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THE GLYCOSIDES

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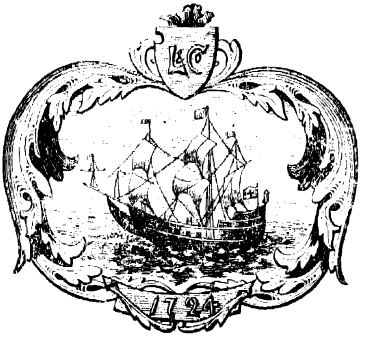

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PREFACE.

THIS monograph is devoted entirely to the glycosides which formed a section only in the writers' former work on the Simple Carbohydrates. During the last seven years the knowledge of the sugars themselves has entered on a new phase; after a period of uncertainty, of conflict between the old and the new ideas, it is settling down to a state when once more clear views of the structure of the sugars can be enunciated.

In the glycoside field very definite progress has been made in almost every direction. The aglucones, i.e. the non-sugar constituents, are so numerous and often so complex that their study takes the reader into almost every branch of organic chemistry, and at the same time amazes him when he considers the complexity of the materials which Nature elaborates. Necessarily the several sections can only be dealt with in brief abstract, but it is believed that the biochemist will be glad to have all the information in one book. The attempt has been made to preserve the balance between the three aspects of the subject: the nature and meaning of the sugars, the constitution of the aglucones, the biological significance of glycosides.

The field of plant chemistry is urgently in need of further workers: the possibility of advance in horticulture, in agriculture, in the understanding of life itself largely depends on exact knowledge in regard to the facts of the plant. In an age of organic synthesis in Industry, we cannot know too much of the methods of the plant, the great worker of synthetic miracles. Solar energy, carbon dioxide, a catalyst and the directive forces of Nature daily perform the task of creating the living world.

The names of two workers stand out prominently in the field during the seven-year period—W. N. Haworth, who has established the pyranose structure of the sugars ; R. Robinson, whose almost daring syntheses of flavones and anthocyanins give us hope that all the secrets of the plant will yield to the chemist's questioning.

E. F. A.
K. F. A.

CHAPTER I.

THE GLYCOSIDES.

THE term glycoside is applied to a large number of substances having the property in common of furnishing a sugar and one or more other products when hydrolysed by acids. Representatives of nearly every class of organic compound occur in plants, chiefly in the fruit, bark and roots, in combination with a sugar which is in most cases dextro-glucose. These compounds correspond in structure to the simple synthetic methyl glucosides, and the general formula of a glycoside is accordingly written

$$CH_2(OH) \cdot CH \cdot (CH \cdot OH)_3 \cdot CH{-}O{-}R$$
$$\qquad\qquad\qquad\qquad\quad \llcorner\!\!\!-\!\!\!-\!\!\!-O\!\!\!-\!\!\!-\!\!\!-\!\!\!\lrcorner$$

where R represents the non-sugar radicle.

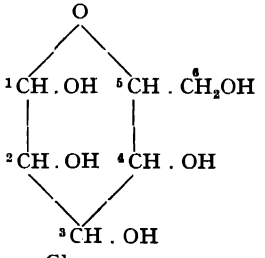
The paired glucuronic acid derivatives so readily formed in the animal and also present in plants are but glucosides oxidised in the side chain; they are therefore brought within the scope of this book. In both instances more or less reactive substances containing an hydroxyl group are combined with a sugar residue to form more stable and usually more soluble substances. Vegetable bases are only seldom found in the form of glycosides, no doubt because they lack hydroxyl groups.

The term glycoside is now used officially as a general name for the group, irrespective of the sugar present; glucoside is the specific name used for those glycosides the sugar constituent of which is glucose.

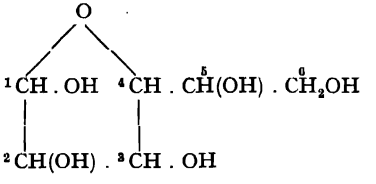
In the past it has been customary to give to glycosides names ending in *-in* based on the plants in which they occur. Latterly it has been proposed in France to substitute the suffix *-oside* to indicate the glycoside nature. Thus Asperulin becomes Asperuloside. For the nonce the older nomenclature will be retained here.

It is becoming usual and very convenient to refer to the non-sugar part of the glycoside as the aglucone, a term originating with the Japanese chemists.

The normal structure of glucose has now been satisfactorily established by the work of Haworth [1] as that of a 1 : 5-glucopyranose ring with a primary alcohol CH_2. OH group as a side chain. All glucosides are regarded as possessing this structure, there being so far no evidence of any natural glucoside having the alternative 1 : 4-glucofuranose structure with a longer side chain; synthetic crystalline methylglucosides and other derivatives having this furanose structure have been prepared.

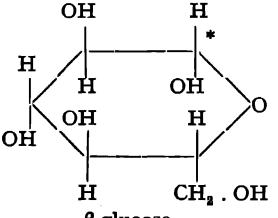


Glucopyranose.



Glucofuranose.

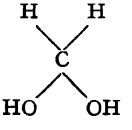
As is well-known, [2] there are two isomeric α- and β-glucoses, α- and β-methylglucosides, and other glucose derivatives, the difference depending merely on the space configuration of the groups on carbon atom 1 marked with * in the formula which represents β-glucose. In α-glucose the positions of the H and OH radicles are reversed, the rest of the molecule remaining unchanged.



β-glucose.

β-glucose is seen to have a completely trans-distribution for its groups, that is to say H and OH alternate above the plane of the carbon ring, a significant fact in view of its being the primary product of photosynthesis [3] and therefore originating from the formaldehyde units whose formation is the first act of photosynthesis by the plant.

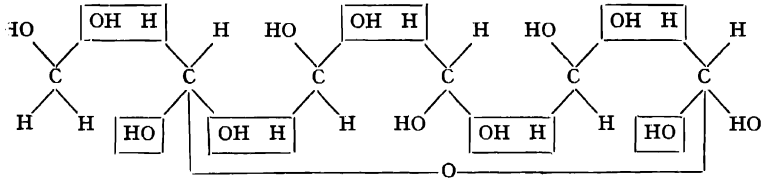
Assuming that hydrated formaldehyde units are to condense together at an active surface, it is obvious that successive units will



place themselves with a definite orientation. This might lead to a *cis*- or to a *trans*-distribution of similar groups, but a haphazard orientation is practically unthinkable.

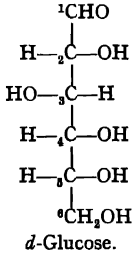
In β-glucose the groups are in fact in the trans position. The isomeric talose in which, when the pyranose ring is closed, the OH groups are all on the same side of the ring is unknown in nature.*

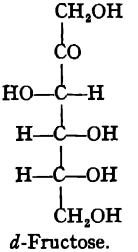
The condensation of six formaldehyde units by loss of six molecules of water, to give the closed ring β-glucose may be represented—

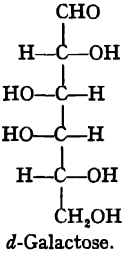


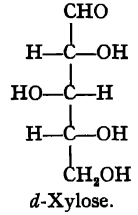
While the mechanism of condensation advanced is not necessarily true, the idea that the units available, by condensation in a regular pattern on a surface, would produce β-glucose, is a useful one.

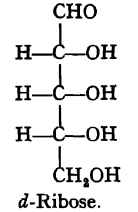
The configuration formulæ of the natural hexose, pentose and methyl pentose sugars, which are components of natural glycosides, are set out below in the old open chain aldehyde form used by Emil Fischer,[4] which most simply indicates their stereochemical relationship to one another :—

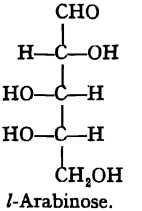

d-Glucose.


d-Fructose.

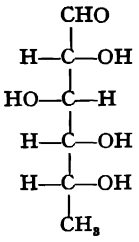

d-Galactose.

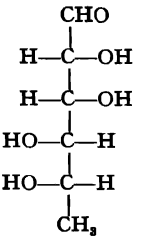

d-Xylose.

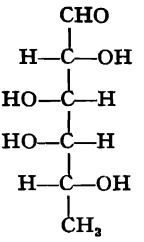

d-Ribose.


l-Arabinose.

* We have adopted the reasoning of Haworth in his " Constitution of the Sugars," page 36, that, when the ring is closed, the position of the hydrogen atom on carbon 5 is reversed. On this basis talose is the true *cis*-isomeride of glucose. According to the open chain formulæ only allose has all the OH groups on the same side of the ring.


d-Epirhamnose.

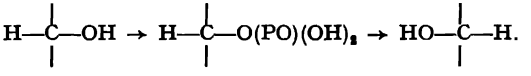

l-Rhamnose.


d-Rhodeose = (*d*-fucose).

Since *d*-glucose is accepted as the primary product of photosynthesis in the plant,[5] the question arises as to how the other natural sugars can be formed from it. The evidence cited in the next chapters will make it clear that these other sugars are far less rare than is perhaps supposed.

The transformation from glucose to mannose and to fructose is readily effected by means of dilute alkali, as was long ago shown by Lobry de Bruyn. The three sugars have a common enolic form, 1. . . . CH . OH
‖ , and the groups on carbon atom 2 undergo inversion.
2. . . . C . OH

One of the most interesting natural transformations of the sugar group is the power possessed by the mammary glands during lactation to convert glucose into galactose. This change involves the inversion of the groups attached to the fourth carbon atom. It always appeared difficult to explain when the 1 : 4-butylene oxide formula was ascribed to glucose, but now that Haworth's pyranose ring structure is accepted, it is possible to explain the change according to Robinson [6] as due to optical inversion taking place on hydrolysis of a glucose-4-phosphoric acid.



Such hexose phosphates are formed as intermediate products in alcoholic fermentation and in many metabolic changes in the animal.

