to take the place of that portion which has distilled over. This process is continued until about 300 c.c. of distillate have been collected. As the distillation proceeds, the furfural can be detected by allowing a drop of the distillate to fall on a filter paper, which is then tested with a drop of dilute aniline acetate solution, containing a small proportion of sodium acetate. When a pink colouration is no longer produced, the distillation is stopped.

The distillate is made up to 400 c.c. with dilute hydrochloric acid (sp. gr. 1.06).

[1] *U. S. Dept. of Agriculture, Bureau of Chemistry*, Oct., 1907, *Bull.* No. 107.
[2] O. Hehner and N. P. Sketchley, *Analyst*, 1899, 24, 178.
[3] R. Adan, *Bull. Soc. Chim Belg.*, 21, 211.

The solution is then slowly neutralised with dry and finely powdered sodium carbonate and then faintly acidified with acetic acid. 10 c.c. or more if required of an acetic acid solution of phenylhydrazine (12 drops of phenylhydrazine to 7.5 c.c. of strong acetic acid) is added and the solution constantly stirred.

The volume is then increased to 500 c.c. with constant stirring for half an hour. The precipitate is collected on an asbestos filter, washed thoroughly and quickly with water and dried in a current of warm air (50° to 60° C.) under slightly reduced pressure.

The weight of precipitate multiplied by 0.516 will give the approximate weight of precipitated or distilled furfural.

An error of about 0.25% may occur if the amount of furfural remaining in the solution and washings has not been included.

The figures for furfural multiplied by 1.88 will give the equivalent of pentosan and is a measure of the amount of husk present in the cocoa preparation.

Theobromine, Caffeine.—There are a large number of different methods advised for the estimation of theobromine and caffeine; the majority of processes are untrustworthy, especially those which rely upon the sublimation of theobromine.

The most systematic results have been obtained by employing the methods described by W. E. Kunze (*Zeitsch. anal. Chemie*, 1894, **33**, 1) and J. Dekker (*Rec. Trav. Chim.*, 1903, **22**, 143–152).

Kunze's Method.—10 grm. of cocoa are boiled for 20 minutes with 150 c.c. of 5% sulphuric acid. The solution is filtered and all the soluble matter is washed out of the residue with boiling water.

The warm extract is precipitated with excess of phospho-molybdic acid, and after 24 hours the precipitate is collected and washed with about 1 litre of 5% sulphuric acid The filter containing the moist precipitate is transferred to a large dish and treated with excess of cold barium hydroxide, after which carbon dioxide is passed through in order to precipitate the barium.

The whole is then dried on a water-bath and extracted with boiling chloroform.

The chloroform is distilled off from the clear filtered extract, when a perfectly white deposit, of the two alkaloids, is left, containing only a trace of ash.

The white residue is weighed, dissolved in ammonia and heated to boiling.

Excess of silver nitrate is then added (11.3 parts of silver to 1 part of theobromine) and the boiling continued until no further ammonia escapes and the liquid is reduced to a few c.c.

An insoluble substitution product of theobromine, $C_7H_7N_4O_2Ag$, is obtained, while the caffeine remains uncombined in solution. The insoluble precipitate is filtered off, washed and weighed.

The silver is estimated in the dried theobromine-silver compound by ignition or by dissolving in nitric acid and precipitating as chloride, by which means the quantity of theobromine, in combination and originally present, can be readily determined.

Dekker's Method.—This process, which has greater brevity in its favour, is capable of giving very accurate results.

10 grm. of powdered cocoa are heated with 5 grm. of magnesium oxide and 300 c.c. of water in a reflux apparatus for 1 hour.

After filtering and draining on a pump, the residue is again boiled with water for 15 minutes and drained. The solutions are evaporated to dryness, the resulting residue being then triturated with fine sand and boiled with 100 c.c. of chloroform. The clear filtered chloroform solution is evaporated to dryness and the white residue, consisting of theobromine and caffeine, weighed.

If it is desired to estimate the two alkaloids separately, the dried residue is treated with 50 c.c. of benzene for 24 hours, which extracts the caffeine. The solution is evaporated to 25 c.c. and filtered; the residue on the filter, consisting of theobromine, is dried and weighed.

Cocoa-red.—The direct estimation of cocoa-red is attended with some difficulty, and is seldom necessary. The following method, approved by Blyth (*Foods*, 1909, 368), has a fair degree of accuracy:

From 2 to 3 grm. of the fat-free cocoa are made into a paste with hydrochloric acid; the acid paste placed in a Soxhlet tube is exhausted with 100 c.c. of absolute alcohol, kept at the b. p. and to which sufficient silver oxide has been added to fix the hydrochloric acid.

The alcoholic liquid is cooled, filtered and then precipitated by an alcoholic solution of lead acetate. The purple-black precipitate obtained is collected on a filter, well washed with boiling water, and transferred to a small flask. Some 70% alcohol is added, and the lead salt decomposed by sulphuretted hydrogen.