A corollary of this suggestion is that the pentose of plant nucleic acid (see p. 74), which has been definitely isolated by Levene in the form of the rare sugar *d*-ribose, is *in situ* the much commoner *d*-xylose. An optical inversion is considered to have followed the removal of the phosphoric acid group attached to carbon 3 of the pentose. It should be said that Levene does not accept this view.

The conversion of glucose to xylose and that of galactose to ara-

binose involves first the oxidation of the groups on carbon 6 to the corresponding uronic acids (see Chapter VII.) and then decarboxylation to the respective pentoses.

The change from hexose to methyl pentose involves in theory the reduction of the primary alcohol group of carbon 6. This theory, however, does not account for the known methyl pentoses, since some of these are related to hexoses which are not known in nature. Thus the common *l*-rhamnose, of which the configuration is well authenticated, is a derivative of *l*-mannose, unknown in nature, and while *l*-fucose, common in fucosans from seaweeds, is a derivative of the unknown *l*-galactose, *d*-fucose derivable from *d*-galactose and *d*-epirhamnose derivable from *d*-glucose are extremely rare. Consequently the reduction of a hexose cannot be the explanation of their origin.[7]

Freudenberg has suggested as more probable the disproportionation of the appropriate hexitols, or the fission of inositols or quercitols, but at the present there is no satisfactory explanation.

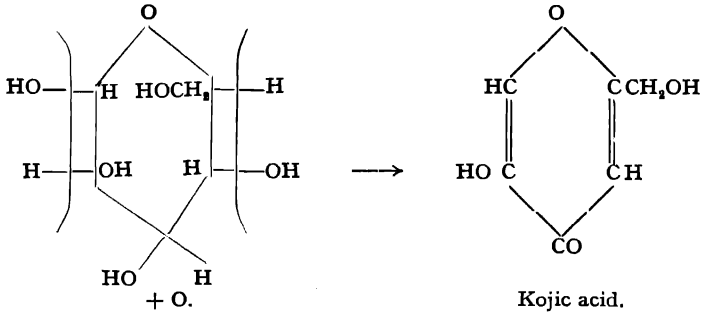
The disaccharides, which are common in the natural glycosides, result from the union of two simple sugars, which may be both the same or different. Consequently they can be either α- or β-glycosides of the sugar whose reducing group forms the disaccharide and they may be joined by elimination of water with the hydroxyl group attached to either carbon 6, 4, 3 or 2 of the second hexose, the reducing group of which eventually unites with the aglucone. A number of isomerides are thus possible in theory, though there seems to be a preference for the junction to be with carbon 6. Isomeric tri- and polysaccharide glycosides may differ in the order in which the respective sugars are joined to each other.

The increased carbohydrate content of the glycoside adds to its solubility.

While considering the derivation of other sugars from glucose it is permissible to digress to consider the possibility of derivation of other substances, in particular aglucones of glycosides which will be described, from hexoses.

β-glucose as a pyranose contains the same ring skeleton as that of the γ-pyrone and pyrylium rings of anthoxanthins and anthocyanins, which contain it fused to an aromatic nucleus.

A pyrone derivative kojic acid is actually formed during the growth of *Aspergillus oryzæ* on glucose, by the loss of two water molecules and oxidation :—



Kojic acid.

Kojic acid has, however, also been shown by Challenger [8] to be formed by the mould from arabinose and xylose, and in best yield from dihydroxyacetone. He assumes a preliminary breakdown of the sugars to a three carbon compound, suggesting that the γ-pyrone is not directly carved out of the pyranose ring.

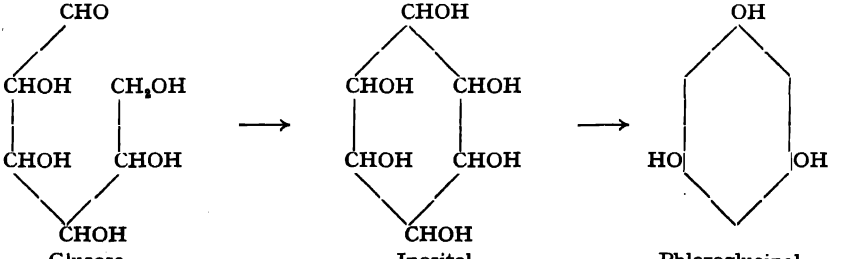
Attention is called to the work of Raistrick [8a] and his collaborators, who have obtained from certain species of *Citromyces* yields, as high as 25 per cent. of the sugar fermented, of a yellow benzopyrone derivative $C_{14}H_{10}O_7$ named citromycetin, which is a dihydroxy-carboxylic acid containing a benzopyrone nucleus.

Robinson [9] has developed a theory for the origin of the C15 skeleton of the anthocyanins and anthoxanthins by condensation of two hexose molecules and a triose.

The idea that a pyrone ring derived from a hexose is significant for the structural development of these compounds is, therefore, unnecessary.

More fruitful, though resting on slender evidence, is Haworth's suggestion of the derivation from glucose of the inositols and from them of poly-hydroxy phenols.

The pyran ring of glucose readily opens to the aldehyde form, which can be formulated as undergoing an aldol condensation to give a ring of 6 carbons, each having an hydroxyl attached to it; by loss of water from inositol the symmetrical trihydroxy-benzene, phloro-glucinol, is produced :—



Glucose Inositol. Phloroglucinol.

The inositols are widely distributed in plants and their occurrence gives support to the above hypothesis.[10, 11] They differ from one another according to the orientation of the hydroxyl groups above and below the plane of the carbon ring: the configuration at present assigned to inactive inositol itself indicates it to be a derivative of α-glucose.

Phloroglucinol is a fundamental unit in the benzopyrone and pyrylium aglucones; all but three of some thirty types have hydroxyl groups in the same positions as in phloroglucinol in the aromatic nucleus which is fused to the oxygen ring.

There is something significant about the occurrence of the symmetrical trihydroxybenzene in these aglucones which suggests a definite mechanism for its production, such as that from a hexahydrohexahydroxy-benzene in the above theory.

Glycosides are obtained by extraction of the plant substance with water or alcohol, an operation conveniently performed in a Soxhlet apparatus. It is usually necessary first to destroy the accompanying enzyme when water is used as solvent. If this operation be omitted, the glycoside is hydrolysed in the process of extraction.

The glycosides as a class are generally crystalline solids, having a bitter taste and lævorotatory optical power.

The glycosides are all hydrolysed by heating with mineral acids to sugar and an organic residue. They are decomposed at very different rates, some glycosides (e.g. gynocardin) being extremely resistant to acid hydrolysis.

The nature of the aglucone has naturally a considerable effect on the stability of a glycoside towards hydrolysis by acid. Thus Moelwyn-Hughes [12] has shown that the change from salicin, which contains a primary alcohol group in the benzene nucleus in the *ortho* position, to arbutin, containing a phenolic group in the *para* position, results in an increase of 158 per cent. in the rate of hydrolysis. The critical increment, which is determined largely by the nature of the link ruptured (fructoside, glucoside or galactoside), is substantially the same in each case. It is suggested by him, in agreement with the conclusion of Armstrong and Glover,[13] that the first step in hydrolysis is the opening of the ring and the momentary formation of a straight chained sugar derivative with the groups —OH and —OR attached to carbon atom 1. It may be assumed that such a compound is so unstable that fission occurs.

In the majority of cases the glycosides are hydrolysed by enzymes. The appropriate enzyme is contained in the same plant tissue, but in different cells, gaining access to the glycoside only when the tissue is destroyed. A great number of such enzymes exist, but it is too much

to say that each glycoside has a special enzyme for its decomposition. The best-known glycoside-splitting enzymes are the emulsin of almonds and the myrosin of black mustard seeds. Both these enzymes can effect hydrolysis of a number of glycosides.

Emulsin is especially wide in its action. Since it is the specific enzyme for β-alkyl glucosides, all glycosides hydrolysed by it are regarded as derivatives of β-glucose, though the fact that emulsin is a mixture of enzymes must not be lost sight of.

The specific β-glucosidase enzyme of emulsin which attacks so large a number of β-glucosides has been termed prunase by Haldane.[14] The term emulsin will be retained here, as it is this mixture which is usually employed in practice.

Active Glucose.

Glucose is one of the most important substances in the economy of the world. In the animal body it is the source of heat: it is the basic material from which starch and cellulose are built: it is the source of alcohol and lactic acid and of many other products of metabolism. The question therefore arises as to the active form which is a precursor to such metabolism, since crystalline glucose in the 1 : 5-amylene oxide or pyranose form is a relatively stable substance.

Less stable is the 1 : 4 ring, or furanose form. There must also be taken into consideration the open chain or aldehyde form and the open chain enolic form common to glucose, mannose and fructose. There further comes into question glucosone, believed to play a part in blood sugar oxidation. The fact that α-glucosan, said to contain a 1 : 2-ethylene oxide ring, is highly reactive and is more easily fermented than glucose[15] is hence of interest. This question has been discussed by Levene,[16] who summarises the position in saying that in aqueous solutions many, if not all, the theoretically possible structures co-exist in equilibrium with each other and that the agents (enzymes) which bring about fermentation or biological changes produce an increase in the amount and concentration of that form which is most apt to cause the required dissociation of the glucose molecule in the particular case. The initial phase in the process is probably the formation of a radicle with free valencies on carbons (1) and (2).

CHAPTER II.

NATURAL GLYCOSIDES.

Phenols, Hydroxyanthraquinones and Hydroxycoumarins.

A SELECTION only of the very many and often but incompletely characterised natural glycosides has been chosen for detailed comment, more particularly for the purpose of showing their relationship in groups according to their aglucones. At present, since the knowledge of the glycosides is chiefly derived from the investigation of substances used for medicinal purposes or of interest as colouring matters, only a beginning has been made in their study.

Many glycosides have been found in several plants and there is no doubt that some are widely distributed. Further work is simplifying the number of different glycosides, as in the saponins.

It is desirable, however, to search for material which will allow the differentiation of species on a purely chemical basis to be attempted as a parallel to the botanical classification, which indeed, as the names of plants show, is partly founded on the characteristic constituents of the plants. An advance in this enquiry in another branch of chemistry has been made by Hilditch in the observed correlation of species and specific fatty acid content.

In time the facts available may make it possible to explain the progressive building up of more complicated aglucones and correlate these perhaps with species or plant habitat. With so few primary materials available, with carbon dioxide as its sole source of carbon, it is an unending source of wonder that the plant has created an apparently limitless range of organic compounds, sometimes of fantastic complexity.

Both on account of the very small quantity of a glycoside usually present in a plant, and the fact that glycosides do not as a rule form insoluble characteristic derivatives which allow of their isolation, it is difficult to discover new glycosides and still more so to determine their nature. The introduction of biochemical methods has much facilitated work of this kind. Bourquelot's [1] biological method, introduced in 1901, has led to the discovery of many new glucosides : thus

at one time out of 281 phanerogams investigated, glucosides were found to be present in 205 and subsequently isolated from fifty-six plants.

Bourquelot's biological method of examining plants for glucosides consists in the addition of emulsin to an extract of the plant and the determination of the changes in optical rotation and cupric reducing power after a period of incubation. A change indicates the presence of β-glucosides and its magnitude gives a rough indication of their quantity.

In some respects too little attention has been paid to the identification of the sugar, often a matter of difficulty, and more thorough investigation of the action of α- and β-enzymes and of enzymes specific to the particular plant is needed.

The nature of the sugar content in several instances has been established by means of the method introduced by Ter Meulen,[2] who makes use of the fact that an enzyme is only compatible with, and therefore only enters into combination with that sugar, the simple glycosidic compounds of which it is able to hydrolyse. He has investigated the rate of hydrolysis of a glycoside by the appropriate enzyme in presence of a number of the simple sugars. Only one of these sugars retards the change; the others are almost without influence. The glycoside in question is considered to be a derivative of that sugar which retarded the hydrolysis.

For instance, rhamninose alone retards the hydrolysis of xanthorhamnin; glucose alone retards the decomposition of salicin.

The Phenolic Glycosides.

TABLE 1.

Glycoside.	Formula.	Sugar.	Aglucone.
Arbutin	$C_{12}H_{16}O_7$	glucose	hydroquinone
Methylarbutin	$C_{13}H_{18}O_7$	glucose	methyl hydroquinone
Salicin	$C_{13}H_{18}O_7$	glucose	*o*-hydroxy-benzyl alcohol
Helicin	$C_{13}H_{16}O_7$	glucose	salicylic aldehyde
Spiraein	$C_{13}H_{16}O_7$	glucose ?	salicylic aldehyde
Populin	$C_{20}H_{22}O_8$	benzoyl-glucose	*o*-hydroxy-benzyl alcohol
Vaccinin	$C_{13}H_{16}O_8$	glucose	benzoic acid.
Picein	$C_{14}H_{16}O_7$	glucose	*p*-hydroxy-acetophenone
Coniferin	$C_{16}H_{22}O_8$	glucose	coniferyl alcohol
Syringin	$C_{17}H_{24}O_9$	glucose	5-methoxy-coniferyl alcohol
Androsin	$C_{15}H_{20}O_8$	glucose	acetovanillone
Gaultherin	$C_{19}H_{26}O_{12}$	primeverose	methyl salicylate
Violutin	$C_{19}H_{26}O_{12}$	vicianose	methyl salicylate
Gein	$C_{21}H_{30}O_{11}$	vicianose	eugenol

ARBUTIN, a colourless, bitter, crystalline substance, is obtained, together with methylarbutin, from the leaves of the bearberry, a

small evergreen shrub (*Arbutus uva ursi*), and from many genera in the *Ericaceæ ;* it yields hydroquinone and glucose when hydrolysed by means of emulsin or mineral acids. It has been obtained from the leaves and also from the bark and roots of many varieties of the pear.[3]

Hydroquinone is a powerful antiseptic ; hence the pharmacological value of arbutin, which has also a diuretic action. Methyl arbutin was one of the first glucosides to be artificially synthesised. Michael [4] prepared it by the interaction of hydroquinone methyl ether and acetochloroglucose. Mannich [5] has synthesised arbutin from acetobromoglucose and quinol.

Commercial arbutin may contain from 5 to 40 per cent. of methyl arbutin ; to purify it, according to Hérissey,[6] it is dissolved in alcohol, precipitated by potassium hydroxide and the precipitate collected, washed and decomposed with calcium carbonate. Mannich states that a better, but still imperfect, method of separation of pure arbutin from the mixture is to take advantage of the additive compound formed by arbutin and hexamethylene tetramine. Bearberry leaves from Tyrol contain much methyl arbutin, whilst Spanish bearberry leaves yield a product containing not more than 5 per cent. of the methyl ether.

When arbutin is hydrolysed by emulsin the quinol formed becomes slightly oxidised by the oxygen present in the solution, which darkens in colour. Methyl arbutin, which yields quinol methyl ether on hydrolysis, does not darken in solution. It is hydrolysed more rapidly than arbutin.

Bourquelot and Fichtenholz [7] have made an extensive study of the distribution of arbutin in the leaves of Pyrus species. Pear leaves (*Pyrus communis*) contain as much as 1·2 to 1·4 per cent. of the glucoside, which can be extracted by ethyl acetate. None could be detected in *Cydonia vulgaris*, *Malus communis*, *Sorbus aucuparia*, or *S. torminalis*, all of which trees were at one time classed with *Pyrus*, but are now regarded by botanists as distinct : the modern classification is thus justified on biochemical as well as on morphological grounds.

The leaves of certain varieties of Pyrus turn black when they fall ; these contain arbutin, which is hydrolysed to quinol by the leaf enzyme, the quinol in turn being acted on by an oxydase to form the black substance. In other varieties a golden yellow tint first appears which then gives place to black. These varieties are shown to contain methyl arbutin ; they produce at first a yellow and not a black oxidation product.

SALICIN, a colourless, crystalline, bitter substance, is the active constituent of willow bark; it has long been used as a remedy for fever and in cases of acute rheumatism. At first thought to be an alkaloid, it was not until 1845 that Piria [8] cleared up its constitution. It is hydrolysed by emulsin to glucose and saligenin (ortho-hydroxy-benzyl alcohol), and has the formula $C_6H_{11}O_5 . O . C_6H_4 . CH_2OH$. Saligenin yields salicylic acid on oxidation, but has the advantage of being less irritant than this acid or its salts, and therefore does not produce digestive disturbances when administered medicinally.

Salicin occurs in many but not all species of *Salix*, also in poplars and in the flower buds of meadow-sweet, *Spiræa ulmaria*. In the willow it is found in the leaves and female flowers as well as in the bark; the leaves and twigs of willows also contain a specific enzyme, salicase,[9] which hydrolyses it.

HELICIN, the glucoside of salicylic aldehyde, $C_6H_{11}O_5.O.C_6H_4.CHO$, is obtained on oxidation of salicin with dilute nitric acid. It has not been found to occur naturally, but was synthesised by Michael [10] from salicylaldehyde and acetochloroglucose. Emulsin hydrolyses helicin and also its hydrazone and oxime. Helicin was coupled by Fischer [11] with hydrogen cyanide to yield a synthetic cyanogenetic glucoside, from which a further series of glucosides were obtained.

Helicin combines with acetaldehyde [12] to form *o*-coumaraldehyde glucoside, $C_6H_{11}O_5 . O . C_6H_4 . CH : CH . CHO$, which is easily reduced to the corresponding alcohol. With acetone the glucoside

$C_6H_{11}O_5 . O . C_6H_4 . CH : CH . CO . CH_3$ is obtained.

SPIRAEIN, an amorphous glucose derivative, present in the roots of *Spiræa kamtschatica* and *S. Ulmaria*, is hydrolysed to glucose and salicylaldehyde by acids but not by emulsin. It is therefore not identical with helicin. However, the enzyme gaultherase hydrolyses it, suggesting that, like gaultherin (see p. 13), it is a derivative of primeverose and not of glucose.

POPULIN, a glucoside present in the bark of a number of species of poplar, is monobenzoyl salicin. It is formed when salicin is shaken with benzoylchloride. It is oxidised to benzoyl helicin and obtained from this compound again on reduction. The benzoyl group is thus in the sugar nucleus and not attached to the alcoholic group of saligenin.

$C_6H_5 . CO . O . C_6H_{10}O_4 . O . C_6H_4 . CH_2 OH.$

Emulsin is without action on it, doubtless because of the position of the benzoyl group. An enzyme present in *Populus monilifera* is said

by Weevers [13] to hydrolyse it to salicin and benzoic acid. Kitasato [14] finds that takadiastase converts it into saligenin and a benzoyl glucose which differs from the natural compound vaccinin. The bacteria in cheese hydrolyse it completely to glucose, saligenin, and benzoic acid.

VACCININ is a constituent of whortleberries (*Vaccinium vitis idæa*), from which it is obtained as a syrup. It was formerly thought to be arbutin. It is an ester and not a glucoside, as it forms a phenyl hydrazone and reduces Fehling's solution. It yields glucose and benzoic acid on hydrolysis.

Another benzoyl ester of the sugars is the dibenzoylglucoxylose $C_{25}H_{28}O_{12}$ obtained by Power and Salway [15] from *Daviesia latifolia*. It is hydrolysed by alkali to benzoic acid and glucoxylose, a non-reducing disaccharide to which a formula analogous to that of trehalose is assigned. Acid hydrolysis leads to the production of glucose and xylose.

An isomeride of higher melting-point which accompanies it has been described by Tutin [16] without any indication as to its composition.

PICEIN, *p*-hydroxyacetophenone-β-glucoside, $C_6H_{11}O_5 - O . C_6H_4 . CO . CH_3$, first discovered by Tanret, is identical with Jowett's salinigrin (originally said by him to be the glucoside of *m*-hydroxybenzaldehyde), with the ameliaroside of Bridel,[17] and with the salicinerin from *Salix cinerea*.[18]

Jowett and Potter [19] found it in only one species of willow and poplar (*Salix discolor*) out of thirty-three species examined by them.