On getting rid of the hydrogen sulphide, filtering and evaporating to dryness, the red colouring matter is obtained in a solid form. By

repetition of the process, purification may be effected and the weight of true cocoa-red ascertained.

A solution of cocoa-red obtained in this way gives a diffuse band in the green, allowing the red, blue and most of the yellow rays to be transmitted.

The solution in alcohol is capable of being estimated on colourimetric principles, but low results are obtained.

Another method of determining cocoa-red and the products of its decomposition has been suggested by Zipperer, but as it entails the use of large quantities of absolute alcohol it is too expensive for ordinary employment.

Starch.—The estimation of starch in cocoa nibs may be made by any of the recognised methods, the nature and origin of any foreign starch that may be present in cocoas or chocolates being detected by the microscope.

As already mentioned, the percentage of natural starch in cocoa is small, variable and of little importance, though the estimation of total starch may be useful where the presence of added starch has already been noticed.

The methods of analysis in which the starch is converted into sugars, capable of direct estimation, by the agency of diastase, dilute mineral acids, etc., will be found satisfactory, after the cocoa material has been freed from fat, sugar and matters soluble in weak alcohol. (See Vol. 1, page 420.)

An excellent general method proposed by Dragendorff is as follows:

2 to 3 grm. of the dry fat-free material are heated with 25 to 30 c.c. of a 5% alcoholic potassium hydroxide solution, for 20 hours on a water-bath; the solution is filtered hot through a weighed ash-free filter-paper.

The residue on the filter is washed first with hot absolute, then with cold ordinary alcohol, and lastly with water; it is then dried and weighed. The loss in weight corresponds to protein matters, sugar, and soluble salts.

The filter and its contents are cut into fine pieces with scissors, and boiled with 5% hydrochloric acid, until the solution no longer gives a blue indication with iodine. The liquid is then filtered through a weighed filter, the residue washed, dried and weighed. The difference between the weights of Nos. 1 and 2 gives very nearly the quantity of starch.

This value may be checked by estimating the dextrose in the filtrate from No. 2, with Fehling's solution.

Dubois'[1] *Method for Cocoa Preparations in the Absence of Sugar.*—2 grm. of the sample are transferred to a 500 c.c. Erlenmeyer flask, to which are added 20 c.c. of water and 12 c.c. of strong sulphuric acid. The latter is cautiously added with slow rotation of the flask. The mixture is heated over a low flame with constant rotation until the colour changes from brown to reddish-black. The time required for this change has been found to be approximately 1 1/4 minutes, so that all samples should be treated for this time.

30 c.c. of water are then added, the mixture heated to boiling and boiled for 15 seconds. A little cold water is poured in, the flask quickly cooled and the acid nearly neutralised with a saturated solution of potassium hydroxide.

The solution is then again cooled and transferred to a 250 c.c. flask, completing the volume with cold water. 50 c.c. of the filtrate are used or the determination of copper-reducing substance as dextrose (100 mgrm. dextrose = 0.2538 grm. copper oxide = 0.2027 grm. of copper).[2]

For Cocoa Preparations Containing Sugar and Soluble Carbohydrates.—4 grm. of the sample are shaken with petroleum ether until the whole of the fat has been removed.

After filtration and a further washing with ether, 100 c.c. of water are added to the contents of the flask and the residue on the filter-paper washed back into the flask. The flask is thoroughly shaken and the contents filtered through a filter-paper. After 3 or 4 washings with water, and after the whole of the sugar has been removed, the filtrate is made up to 500 c.c. and may be used for sugar estimation, while the residue is washed into an Erlenmeyer flask and treated as described in Dubois' method for estimation of the starch.

Dubois by employing these methods obtained 10.77% to 13.05% of starch in cacao nibs, 11.38% to 13.64% in bitter chocolate and 7.4% to 8.5% in sweet chocolate. In later experiments the same author obtained 16.3% to 19.8% of starch in cocoas and 10.4% to 18.2% in bitter chocolates.

Welmans[3] has estimated the quantity of commercial dextrin added to chocolate. The dextrin is detected by treating the cacao preparation after extraction of fat, with water.

[1] W. L. Dubois, U. S Dept. of Agric., 1901, *Bull.* 132.
[2] Brown, Morris and Millar, *Trans*., 1897, 71, 281.
[3] P. Welmans, *Zeit. öffent. Chem.*, 5, 478.

The solution is filtered and to 10 c.c. of the filtrate 40 c.c. of 90% alcohol added, when, if dextrin is present, an immediate turbidity is produced. The dextrin may be quantitatively precipitated by lead acetate in the presence of ammonia. (See also Vol. 2, page 420, etc.)