CONIFERIN, the glucoside of the fir-tree, was of importance as the starting-point for the synthesis of vanillin, which is formed from it by oxidation with chromic acid.

It yields glucose and coniferyl alcohol when hydrolysed by emulsin, and has the formula

$$C_6H_{11}O_5 . O . C_6H_3(OMe) . CH : CH . CH_2OH.$$

By careful oxidation with chromic acid, glucovanillin is formed, and this may be oxidised to glucovanillic acid or reduced to glucovanillyl alcohol. All three glucosides are hydrolysed by emulsin.

The synthesis of coniferin has been effected by Pauly and Feuerstein,[20] who coupled 4-hydroxy-3-methoxycinnamic-aldehyde with acetobromoglucose to give glucoconiferylaldehyde. This could not be reduced by purely chemical means, but reduction was smoothly effected biologically by adding it to a solution of sugar undergoing brisk fermentation. This method is also effective in the reduction of chloral to chloral alcohol.

SYRINGIN, $C_{17}H_{24}O_9$, the glucoside of *Syringa vulgaris*, and also *Ligustrum vulgare*, etc., is 5-methoxyconiferin. It is likewise hydrolysed by emulsin. The ketoglucoside α-glucoacetosyringone has been synthesised by Mauthner [21] from 4-hydroxy-3 : 5-dimethoxyacetophenone ; this glucoside does not occur naturally. The corresponding glucosyringaldehyde has also been synthesised from 4-hydroxy-3 : 5-dimethoxybenzaldehyde and is identical with the oxidation product of syringin prepared by Körner.[22]

It is of interest that syringenin, the aglucone of syringin is the alcohol corresponding to sinapinic acid, which is contained in sinapin, the aglucone of the mustard oil glucoside sinalbin (*q.v.*).

Syringaic acid, $C_9H_{10}O_5$, which is 3 : 5-dimethylgallic acid, found by Power in *Robinia pseudacacia*, is perhaps present there as glycoside. Its glucoside was synthesised by Mauthner [23] and by Fischer and Bergmann.

ANDROSIN, $C_{15}H_{20}O_8$, the glucoside of acetovanillone, $C_6H_{11}O_5 . O . C_6H_3 (OMe) . CO . CH_3$, was isolated by Moore [24] from the rhizome of *Apocynum androsemifolium*.

GAULTHERIN (*Monotropitin*) is the parent glycoside of methyl salicylate, commonly found in plants, especially in the roots of *Gaultheria* and *Spiræa* species.

Emulsin is without action, but a synthetic glucoside,[25] obtained by the action of diazomethane on glucosidosalicylic acid, is hydrolysed by emulsin and is therefore a β-glucoside and is not identical with gaultherin.

Gaultherin is hydrolysed by mineral acids: and the enzyme gaultherase which accompanies it in plants hydrolyses it to the biose primeverose, a glucoxyloside (see p. 76). Gaultherin is therefore a primeveroside, $CH_3OOC—C_6H_4—O—C_6H_{10}O_4—O—C_5H_9O_4$.

According to Bridel it is also identical with *Monotropitin* from *Monotropa Hypopitys* and *Betula lenta*.

The synthesis of gaultherin has been achieved by Robertson and Waters,[26] who have also prepared the rhamnoside and xyloside of methyl salicylate.

The methyl-salicylate glycoside *Violutin*, extracted from *Viola cornuta*,[27] is stated to contain glucose and *l*-arabinose probably combined as vicianose.

GEIN, from *Geum urbanum*, is hydrolysed to *l*-arabinose, glucose, and eugenol.[28] The aglucone is attached to glucose, and the enzyme, gease, of the plant hydrolyses the glycoside to eugenol and the disaccharide vicianose previously found in vicianin. It has the structure

$$C_5H_9O_4—O—C_6H_{10}O_4—O—C_6H_3(OMe) . CH_2 . CH : CH_2.$$

PHLORIDZIN, the glucoside found in the bark and particularly the root bark, whence its name, of the apple, pear, cherry, plum, is remarkable in many ways. It is also present in the leaves of the apple.

It causes severe glycosuria in animals when injected internally, as also does synthetic phloroglucinol β-glucoside.

Towards acid hydrolysis it is the most labile glucoside hitherto examined.[29] The critical increment of the reaction is materially smaller than that of other glucosides and shows an analogy to that of the γ-fructosides.

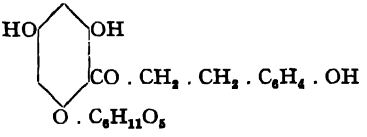
Emulsin is usually stated to be without action on it, but according to Bourquelot and Hérissey [30] it is decomposed both by almond emulsin and by *Aspergillus niger*, as well as by a number of animal extracts.

Owing to its very low solubility it is difficult to examine the enzymic hydrolysis of phloridzin, but Moelwyn Hughes [31] has shown that emulsin is definitely without action at various values of pH and of temperature and that it is hydrolysed by maltase free saccharase—that is presumably by a fructosidase.

The identification of the sugar as glucose is questioned as the specific rotation is lower, and comparison of the action of various bacilli on glucose and phloridzin sugar does not confirm their identity.

On the other hand, Bridel,[32] in a recent paper, asserts that phloridzin is hydrolysable by emulsin, but owing to the extremely small solubility in water, at a very small rate, which is only made manifest to polarimetric observation after a very long time.

The constitution of phloridzin is



Mineral acids form glucose and phloretin [33] $C_{15}H_{14}O_5$. The constitution of phloretin was established by its synthesis by Fischer and Nouri,[34] whilst Cremer and Seuffert [35] showed that the glucose residue was attached to the phloroglucinol nucleus, since on warming phloridzin with baryta water they obtained phloroglucinol β-glucoside, previously synthesised by Fischer and Strauss,[36] and phloretic acid, parahydroxyhydrocinnamic acid. Wessely and Sturm [37] confirmed the above constitution by methylation methods. The final proof as to which hydroxyl is concerned has been given by Johnson and Robertson.[38]

Zemplen,[39] who has prepared phloretin from purely synthetic materials, sought to effect the condensation with acetobromoglucose, which is able, however, to react with at least three different hydroxyl groups and so furnish a number of isomerides. He failed to isolate phloridzin.

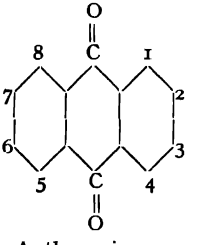
The direct evidence that phloridzin sugar is glucose is unsatisfactory. It may be a derivative of fructose or of γ-glucose, and further investigation is required as it cannot yet be regarded as a normal β-glucoside.

GLYCYPHILLIN, the glycoside of the leaves of *Smilax Glycyphylla*,[40] also contains phloretin, the sugar being rhamnose.

The phloroglucinol aglucone is present in a large number of glycosides, often as part of a larger complex.

Hydroxyanthraquinone Glucosides.

Hydroxy compounds of anthraquinone are widely distributed in plants both as glycosides and in the free state. They are yellow or red colouring matters used with mordants and formerly were of great importance in dyeing. The emodins are of medicinal importance, being constituents of rhubarb root.



Anthraquinone.

TABLE 2.

Ruberythric acid	2 glucose	1 : 2-dihydroxyanthraquinone.
Rubiadin .	1 glucose	1 : 3-dihydroxy-2-methylanthraquinone.
Purpurin . .	1 glucose	1 : 2 : 4-trihydroxyanthraquinone.
Xanthopurpurin	1 glucose	1 : 3-dihydroxyanthraquinone.
Hystazarin .	1 glucose	2 : 3-dihydroxyanthraquinone.
Munjistin .	1 glucose	1 : 3-dihydroxy-4-carboxyanthraquinone.
Frangulin .	1 rhamnose	emodin = 1 : 6 : 8-trihydroxy-2-methylanthraquinone.
Morindin . .	2 glucose	isomer of emodin.
Polygonin .	glucose	emodin.
Rheochrysin .	glucose	emodin methyl ether.
Chrysophanin .	glucose	1 : 8-dihydroxy-3-methylanthraquinone.
Barbaloin .	glucose	aloemodin.

Madder, the ground root of *Rubia tinctorum*, which was long considered as a most important dye-stuff and has been cultivated from

remote antiquity, consists of a number of glycosides, of which the most important is ruberythric acid. This is composed of two molecules of glucose and alizarin, i.e. 1 : 2-dihydroxyanthraquinone. The glucose molecules are probably united as a disaccharide, since the glucoside is strongly acid and forms red coloured salts, indicating the presence of a free hydroxyl group. After its synthesis by Graebe and Liebermann, alizarin has been manufactured entirely from anthraquinone and the madder industry destroyed.

The madder contains an enzyme, erythrozyme, which hydrolyses the glycoside.

Zemplen and Müller[1] have prepared synthetic glycosides from alizarin and glucose and also from the disaccharides cellobiose and gentiobiose; with maltose no crystalline compounds could be obtained. They conclude that ruberythric acid is not a gentiobioside, and though there were certain analogies in melting-point with the cellobioside the two compounds could not be regarded as identical. A monoglucoside of alizarin with the sugar residue in position 2 has been synthesised.[2, 3]

Müller,[4] who has combined acetobromoglucose with a number of hydroxyanthraquinones, has established the existence of dihydroxyanthraquinone diglucosides, including those containing the sugar residues attached to the same benzene nucleus. α-hydroxyl groups, when protected by β-hydroxyl groups, will not, however, react under any conditions with excess of the halogenated sugar. There is strong presumption, therefore, that ruberythric acid is a bioside.

The possibility that two sugars may at the same time be attached to different hydroxyls in the polyhydroxy aglucones has always to be borne in mind—particularly when considering the structure of the anthoxanthins. So far no evidence of such compounds in nature has been found, but it will be well to search for them.

Other glucosides present in madder are as follows :—

PURPURIN, the glucoside of 1 : 2 : 4-trihydroxyanthraquinone, is more easily hydrolysed than ruberythric acid.

RUBIADIN is the glucoside of 1 : 3-dihydroxy-2-methyl-anthraquinone.

The question whether the methyl group was in position 2 or 4 has been the subject of considerable enquiry; it has been finally set at rest by the synthesis of rubiadin by Jones and Robertson,[5] who have also established that the sugar is attached at position 3. It is of interest that when acetobromoglucose was used in excess, an octa-acetyl-β-diglucorubiadin was obtained.

Closely related is MUNJISTIN, which is composed of 1 : 3-dihydroxy-4-carboxyanthraquinone and glucose; it occurs in *Rubia munjista.*

XANTHOPURPURIN, or the glucoside of 1 : 3-dihydroxyanthraquinone, which is isomeric with alizarin.

HYSTAZARIN, or 2 : 3-dihydroxyanthraquinone, is found as a monomethyl ether.

Three dimethyl ethers of anthragallol—which is the synthetic anthracene brown dyestuff of commerce and has the structure of a 1 : 2 : 3-trihydroxyanthraquinone—exist in nature. Lastly, a trihydroxy methylanthraquinone is known.

It is evident that practically all the possible hydroxyanthraquinones exist as glucosides in the madder group of plants. As a consequence of the mixture, natural alizarin has always dyed a bluer shade than the commercially pure synthetic product.

FRANGULIN, from the bark of *Rhamnus frangula*, is hydrolysed to rhamnose and emodin, which is 1 : 6 : 8-trihydroxy-2-methylanthraquinone.

MORINDIN is the glucoside of morindon, the colouring matter of various *Morinda* species used in Bengal and Java as dyes. The aglucone is isomeric with emodin.

POLYGONIN, isolated by Perkin from *P. cuspidatum*, is hydrolysed to glucose and emodin. The plant contains a second glucoside of emodin dimethylether.

RHEOCHRYSIN is the glucoside of emodin methyl ether; it occurs in Chinese rhubarb.

CHRYSOPHANIN is the glucoside of chrysophanic acid, which is 1 : 8-dihydroxy-3-methylanthraquinone. Related to it is aloe-emodin, in which the CH_3 group has become $—CH_2OH$, and rhein where it is —COOH. The latter occurs in Chinese rhubarb and the former is a product of hydrolysis of the aloins from Barbadoes and Curaçao.

The aloins are compounds of pentoses and hydroxyanthraquinone derivatives in which it is suggested the aldehyde group of the sugar is free, the terminal hydroxyl being linked to a phenolic hydroxyl. They have been investigated by Tschirch and by Léger.

BARBALOIN is hydrolysed to aloemodin and *d*-arabinose. This pentose was at first described under the name aloinose[6]: it affords one of the rare instances of the natural occurrence of both *d* and *l* modifications of a carbohydrate.

Gibson and Simonsen [7] have confirmed the production of *d*-arabinose and expand the formula to $C_{20}H_{12}O_3(OH)_6$. They show that the constitutions of barbaloin and of the aloins is far more complex than hitherto assumed, as evidenced by the extreme resistance to hydrolytic agents.[7]

Coumarin Glycosides.

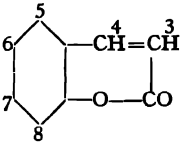
TABLE 3.

Glucoside.	Formula.	Sugar.	Aglucone.
Skimmin . .	$C_{15}H_{16}O_8$	glucose	7-hydroxycoumarin
Æsculin . .	$C_{15}H_{16}O_9$	glucose	6 : 7-dihydroxycoumarin
Daphnin . .	$C_{15}H_{16}O_9$	glucose	7 : 8-dihydroxycoumarin
Scopolin . .	$C_{22}H_{26}O_{14}$	2 glucose	6-methylæsculetin
Fabiatrin . .	$C_{16}H_{18}O_9$	glucose	6-methylæsculetin
Fraxin . .	$C_{16}H_{18}O_{10}$	glucose	6-methoxy-7 : 8-dihydroxycoumarin

Coumarin itself is very widely distributed in plants and it is usually considered that it is present in the form of a glucoside. The odour is as a rule only perceptible in the dried leaf, but it is rapidly developed in the living leaves when these are submitted to the action of anæsthetics or of cold,[1] a phenomenon which is generally characteristic of the hydrolysis of glucosides. Bourquelot and Hérissey [2] found that species of Melilotus and of Asperula gave coumarin under the influence of emulsin, an observation extended to *Melittis melissophyllum* by Guérin and Goris,[3] and to *Melilotus arvensis* by von Lippmann. As coumarin itself has no free hydroxyl group, it is possibly present originally as *o*-coumaric acid, which readily changes into the *cis*-form coumarinic acid,

$$\begin{array}{l} H \cdot C \cdot C_6H_4 \cdot OH \\ \quad\quad \| \\ H \cdot C \cdot COOH \end{array}$$

and this into the lactone coumarin,



Melilotin, the glucoside of coumaric acid, has actually been isolated by Charaux [4] from the flowers of *Melilotus altissima* and *arvensis*. When acted upon by emulsin, or by heating with dilute

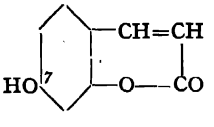
mineral acids, it is hydrolysed to glucose and coumaric acid and therefore can be formulated

$$C_6H_{11}O_5—O—C_6H_4 . CH : CH . CO_2H.$$

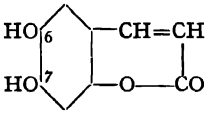
It is well known that the woodruff (*Asperula odorata*) loses its odour of coumarin, which is so marked in the spring, in summer. Herboth [5] has measured the amount of glucoside by extracting the plants freed of the roots with 95 per cent. alcohol, then hydrolysing the extract with emulsin. The change measured with the polarimeter is regarded as indicating coumarin glucoside, and he shows that the amounts present in the plant in August and May bear to one another the relation 1 : 6.

Several glycosides containing hydroxycoumarins are known in a variety of plants. They contain one, two, or three hydroxy groups or their methyl ethers. In many cases the point of attachment of the sugar has been established.

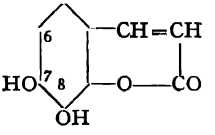
The following formulæ show the relation of these hydroxycoumarins :—



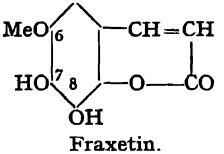
Skimmetin.



Æsculetin.



Daphnetin.



Fraxetin.

Skimmin, $C_{15}H_{16}O_8$, a constituent of *Skimmia japonica*, is the glucoside of 7-hydroxycoumarin or skimmetin, which is identical with umbelliferone, a widely distributed plant product especially in the resins of the *Umbelliferæ*.

The methyl ether of skimmetin—herniarin—was obtained by Power and Browning [6] from the flower heads of German chamomile (*Matricaria Chamomilla*).

Isomeric with skimmin is the synthetic gluco-oxycoumarin of Mauthner.[7]

Æsculin, found in horse chestnut bark (*Æsculus hippocastanum*), and Daphnin, a constituent of several species of *Daphne*, are glucosides of the isomeric 6 : 7 and 7 : 8-dihydroxycoumarins named æsculetin

and daphnetin respectively. In æsculin the glucose residue is shown by Head and Robertson [8] to be located in position 6.

In daphnin the sugar residue is considered by Wessely and Sturm [9] to be attached to position 7, although previously they had thought position 8 possible [10] by analogy with the synthetic daphnin obtained by Leone [11] from acetobromoglucose and 7 : 8-dihydroxycoumarin which has the glucose in the 8-position and which Hattori [12] has proved not to be identical with daphnin.

The methyl ethers of æsculin are known in glucosides.

SCOPOLIN is the glucoside of scopoletin, first isolated from the rhizome of *Scopolia japonica* and also known as gelseminic acid from *Gelsemium sempervirens*.

Moore [13] identified scopoletin as 6-methoxy-7-hydroxy coumarin, so that the sugar is in position 7.[14, 15]

Scopolin is said to contain two molecules of glucose as a disaccharide.

FABIATRIN, from *F. imbricata*, is a simple glucoside of scopoletin.[16]

Limettin, the dimethyl ether of æsculin, is found in various oils of citrus species.

FRAXIN, found in the ash (*Fraxinus excelsior*), particularly in the young bark, in species of *Æsculus* and in *Diervilla*, is the glucoside of 6-methoxy-7 : 8-dihydroxycoumarin, termed fraxetin. It is hydrolysed by emulsin. Wessely and Demmer [17] have shown the glucose molecule to be attached at position 8, for it is converted by methylation and hydrolysis and ethylation into 6 : 7-dimethoxy-8-ethoxy coumarin.[18]

AUCUBIN is the characteristic glycoside of the spotted laurel,[19] which causes the leaf to go black when injured or exposed to organic vapours, owing first to hydrolysis of the glycoside and then to oxidation of the aucubigenin. It is easily prepared from the ripe berries of the aucuba. It appears to be generally distributed, as it has been obtained in pure condition from species of *Melampyrum*,[20] from the seeds of *Veronica hederæ folia*,[21] and from the stems and seed of *Rhianthus*.[22]

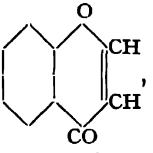
Bergmann and Michalis,[23] who obtained it from the seeds of *Plantago lanceolata*, gave it the composition $C_{15}H_{22}O_9$. It is identical with meliatin from *Lathroea claudestine*.

ASPERULIN, m.p. 126° $(\alpha)_D$-204°, from the woodruff (*Asperulera odorata*), is hydrolysed by emulsin to glucose and an aglucone. It is said by Hérissey [24] to have similar properties to aucubin.

CHAPTER III.

GLYCOSIDES OF THE SOLUBLE PLANT PIGMENTS.

The Anthoxanthin Glycosides.