Sugar.—The quantity of sugar present in cocoa nibs is small and unimportant. In chocolate it is very necessary, however, to estimate the amount of added cane-sugar, and in milk chocolate it may also be required to estimate lactose.

The average cupric reducing power of cocoa nibs expressed as CuO is found to be about 5 per 100 parts by weight of dry and fat-free cocoa matter. As lactose, in milk chocolates, is likely to be the only reducing sugar present, its estimation may be made by finding the cupric reducing power of the sample under examination, and allowing for the reducing power of the cold water extract of the nib.

The following process for estimating sucrose and lactose (Dubois' method, *J. Amer. Chem. Soc.*, 1907, **29**, 556) is advised by the Association of Official Agricultural Chemists, U. S. A.:

13 grm. of sample are extracted with petroleum ether to remove the fat.

The residue is shaken with 100 c.c. of water for 10 minutes; 5 c.c. of basic lead acetate solution is added, the precipitate filtered off and excess of lead removed.

25 c.c. of this solution are allowed to stand overnight, then examined polarimetrically. Multiply the readings obtained by $2(=a)$. 50 c.c. of the solution are inverted by the method mentioned later, and after cooling and nearly neutralising with sodium hydroxide solution, is made up to 100 c.c. This solution, after being brought to the temperature at which the direct readings were made, is examined in the polarimeter, and the readings multiplied by $4(=b)$. The same solution is also examined at 86° in a water-jacketed tube; the readings are multiplied by $4(=c)$.

The approximate weights of sucrose and lactose present in the 13 grm. of sample are calculated by the following formula:

$$\text{grm. of sucrose} = \frac{(a-b)\ 1.05 \times 13}{142.66 - \frac{1}{2}}$$

$$\text{grm. of lactose} = \frac{c \times 1.264 \times 1.11 \times 1.05 \times 13}{100} = \frac{19.152\ c}{100}$$

(The lead acetate solution is prepared by boiling 430 grm. of normal lead acetate, 130 grm. of lead oxide and 1,000 c.c. of water together for 30 minutes. The mixture is allowed to cool and settle, and the supernatant liquor diluted with freshly boiled water to a sp. gr. of 1.25.)

The factor 1.264 in the estimation of lactose is the allowance made for difference in normal weights of sucrose and lactose.

$$\left(\frac{32.884}{26.00} = 1.264\right)$$

From the total amount of sugar found by the above methods obtain the value x, the volume correction for solutions containing varying amounts of sugar, from the following table:

2	grm.	of	sugar	in	sample	$x = 101.2$
4	"	"	"	"	"	$x = 102.5$
6	"	"	"	"	"	$x = 103.6$
8	"	"	"	"	"	$x = 104.8$
10	"	"	"	"	"	$x = 106.05$
15	"	"	"	"	"	$x = 109.40$
20	"	"	"	"	"	$x = 112.40$

Then the

$$\text{Percentage of sucrose} = \frac{(a-b)\ 1.05\,x}{142.66 - \frac{t}{2}}$$

$$\text{Percentage of lactose} = 1.473\,x$$

In the estimation of the percentage of sucrose, the factor 1.05 is the allowance made for the dilution due to the addition of 5 c.c. of basic lead acetate solution.

The method recommended for the inversion of the sugar solution is as follows:

To 50 c.c. of the clarified solution free from lead, add 5 c.c. of 38.8% hydrochloric acid and set aside for 24 hours at 20°; if the temperature is above 25°, set aside for 10 hours.

The official method for estimation of sugar in chocolate, according to the German decree of May 31, 1891, is as follows:

13.024 grm. of chocolate are dampened with alcohol, then warmed for 15 minutes with 30 c.c. of water on the water-bath; while still hot it is poured on to a wet filter, the residue being again treated with hot water until the filtrate nearly equals 100 c.c. The filtrate is mixed with 5 c.c. of basic lead acetate solution, allowed to stand for 15 minutes,

then clarified with alum and a little alumina, made up to a definite volume (110 c.c.) and examined in the polarimeter. (See also Vol. 1, pages 338, 365.)

Fat.—Cacao butter is readily soluble in ether and petroleum ether, and its estimation is made by exhausting a finely powdered weighed portion in a Soxhlet tube with either solvent: 5 grm. is a convenient quantity of sample to take for this determination.

Cocoa nibs contain 45 to 55% of fat; cocoa-powder should contain at least 20%, and sweet chocolate from 25 to 30% of fat.

Besides the mere estimation of quantity, the quality of the fat present in chocolate is of great importance, as paraffin wax, beeswax, tallow, Illipé fat, and others, are used from time to time for their hardening properties, coconut fat, arachis nut, cotton-seed, sesame, and other vegetable oils being used for cheapening purposes.

Determinations of the saponification and iodine values and refractive index of the extracted fat (see Vol. 2, pages 3–89) are essential for the detection of foreign fats.