THE great majority of soluble yellow plant pigments are derived from benzopyrone or chromone,  , to the aromatic nucleus of which are attached one or more hydroxyl groups, and to the pyrone nucleus one of the simple aromatic compounds benzene, phenol, resorcinol, catechol or pyrogallol ; the hydroxyl groups are often methylated. The xanthones are derived from dibenzopyrone.

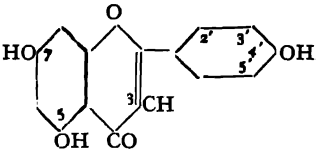
They may be conveniently grouped under the name " anthoxanthins," proposed by Willstätter and Everest [1] on account of their structural similarity to the anthocyanins.

These pigments, before the days of synthetic dyestuffs, were of great importance, being obtained from barks and dyewoods and used with copper, iron, tin, or aluminium mordants to yield comparatively fast green, yellow, and red dyes.

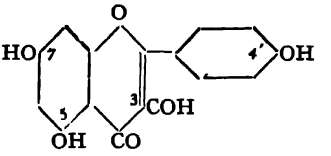
In the following no attempt has been made to do more than list the various glycosides and indicate their structure and relationships. The physical and chemical and tinctorial properties of the aglucones and their glycosides form a special section of the subject with a considerable literature of its own.

The structure of the pigments has been deduced from the study of their decomposition products and in many instances confirmed by synthesis : much of the progress in this group is due to the work of A. G. Perkin.[2]

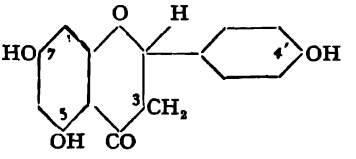
Five groups will be considered, the flavones, the flavonols, the flavanones, the isoflavones and the xanthones :—



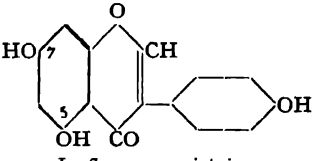
Flavone-apigenin.



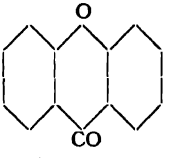
Flavonol-kæmpferol.



Flavanone-naringenin.



Isoflavone-genistein.



Xanthone.

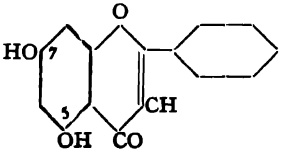
All occur in plants as glycosides in which the sugar residue may be either glucose or rhamnose, or some other monosaccharide, a disaccharide, or even a trisaccharide. In addition to the differences in the aglucone, there are several isomerides possible according to which hydroxyl group is concerned in the attachment of the sugar: such differences in constitution correspond with differences in the properties of the several glycosides, especially in their behaviour towards acids.

The glycosides are in general colourless or nearly so, and it would appear that when a yellow tint is attributed to them, it is to be traced, as in the yellow cotton flower, to their presence as a potassium or other salt, or to the sugar-free pigments, which occur also in the free state alongside the glycosides, and are yellow.[3]

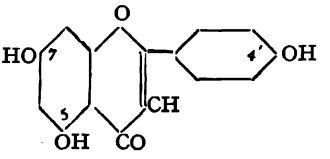
The following flavones and flavonols and their glycosides have been isolated from plants:—

Flavones.

The glycoside of *Chrysin*, 5 : 7-dihydroxy-flavone, has not itself been isolated from plants, but the flavone occurs in various species of poplar and mallows:—



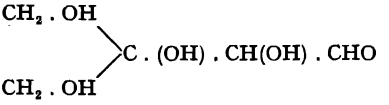
APIIN, the glycoside present in the leaves and seeds of parsley, is hydrolysed to glucose, apiose (a sugar of abnormal constitution), and apigenin, 5 : 7 : 4′-trihydroxyflavone :—



According to Perkin the sugar glucoapiose is attached to the 7-hydroxyl group through the glucose portion of its molecule.

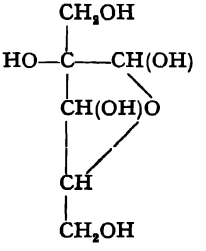
Apigenin also occurs as a glucoside in chamomile flowers.

The sugar apiose, which has so far only been found as a constituent of apiin, has a branched chain of carbon atoms and was shown by Vongerichten [4] and recently confirmed by Schmidt [4] as possessing the formula



It is oxidised to apionic acid, which, when reduced by hydriodic acid and phosphorus, yields isovaleric acid.

The only other sugar known with a branched chain is hamamelose, from hamameli tannin, for which Schmidt [4a] gives the structure of a γ-sugar :—

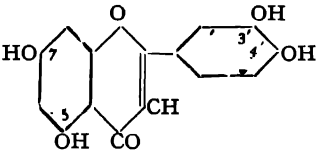


It seems probable that further abnormal sugars will be isolated as the detailed examination of plant products proceeds.

ACACIIN, from the leaves of *Robinia pseudacacia*, is hydrolysed to two molecules of rhamnose and acacetin, which is 4′-methyl-apigenin. Hattori [5] deduces from the absorption spectrum that the sugar is attached to the 7-hydroxyl group.

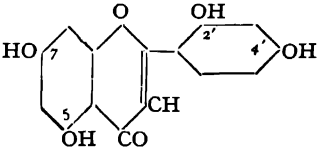
GALUTEOLIN,[6] from the seeds of *Galega officinalis*, yields glucose and luteolin, which is also the colouring matter of Weld (*Reseda*

luteola), and of *Genista tinctoria*. Luteolin is 5 : 7 : 3′ : 4′-tetrahydroxy-flavone :—



Diosmin,[7] the glycoside from *Scrophularia nodosa* and a variety of plants, is hydrolysed to rhamnose, two molecules of glucose and diosmetin, which is 4′-methyl-luteolin.

Lotusin is a cyanogenetic glycoside occurring in the Northern African *Lotus Arabicus*. It is hydrolysed to two molecules of glucose, hydrocyanic acid and to lotoflavin, considered by Dunstan and Henry [8] to be 5-7-2′-4′-tetrahydroxy-flavone, isomeric with luteolin :—

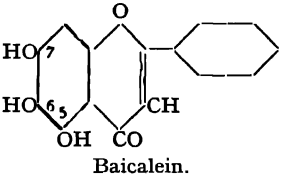


A synthesis of this compound by Robinson and Venkataraman, and by Cullinane, Agar and Ryan, and the comparison of its properties with the natural product, did not, however, confirm their identity.

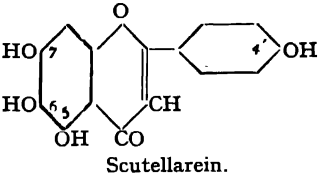
Orobosin,[9] from *Orobus tuberosa*, is a β-glucoside hydrolysed by emulsin, yielding glucose and orobol, a tetrahydroxy-flavone.

The two following interesting flavone derivatives contain their sugar in the form of glucuronic acid instead of glucose.

The roots of *Scutellaria baicalensis* contain Baicalin,[10] which yields glucuronic acid and baicalein, 5-6-7-trihydroxyflavone :—



Baicalein.

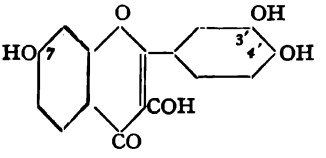


Scutellarein.

The leaves of *Scutellaria baicalensis*, and the flowers of *Scutellaria altissima*, contain Scutellarin,[11] which, on dissolving in concentrated sulphuric acid and pouring into water, is hydrolysed to glucuronic acid and scutellarein, 4′-hydroxy-baicalein. The glucuronic acid is in both cases thought to be attached in position 6. The occurrence of the one compound in the roots and the other with an additional hydroxyl group in the leaves of the same plant is of great interest.

Flavonols.

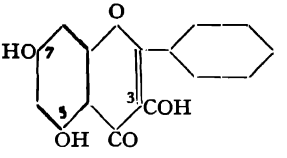
FUSTIN, the glycoside from the stems and branches of young fustic (*Rhus Cotinus*) and from *Quebracho Colorado*, is hydrolysed to rhamnose and fisetin, 7-3′-4′-trihydroxy-flavonol:—



The leaves of young fustic which constitute the tanning material, Venetian Sumach, contain myricetin.

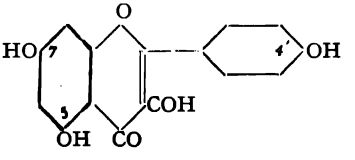
Fisetin is unique in being the one flavonol which does not possess a 5-hydroxyl group; it is also one of the few fluorescent flavonols.

The glycoside of Galanga root (the rhizomes of *Alpina officinarum*) yields GALANGIN, or 5-7-dihydroxy-flavonol:—



The 3-methyl ether of galangin accompanies it in the root.

KÆMPFERITRIN is a glycoside of Java indigo (*Indigofera arrecta*), originally introduced into Java from Natal. It yields two molecules of rhamnose, attached in the glycoside as a disaccharide to the 3-hydroxyl group, and kæmpferol which is 5-7-4′-trihydroxy-flavonol:



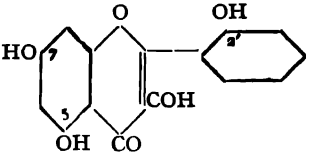
KÆMPFERIN, from Senna leaves, contains two molecules of glucose instead of rhamnose: a rhamnoside of kæmpferol was proved by Petrie [12] to be the inflorescence found on four species of acacia in New South Wales.

Galanga root also contains a kæmpferol 4′-monomethyl ether known as kæmpferide.

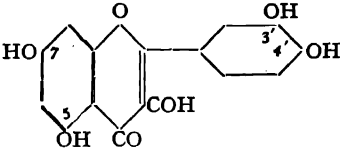
ROBININ [13] is a glycoside from the flowers of *Robinia pseudacacia*, yielding kæmpferol and a trisaccharide robinose, composed of two

rhamnose molecules and one of galactose; it is an isomer of rhamninose.

DATISCIN,[14] the glycoside of the Bastard Hemp (*Datisca cannabina*) yields on hydrolysis a rhamnoglucose known as rutinose, and datiscetin, 5-7-2′-trihydroxy-flavonol. The 2′-hydroxy group is uncommon in these compounds:—



QUERCITRIN is the glycoside of the oak bark. It is easily hydrolysed by acids to rhamnose and quercetin, 5-7-3′-4′-tetrahydroxy-flavonol:—



QUERCETIN is very widely distributed in plants, from many of which the glycoside itself has not been isolated. It occurs frequently together with other pigments of the group, and formerly followed indigo and madder in importance as a natural dyestuff. Complete methylation of quercitrin with diazomethane and subsequent hydrolysis of the sugar group gives 5-7-3′-4′-tetramethyl flavonol, proving the sugar to have been attached in position 3. A similar method shows rutin, xanthorhamnin and isoquercitrin to be 3-glycosides.[15]

INCARNATRIN, the glycoside of Crimson Clover (*Trifolium incarnatum*) is hydrolysed to glucose and quercetin by emulsin.

QUERCIMERITRIN, obtained from the flowers of the Indian cotton flower (*Gossypium herbaceum*), is hydrolysed with difficulty by acids to glucose and quercetin. It is the 7-β-glucoside.

ISOQUERCITRIN accompanies quercimeritrin in cotton flowers. It dyes different shades, more like those of quercitrin and also differs from quercimeritrin in being more easily hydrolysed by acids to glucose and quercetin. It is a 3-β-glucoside.

SEROTIN, present in *Prunus Serotina*, the wild black cherry, is easily hydrolysed by acids to glucose and quercetin.

RUTIN, which is widely distributed in plants, is hydrolysed with difficulty by acids to quercetin, glucose and rhamnose; the sugar is the disaccharide rutinose attached to the 3-hydroxyl group.

The various quercetin glycosides illustrate the introductory remarks on the different properties of isomeric glycosides formed from different sugars, or with the sugars differently attached.

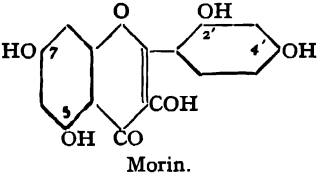
Methyl ethers of quercetin also occur as glycosides.

XANTHORHAMNIN,[16] the glycoside of Persian berries and other *Rhamnus* species, is hydrolysed by the accompanying enzyme rhamninase of the plant to rhamnetin and rhamninose, a trisaccharide which is hydrolysed by acids to galactose and two molecules of rhamnose and is apparently an isomer of robinose. Rhamnetin is quercetin 7-methyl ether and the sugar in the glycoside is attached in position 3.

An ISORHAMNETIN was isolated from an Indian dye, asbarg, the flowers of *Delphinium zalil*, where it occurs as a glucoside, by Perkin. It also occurs in the wallflower and is 3′-methyl-quercetin.

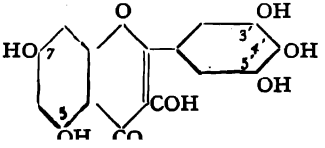
RHAMNAZIN, also from Persian berries, is quercetin 3′-7-dimethyl ether.

The wood of the tree *Chlorophora tinctoria*, formerly known as *Morus tinctoria*, the old fustic tree, contains as a glycoside an isomer of quercetin *Morin*, 5-7-2′-4′-tetrahydroxy-flavonol, corresponding to the flavone lotoflavin. Old fustic also contains the interesting derivative maclurin, a pentahydroxy benzophenone, which probably occurs in a protected form as a glycoside in the wood; of further interest in considering structural relationships is the occurrence of cyanomaclurin.

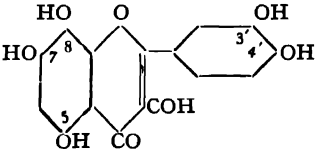


Morin.

The glycoside MYRICITRIN, found in the leaves of Rhus species and in the bark of *Myrica rubra*, the box myrtle, and in other dye-woods, yields rhamnose and myricetin 5-7-3′-4′-5′-pentahydroxy-flavonol:—

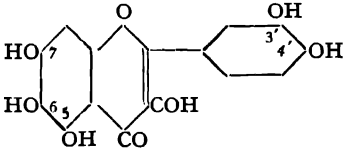


GOSSYPITRIN,[17] one of the glycosides of Egyptian cotton flowers, is hydrolysed to glucose and *gossypetin*, which is 5-7-8-3′-4′-pentahydroxy-flavonol :—



Gossypetin forms a quinone, gossypetone, on exposure of its alkaline solution to oxidation by the air, for which Perkin suggested a para-quinonoid structure. It dyes identical shades to gossypetin itself, suggesting that the latter is oxidised in the normal dyeing process.

A third isomeric pentahydroxy-flavonol is QUERCETAGETIN,[18] which occurs as a glucoside in the flower of the African marigold (*Tagetes patula*). It is 5-6-7-3′-4′-pentahydroxy-flavonol :—



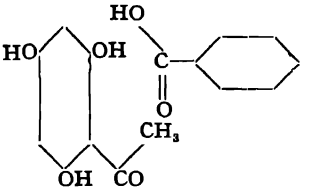
Perkin has compared the glycosides of a number of varieties of cotton flowers.[19] The red flowers of *Gossypium arboreum* contain isoquercitrin ; the yellow flowers of *G. neglectum* and the ordinary Indian cotton flower, *G. herbaceum*, contain gossypitrin and isoquercitrin, whereas the yellow flowered Egyptian *G. barbadense* contains quercimeritrin as well. White flowered varieties of *G. neglectum* gave only very small quantities of a glycoside resembling apiin, and the pink flowers of *G. sanguineum* contain only traces of flavones.

The leaves and flowers of Upland cotton, *G. hirsutum*, grown in U.S.A., contain quercimeritrin and isoquercitrin, the latter being present in the petals only.

Different parts of a plant sometimes contain glycosides of different flavones. Thus the bark of *Robinia pseudacacia* contains acacetin and the flowers apigenin ; the stem of the yellow cedar contains fisetin and the leaves quercetin ; the Venetian sumach contains fisetin in the stem and myricetin in the leaves. Other plants contain several derivatives of a single flavone : Persian berries contain both quercetin and its methyl ethers, rhamnetin and rhamnazin. Proof cannot yet be given indicating a progressive building up of a compound as this would suggest.

Syntheses of many of the flavones and flavonols have been accomplished by methods due to Kostanecki, and more recently by Robinson and co-workers whose methods are of general application and unambiguous.

The most successful method of synthesis [20] is to condense phloracetophenone with the anhydride and the sodium salt of the appropriate acid. The acyloxy compound thus obtained is then hydrolysed.



Phloracetophenone + benzoic acid → Chrysin.

If *w*-methoxy-phloracetophenone is used instead and the product demethylated, flavonols are obtained. In this way were synthesised myricetin, datiscetin, kæmpferol, fisetin, quercetin and morin. The use of *w*-benzoyloxy derivatives enabled methoxy-flavonols to be prepared on direct hydrolysis : in this way were made kæmpferide, isorhamnetin, gossypetin and quercetagetin.

No naturally occurring flavone glycoside has yet been synthesised except by the enzyme method, with the exception of acetyl derivatives prepared by hydrolysis of an acetylated glycoside and recombination of the aglucone with acetobromoglucose.

The investigation of the flavone and flavonol glycosides has led to the discovery of several interesting di- and tri-saccharides containing methyl pentoses. Robinose containing two molecules of rhamnose and one of galactose, the isomeric rhamninose, rutinose consisting of glucose and rhamnose, and the sugar from diosmin yielding two molecules of glucose and one rhamnose, are referred to more fully in Chapter VIII.

No glycoside containing sugar residues which are proved to be attached to two different hydroxyls has yet been found, perhaps only because of the difficulty of characterisation ; glycosides with the sugar attached to the single aromatic nucleus have not been isolated in this group nor in the anthocyanins, Nature's method of protecting this part of the molecules being apparently to form methoxy derivatives. The position of the sugar in the flavone glycosides has in several cases been ascertained by means of the method of methylation and disruption mentioned for quercitrin, and by comparison of the dyeing properties of the glycosides and the free

pigments, the colours obtained with mordants depending upon the number and position of the hydroxyl groups according to well-defined rules, so that when a hydroxyl group is disabled by being the point of attachment of the sugar its position can be identified. The bands in the absorption spectrum are also fixed by the number and position of the hydroxyl groups, and acetylation or glycoside formation shifts the band, nullifying the effect of the hydroxyl group whose position may be thus estimated. Japanese workers have developed this method.[5, 21]

The flavone glycosides are so widely distributed in plants in the form of colouring matters in the cell sap that the occurrence of the parent substance, flavone, is of quite special interest. It is present as the farina of many species of primula in almost pure condition, as was shown by Hugo Müller.[22] No opinion was expressed by him as to the physiological function which flavone exercises in the economy of the plant, though the fact that it is excreted so freely would seem to imply that it is of no further use in the life processes, although its repellant action towards water is probably of importance. The observation that the pigment of the wings of the marbled white butterfly, *Melanargia galatæa*, contains a flavone which occurs also as a glycoside in the grass which is its food plant,[23] confirms the idea that flavones are unwanted by the living organism, and are excreted or secreted.