In the absence of cacao butter substitutes containing glycerides of volatile fatty acids, the Reichert-Meissl process will give the amount of milk fat present in milk chocolates with reasonable accuracy.

Saponification Value.—The presence of coconut fat will cause an increase, paraffin wax and beeswax a decrease in this value. The saponification of cacao butter is fairly constant, varying 1 or 2 units on either side of 193.0.

2 or 3 grm. of the extracted fat are accurately weighed in a flask; about 25 c.c. of approximately $N/2$ alcoholic potassium hydroxide added, and the whole is heated on a water-bath under a reflux condenser until complete solution has taken place.

The unneutralised alkali is titrated with $N/2$ hydrochloric acid, using phenolphthaleïn as indicator.

The standardisation of the $N/2$ alcoholic potassium hydroxide is preferably effected by heating 25 c.c. on the water-bath under a reflux condenser, side by side with the fat examined and subsequently titrating.

The difference between the amounts of acid required corresponds to the amount of alkali required for the saponification, which is expressed in milligrams, for 1 grm. of fat, thus:

$$K = \frac{n}{w} \times 56{,}100.$$

Where K = saponification value, n = c.c. of normal potassium hydroxide solution, and w = weight of fat taken; 56.1 is the molecular equiualent of potassium hydroxide.

Iodine Value[1]—Hubl's Method.—A low iodine value will point to the presence of coconut fat; a decrease will also be followed by the addition of beeswax. The iodine value of cacao butter is about 35.

50 grm. of iodine are dissolved in 1 litre of alcohol, and 60 grm. of mercuric chloride in another.

1 grm. of cacao butter is weighed out and dissolved in 10 c.c. of pure chloroform, and to the solution 30 or 40 c.c. of a solution containing equal volumes of the iodine and mercuric chloride solutions are added.

The mixture is allowed to stand for several hours in the dark or overnight, when after the addition of 10 or 15 c.c. of a 10% solution of potassium iodide with about 150 c.c. of water, the excess of iodine is titrated with a solution of sodium thiosulphate (about 24 grm. per litre) previously standardised on pure resublimed iodine or potassium dichromate, a little starch paste being used as indicator.

The iodine value is expressed in grm. of iodine absorbed by 100 grm. of fat.

Precautions.—The iodine and mercuric chloride solutions must not be mixed many hours previous to the estimation, and a blank must be conducted side by side with the actual experiment.

The chloroform used should be free from iodine absorbing impurities.

Reichert-Meissl Value.—This value is about 0.5 for pure cacao butter; a large increase on this in a fat extracted from plain sweet chocolate shows the probable addition of coconut fat. In a milk chocolate an increase will be noticed, due to the presence of milk fat.

5 grm. of fat are saponified by 2 grm. of stick potassium hydroxide and 50 c.c. of 70% alcohol, by heating together on the water-bath.

The evaporated alcoholic soap is dissolved in 100 c.c. of water and acidified with 40 c.c. of 10% sulphuric acid solution. 110 c.c. are slowly distilled off, of which 100 c.c. are filtered through a dry filter and titrated; the $N/10$ alkali consumed is increased by 1/10 to allow for the 10 c.c. not used.

Coconut fat requires about 7.0 c.c. of $N/10$ alkali; arachis or earth nut oil, about 0.5 c.c.; milk fat, 20–30 c.c.

[1] (Am. Ed.) The Hanus solution is so rapidly gaining in favour in the United States that many laboratories only use the Hübl solution in special cases. This is true of the writer's laboratory.

Björklund's Ether Test.—The ether test, which in a modified form, constitutes the British Pharmacopœia test of purity, consists in shaking a quantity of the fat (3 grm.) in a test-tube with twice its weight of ether at 18° C. Pure cacao butter produces a clear solution, while the presence of wax will at once cause turbidity, which will not clear even on warming.

If a clear solution is obtained, the test-tube is immersed in water at 0° C., and the minutes which elapse before turbidity occurs noted.

Bjorklund made the following observations:

	Turbidity at 0° after minutes	Clear solution at
Pure cacao butter	10–15	19–20°
Cacao butter + 5% beef tallow	8	22°
Cacao butter + 10% beef tallow	7	25°

It has been observed also that the form of the crystals in the chilled ethereal solution is a useful indication of the addition of tallow, pure cacoa butter crystallising in well-defined shapes at the bottom and sides of the tube, while a small percentage of tallow renders the solution cloudy and flocculent.

Refractive Index on Zeiss Butyro-refractometer.—The determination of the refractive index of a fat has long been known as a useful indication for the state of purity.

The instrument in general use is the Zeiss butyro-refractometer which from its handiness and for general utility is most to be recommended. When the Abbé refractometer only is available readings can be calculated to the butyro-scale (see Leach, *Food Inspection and Analysis*, and elsewhere).

There are a few oils, however, such as Tung oil and the rosin oils, which fall without the scale (5 to 105° on the butyrometer) and for these the Abbé refractometer or the oleorefractometer of Amagat and Jean may be used.

The instrument, which consists of an Abbé double prism capable of being heated by a current of hot water and a permanently attached telescope, the objective of which is adjusted by a micrometer screw, does not need further description, the method of working being one of greatest simiplicity. (See Vol. 1, page 22.)

Some figures of the value for the detection of adulteration of cacao butter obtained by the writer are given:

	Readings on Zeiss butyro-refractometer at 40° C.
Cacao butter	46 –47
Coconut stearin	35 –36
Beef tallow	48 5–49 0
Palm butter (hard)	47 0
Palm kernel oil	38 –39
Earth nut oil	55 –56
Butter fat	42 –45 (usually 44.5)
Hazel nut oil	54 2
Almond oil	57 2
Fat from Cailliér's milk chocolate	46 0
Fat from Nestlé's milk chocolate	46 0
Fat from Peter's milk chocolate	46 2
Fat from "Nuttis" (Peek Frean's nut chocolate)	50 0

In the table below will be found physical and chemical constants for cacao butter, and for those fats and oils with which it is likely to be adulterated. There are also included in the same table, constants for those fats and oils which may be found in conjunction with cocao butter in the fat extracted by a solvent from commercial chocolates other than plain, such as milk and nut chocolates, etc.

The determination of the constants, the values of which can be explained by the composition of the fat under examination, affords an indication of the nature of the adulterant. Thus, a high iodine value suggests the presence of certain foreign vegetable oils, such as sesame, arachis or almond. A low saponification value will suggest beeswax or paraffin wax. A high saponification value will suggest coconut oil or palm kernel oil stearins. A low iodine value will be found if coconut oil or stearin has been substituted for cacao butter.

In general the vegetable oils increase the iodine absorption value and lower the m. p. of the insoluble fatty acids obtained from the mixed fats.

Cacao butter may show a high acid value if the fat has been extracted from shells (Dutch IIa butter) or unduly exposed to the oxidising influence of air and light. The acid value given by Matthes and Rohdlich[1] for cacao butter is 1.1 to 1.95.

There have been many researches upon the detection of cacao butter substitutes in chocolate. Coconut fat or stearin, after a great part of the olein has been removed, is especially valuable as a cacao butter substitute. Wauters[2] suggests the following method for the detection of coconut fat in cacao butter:

[1] Matthes and Rohdlich, *Ber.*, 1908.
[2] Wauters, *Analyst*, 1901, 26, 128, 292.

Fat or oil	Sp gr	M P	M P of fatty acid	Saponification value	Reichert-Meissl value	Iodine value
Cacao butter	0 964–0 974 at 15° 0 8577 at 98°	30–34°	48 53°	192–195	0 2–0.9	32–42
Possible adulterants or substitutes						
Bassia tallow	0 9175 at 15°	25–42°	39 5–45°	187–194	0 5–0 8	54–68
(Mixture of Mohwah, and Mahua butters)	0 8943–0.8981 at 100°					
Borneo tallow	0 892 at 100°	37 5°	53 5°	192 4–196	0 3–0 5	30–31
Chinese tallow	0 9180–0 9217 at 15°	36–46°	39–57°	179–203	0 2	23–38
Cotton-seed stearin	0 867 at 100°	30–40°	27–45°	194 5	0 8–1 0	89–93
Goa butter	0 911 at 50°	41–43°	61 0°	187–191.5	0 1–1 5	25–34
(Kokum butter)	0 8889 at 100°					
Mafura tallow	0 902 at 40°/15°	29–40°	51–55°	201–221	1 5	43 5–56
Nutmeg butter	0 945–0 996 at 15°	43–51°	42 5°	154–161	1–4 2	48–85
Palm oil	0 9210–0 9245 at 15°	27–43°	48–50°	200–205	0 8–1 9	53–58
Phulwara butter	0 8970 at 100°/100°	39°		191	0 4	42
(Karité fat)						
Piney tallow	0 915 at 15°	36–42°	56°	189–191	0.2–0 4	38–39
(Malabar tallow)	0 8900 at 100°					
Shea butter	0 9175 at 15°	23–28°	39 5–56°	179–192	. . . .	56–67
(Galam butter)	0 859 at 99 5°/15 5°					
Coconut oil	0 9259 at 15° 0 8736 at 100°	20–28°	24–25°	246–262	6 6–8 4	8.2–9.5
Cocoanut stearin	0 8700 at 100°	29 3–29 5°	28 1°	252	3 4	4 0–4 5
Palm nut	0 8731 at 99°/15 5°	23–30°	21–28 5°	243–255	5–6 8	10 5–17.5
(Palm-kernel oil)						
Palm nut stearin	0 8700 at 100°	31 5–32°	28 5–29 5°	242	2 2	8
Beef fat	0 8950 at 15 5°	42–50°	41–47 5°	196	0 3–0 5	36–42
	0 8626 at 98–100°					
Lard	0 8600 at 98–100°	30–44°	37–47°	195–203	0 2	47.5–64
Mutton fat	0 937–0 953 at 15 5°	47–49°	34–36°	196	0 3	33–50
Tallow	0 925–0 940 at 15 5°	38–50°	41–49°	193–198	0 2	33.48
Beeswax (normal)	0 959–0.970 at 15 5° 0 962–0 966	62–66°		88–98	. .	8 5–11.5
Paraffin wax	0 824–0 940 at 15 5° (according to melting-point)	36 7–58 3			. .	3.9–4.0
Possibly present, due to addition of nuts, milk, etc.		Solidification point				
Almond oil	0 914–0 920 at 15 5°	−10 to −20°	13–14°	188–192	0 5	93–100
Arachis (earth nut oil)	0 911–0 926 at 15 5°	+3 to +10°	27–30°	186–194	0 5	83–101
Hazelnut oil	0 916–0 917 at 15 5°	−10 to −20°	20–25°	191–197	0 9–1.0	83–90
Pinenut oil	0 9215–0 9250 at 15 5°	−18 to −20°	16–19°	191–193	. .	106–120
Walnut oil	0 9240–0 9268 at 15 5°	−12 to −24°	15–20°	190–197	0.0	139–148
Butter fat	0.900–0.913 at 38 5°	28–36°	38–42	215 8–241.1	21.0–33 4	28–42

5 grm. of the extracted fat (if from chocolate) is saponified and the soap dissolved in 150 c.c. of boiling water; 50 c.c. of 5% sulphuric acid solution are then added and the whole distilled so that 100 c.c. pass over in 30 to 35 minutes. After the first distillation, another 100 c.c. of water are added and the distillation repeated. The two distillates are separately filtered and 50 c.c. of each filtrate titrated against a $N/10$ solution of sodium hydroxide. The filters are washed with 50 c.c. of ethyl alcohol and the washings mixed with 50 c.c. of the filtrate and again titrated.

By this method, which is really an extension of that for estimation of Reichert-Meissl value, Wauters obtained the following results, the figures being expressed as c.c. of $N/10$ sodium hydroxide solution required for neutralisation:

	Soluble volatile acids			Insoluble volatile acids		
	First	Second	Total	First	Second	Total
Coconut oil ..	7 1	4 3	11 4	7 85	7 55	15 4
Cacao butter.	0 1	0 0	0 1	0 25	0 15	0 4

Sachs[1] has examined many exotic vegetable fats which have been used as substitutes for cacao butter. Dika or Gaboon fat, Tankawang fat or Borneo tallow and Illipé fat have been examined by this author, who gives also the constants he obtained for them.

The same author has examined the stearin obtained from coconut and palm nut fats. He suggests the admixture of 75% coconut stearin with 25% Japan wax for a good cacao butter substitute. This mixture gives the following constants: m. p. 34° to 35.5°, iodine value 7.8, saponification value 237, Reichert-Meissl value 5.5.

Other mixtures recommended by Sachs are 2/3 palm nut stearin with 1/3 coconut stearin, and 40% Tankawang fat with 60% coconut stearin.

The decisions of the recent congresses at Geneva, 1908, Paris, 1909, and Berne, 1911, have all been in the direction of suppressing the addition of foreign fats to chocolate and in certain countries other legal standards for cocoa and chocolate maintain.

English chocolate manufacturers, however, have not yet committed themselves to legal standardisation of their goods and it is undoubtedly

10. Sachs, *Analyst*, 1908, 123.

true that many articles appear on the home market as cocoa and chocolate that would not be permitted in other countries such as the United States, Germany, Switzerland, Belgium, Roumania, etc.

In the *Times* of June 10, 1910, two cases of adulteration of cocoa powder were quoted; in the one, a powder containing 60% husk was being sold as cocoa and though it was explained that the presence of so large a quantity was undesirable and unnecessary, the case was adjourned for further investigation. The second case, dealing with the sale of cocoa containing 20% of husk was dismissed.

INDEX.

ACET-phenetidin, 99
Acetanilide, 83
Acolyctine, 275
Aconines, 264
Aconite, toxicology of, 283
—— toxicological detection of, 285
—— pharmacology of, 287
—— root and its preparations, 279
—— bases, constitution and characters of the, 256
Aconitine, 256, 258
—— derivatives of, 262
—— salts of, 260
Acridine, 126
Alkaloids, isolation and purification of 171, 177
—— with acids, reaction of the, 202
—— properties of the, 183
—— classification of the, 206
Amines, physical properties of, 12
—— separation of, Hoffman's method, 5
Amines, detection and separation of 4
—— separation of Delepine's method, 7
Aminonaptholdisulphonic acids, 127
Aminonaptholsulphonic acids, 126
Aminonapthols, 125
Aminophenacetin, 104
Aminophenols, 97
Ammonia, estimation of pyridine in, 137
—— separation and estimation of the three methylamines from, 18
Analgen, 159
Aniline, 53
—— salts of, 59
—— oils, 74
Anhydro-bases, 545
—— ecgonine, 339
Antifebrin, 103
Antipyrine, 36
Apocodeine, 395
Apomorphine, 387
—— hydrochloride, 388
Areca or betel-nut alkaloids, 208
Arecaidine, 211
Arecaine, 210
Arecoline, 208
Argentol, 160
Atroglyceryl tropeine, 302
Atisine, 277
Atrolactyl tropeine, 302
Atropamine, 291, 298
Atropine, 294
—— and its allies, toxicological detection of, 309
Atroscine, 300
Azoimide, 27

BELLADONNA plaster, 315
—— root, 311
Belladonnine, 291, 299
Benzanilide, 87
Benzidine, 108
Benzoyl-pseudotropine, 341
Benzyl-naphthylamine, 116
Berbamine, 563
Berberine, 551
—— salts of, 559
Bikhaconitine, 256, 271
Brom-acetanilide, 86
Brucine, 464
—— analytical reactions of, 465
—— toxicology of, 466
Butylhypnal, 46

CACOA, 685
—— analyses of "nibs" and husks, 68
—— butter, 715
Caffeine, 579,
—— isolation and estimation of, 606
—— salts of, 588
Caffetannic acid, 645
——— estimation of, 657
Canadine, 574
Caper tea, 638
Chicory, 674
Chocolate, 685
Cinchona bases, amorphous, 543
——— table of, 499
——— general properties of, 503
—— barks, 479
——— composition of, 481
——— assay of, 486
——bases separation of, 490
—— alkaloids, separation of, 492
——— titration of, 496
Cinchonamine, 547
Cinchonicine, 544

Cinchonidine, 537
Cinchonine, 540
—— sulphate, 542
—— hydrochloride, 542
Cinchonine, 522
Cinchonidine, 522
Cinnamyl-cocaine, 339
Coca, amorphous bases of, 341
—— assay of fluidextract of, 344
—— leaves, 344
—— alkaloid from Java, 348
Cocaic acid, 341
Cocaine, 321
—— bases allied to, 338
—— qualitative tests for, 322
—— salts of, 326
—— crude, 328
—— in the presence of the eucaines, tests for, 334
—— decomposition-products of, 336
Cocamine, 340
Cocatannic acid, 344
Cocethyline, homococaine, 339
Cocoa-red, 699
—— powder, 690
—— and chocolate, 685
Codamine, 396
Codeine, 356, 390
—— detection and estimation of, 392
Coffee, 642
—— roasting of, 648
—— analysis of, 651
—— adulterations of, 663
—— extracts, 678
Collidine, 144
Columbamine, 575
Columbic acid, 577
Columbin, 576
Conhydrine, 214
Coniceines, 215
Coniine, 211
Conquinamine, 537
Cryptopine, 396
Cumidines, 73
Cupreine, 522, 548
Curare, alkaloids of, 474
Curarine, 476
Curine, 475

Datura, 319
Dextro-cocaine, 338
Diaminophenols, 105
Diaminonaphthalenedisulphonic acids, 124
Diaminonaphthalenesulphonic acid, 124
Diaphtherin, 160
Diethylaniline, 95
Diethylnaphthylamine, 116
Dimethylamine, 14
Dimethylnaphthylamines, 116
Dimethyl-phenylene, 107
Dimethylpyridines, 143
Dinapathylamine, 116
Diphenylamine, 95
Diethylaniline, 89
Diuretin, 594
Dragendorff's reaction, 190

Ecgonine, 337
Ethylamine, 19
Ethylnaphthylamines, 114
Ethylpyridine, 144
Ethylhydrazine, 29
Exalgin, 103

Formyl-phenetidine, 104

Gnoscopine, 397
Griserin, 160
Guarana, 683
Guvacine, 211

Hemlock and its preparations, assay of, 221
Heroin, 389
Homatropine, 302
Homocinchonidine, 540
Homoquinine, 549
Homoarecoline, 210
Hydrastine, 564
Hydrastinine, 572
Hydrazone, 34
Hydrazine, 23
Hydroacridine, 164
Hydrocinchonidine, 540
Hydrocinchonine, 542
Hydrocotarnine, 397
Hydroquinine, 533
Hydroquinicine, 534
Hyoscyamine, 297
Hyoscine, 299
Hyoscyamine (amorphous), 300
Hyoscyamine, 291
Hyoscine, 291
Hyoscyamus, 316

Indaconitine, 256
Insecticides and germicides from tobacco, 250
Isatropic acid, 293

Japaconitine, 266
Jateorrhizine, 575
Jesaconitine, 256, 273

Kairine, 159

Kola, 680
Kolatein, 682

Lanthopine, 398
Lapaconitine, 277
Laudanine, 398
Laudanidine, 398
Laudanosine, 361, 399
Laudanum, 429
Lie tea, 639
Loritin, 159
Lobeline, 223
Lupine alkaloids, 225
Lycaconine, 274
Lycaconitine, 274

Mandragorine, 300
Marme's reagent, 190
Mate, Paraguay tea, 641
Mayer's reagent, 191
Meconidine, 399
Meconic acid, 411
Meconin, 410
Meconoisin, 411
Meteloidine, 291, 301
Methylaniline-nitrosamine, 89
Migraufine, 47
Methylphenyl-naphthylamine, 116
Methacetin, 104
Methylamine, 12
Methylacetanilide, 86
Methyl-phenacetin, 104
Methylpyrroles, 149
Methylaniline, 88
Methyl-coniine, 216
Monamines, 2, 10
Morphine, 374
—— basic association of, 395
—— salts of, 376
—— detection of, 379
—— quantitative estimation of, 383
Myoctonine, 275

Naphthylamines, 111
Naphthylaminedisulphonic acids, 122
Naphthylaminesulphonic acids, 117–121
Naphthylaminetrisulphonic acids, 123
Naphthylaminesulphonic acids, analysis of, 124
Narceine, 360, 399
Narcotine, 400
—— meconate, 402
Narcotine, 357, 400, 409
Nicotine, 237
—— and tobacco, poisoning by, 241
Nitraniles, 62
Nux vomica, 467
—— preparations of, 472

Opionin, 411
Opium, 407
—— bases, constitution of, 355
—— and morphine, toxicology of, 434
—— bases, general characters of, 361
———— with solvents, behaviour of, 362
———— estimation and separation of, 370
———— colour reactions of, 366
—— actions of solvents on, 414
—— estimation of morphine in, 417
—— the sampling of, 418
—— extract of, 429

Orexin, 161
Osazones, 35
Oxyacanthyne, 361

Palmatine, 576
Palmatisine, 279
Papaverine, 359, 403
Papaverosine, 403
Paranitrosodimethylaniline 90
Paregoric, 429
Piperidine, 141
Phenacetins, 99
Phenylnaphthylamine, 115
Phenylenediamines, 105
Phenylhydrazides, 32
Phenylpyrazolones, 36
Phenylmethane, 87
Piturine, 229
Picolines, 142
Pomegranate-alkaloids, 230
Protopine, 404
Pseudoconhydrine, 215
Pseudaconitine, 256, 270
Pseudocodeine, 395
Pseudohyoscyamine, 298
Pseudohyoscyamine, 291
Pseudomorphine, 357, 404
Pseudotropine, 294
Pyraconine, 265
Pyraconitine, 265
Pyramidone, 47
Pyrazolines, 35
Pyridine, 131
—— bases, isolation of, 130
Pyridinecarboxylic acids. 145
Pyrro, 148

Quinamine, 536
Quinazoline, 160
Quinic acid, 483

Quinidine, test for, 522
Quinidine, 535
Quinine, 507
—— detection and estimation of, 510
—— salts of, 514
—— sulphate, 515
—— hydrochloride, 527
—— tincture of, 533
Quinoline 151
Quinosol, 160
Quinovic acid, 482

REMIJIA barks, alkaloids of, 546
Renalgen, 45
Rohoeadine, 405

SALIPYRINE, 45
Scopolamine, 291
Scopolia, 320
Scopoline, 294
Septentrionaline, 277
Spigeline, 235
Stramonium seeds, 319
Strychnine, 441
—— salts of, 443
—— analytical reactions of, 444
—— toxicology of, 455
—— preparations of, 459

TEA, 597
—— analysis of, 602
Tea, adulterations of, 629
Tetralkylammonium bases, 20
Thalline, 157
Thebaine, 405
Theobroma cacao 685
Theobromine, 591, 682,
Theophyline, 595
Thermifugen, 159
Tobacco, 237
Toluidines, 63
Toluylenediamines, 107
Tolynaphthylamines, 115
Tolypyrine, 47
Triaminophenol, 105
Trimethylamine, 14
Triphenylamine, 97
Tritopine, 406
Tropeines, artificial, 301
—— detection and estimation of, 303
Tropine, 293
Tropic acids, 292
Tubocurarine, 476
Tussol, 46

VEGETABLE alkaloids, the, 167
Volatile bases of vegetable origin, 207

XANTHALINE, 406
Xanthine, 580
Xylidines, 71