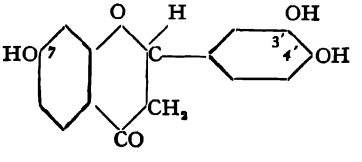
Shibata and Nagai [24] examined the leaves, flowers and bark of a large number of alpine and tropical plants and found that flavones were widely spread and almost invariably present: they are confined mainly to the epidermis and peripheral parenchymatous layer of aerial parts. They suggest that the flavone glycosides exert a protective action against the solar rays, especially those of short wave-length, which are injurious to the living protoplasm, and evidence the fact that plants grown in the shade contain less flavone glycoside than those grown in the open. Similarly plants provided with a heavy cuticle are usually poor in flavones. Increased insolation at higher altitudes or in a more sunny habitat leads to increased flavone production over that in the former habitat. They also found that flavones and anthocyanins often interchange, showing they have a similar protective function: young shoots contain red anthocyanin which changes into the colourless flavone in the green organ and back to the anthocyanin before the autumn fall of the leaves. To detect the flavone, the tissue was extracted with hot alcohol and a few cubic centimetres of the extract were heated with a drop of mercury, a little magnesium

powder and a few drops of concentrated hydrochloric acid in a test tube. The production of a red colour, due, they thought, to production of anthocyanidin, proved the presence of flavone. The absolute value of the method is limited because it is known that other anthoxanthins are reduced to red colouring matters and also the red colouring matters are not necessarily anthocyanins but open-chain compounds. The method is useful for showing general distribution of anthoxanthins in the plant.

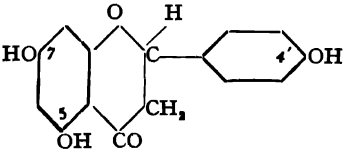
Flavanone Glycosides.

A number of glycosides whose exact constitution was unsettled, but were considered to be, on the evidence available, hydroxy-phenyl-styryl ketones, have recently been shown by Asahina, Shinoda and other Japanese workers[1] to be derivatives of flavanone or dihydroflavones. A proof of this structure is given by their reduction with magnesium or sodium amalgam and hydrochloric acid to red anthocyanidins, and further confirmation is provided by their successful synthesis in several instances.

The orange flowers of the common Indian tree, *Butea frondosa*, contain a glucoside of *butin*, the structure of which, as also that of *butein*, the chalkone into which it readily isomerises, has been known for many years. Butin is 7-3′-4′-trihydroxy flavanone:—



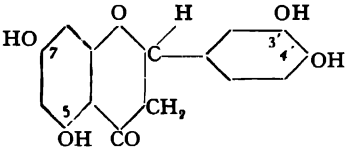
NARINGIN,[2] from the blossoms of *Citrus decumana*, is hydrolysed to naringenin and rhamnose and perhaps also glucose. Naringenin is 5-7-4′-trihydroxy flavanone:—



Its 7-methyl ether is sakuranetin, obtained together with glucose from SAKURANIN, probably a 5-glucoside, in the bark of *Prunus yedoensis*, *P. serulata* and *P. paniculata* (the Japanese cherry).

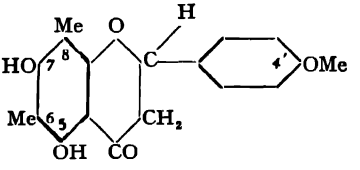
The dried leaves of *Eriodictyon californicum* yield a considerable quantity of glucose, together with *eriodictyol* and *homoeriodictyol*.[3]

The former is 5-7-3′-4′-tetrahydroxy-flavanone and the latter its 3′-methyl ether:—

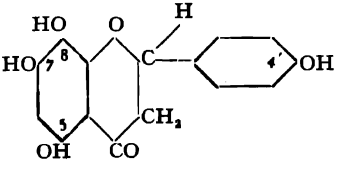


HESPERIDIN is a glycoside occurring in the peel of the orange, lemon and most citrus fruits except *Citrus decumana;* it hydrolyses to one molecule of glucose, one of rhamnose and hesperitin, which is 4′-methoxy 3′-5-7-trihydroxy flavanone, isomeric with eriodictyol.

A remarkable flavanone is *matteucinol* from the rhizomes and stipules of *Matteucia orientalis.* It has been proved by Fujise[4] to possess the structure 5-7-dihydroxy-4′-methoxy-6-8-dimethyl flavanone:—

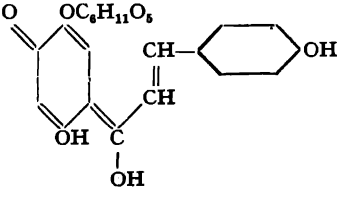


The safflower, once a natural colouring matter of importance, yields a monoglucoside CARTHAMIN,[5] which hydrolyses to carthamidin and an isocarthamidin. The former is given the structure



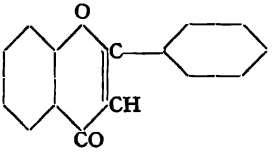
and the latter has been given the 5-6-7-position for the hydroxyl groups.

To carthamin itself is assigned the chalkone structure

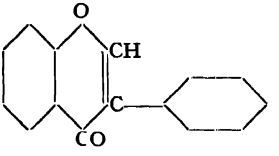


The Isoflavones.

The carbon skeleton of the isoflavones may be compared with that of the flavones which they resemble in reactions and dyeing properties :—



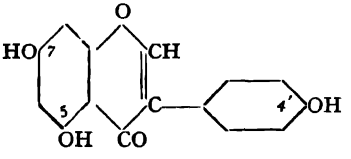
Flavone skeleton.



Isoflavone skeleton.

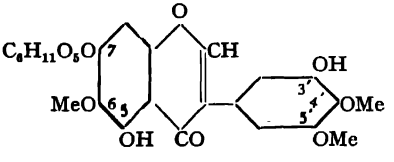
They occur as glycosides, but are much rarer and but four are known.

GENISTEIN, the colouring matter of Dyer's broom, *Genista tinctoria*, is 5-7-4′ trihydroxy-isoflavone, and has been synthesised [1, 3] :—

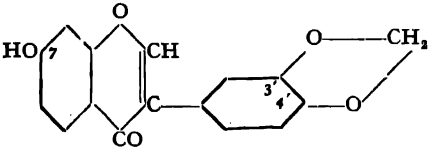


The glucoside PRUNETIN,[5] from Prunus bark, yields an aglucone, which is genistein 4′-methyl ether.

The glucoside IRIDIN,[2] occurring as 1 per cent. of the dried rhizomes of *Iris germanica*, *pallida* and *florentina* of commercial " Florentine Iris root," yields glucose and irigenin, and has the formula 3′-5-dihydroxy 4′-5′-6-trimethoxy-isoflavone 7-glucoside :—



ψ-BAPTISIN,[6] from the roots of *Baptisia tinctoria*, hydrolyses to glucose, rhamnose and ψ-baptigenin, 7-hydroxy 3′-4′-methylenedioxy-isoflavone :—



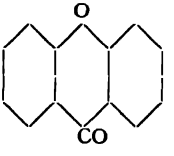
In view of the resemblance of isoflavones to flavones the possibility of their common origin must be considered. Catechin tetramethyl ether on dehydration undergoes a migration to give a deri-

vative containing the isoflavone carbon skeleton. Baker[4] has tried unsuccessfully to oxidise the methylene group in this compound to give an isoflavone, and so concludes that as no isoflavones corresponding to known catechins are known, that they are not produced from catechin-like substances.

Their rarity, and the fact that they do not occur together with the isomeric flavones, suggests an independent synthesis by the plant and not an alternative synthesis to flavones from the same materials.

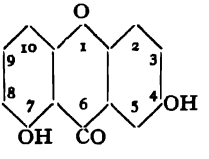
The Xanthones.

The xanthone colouring matters are included with the anthoxanthins as derivatives of dibenzo-γ-pyrone:—



or xanthone.

EUXANTHONE is formed when cattle are fed with mango leaves. The urine contains euxanthic acid (Indian yellow) which is a combination of glucuronic acid with euxanthone. The pigment is made in Bengal and largely used in India. Euxanthone is 4-7-dihydroxy-xanthone, the glucuronic acid is attached at position 4.



Gentisin, the yellow pigment present in *Gentiana lutea*, no doubt originally in the form of a glycoside, is 9-methoxy-4 : 7-dihydroxy-xanthone.

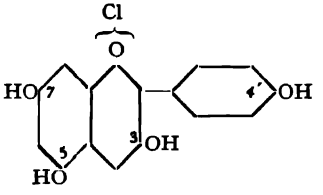
The Anthocyanin Glycosides.

The soluble red, violet and blue pigments of flowers, fruits and leaves known as anthocyanins,[1,7] are all glycosides. On hydrolysis they yield a sugar, in some cases other residues, and an anthocyanidin.

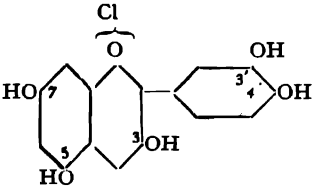
The anthocyanidins are all derived from three typical substances. They are simply these or their *o*-methyl derivatives. The wide range of hues obtained in the plant is partly produced by variations in the acidity of the cell sap,[2] the anthocyanins forming red oxonium

salts in acid solution, which change through the violet neutral colour bases to the blue sodium and potassium salts of the quinonoid forms as the sap is rendered alkaline. These alkaline solutions are very susceptible to atmospheric oxidation and are unstable. If aqueous solutions of the salts of anthocyanidins are much diluted the insoluble colourless pseudo-bases of the pigments are precipitated, which in acid solution are reconverted to the coloured forms once more.

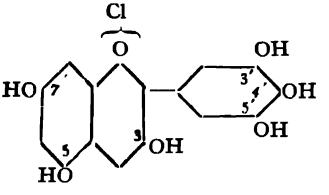
Willstätter [3] showed the anthocyanidins to be benzopyrylium derivatives, and gave the annexed formulæ to the three typical substances :—



Pelargonidin.



Cyanidin.



Delphinidin.

Anthocyanins may be monoglycosides, diglycosides or complex diglycosides. In monoglycosides are found glucose or galactose, and in diglycosides two molecules of glucose or one of glucose and one of rhamnose, or a pentose.

In the extraction of anthocyanins from the plant material, use is made of their power of oxonium salt formation.[1, 3] Cyanin may be extracted from red dahlia petals in the following way : the fresh flowers are extracted with glacial acetic acid or with methyl-alcohol saturated with hydrochloric acid and the pigment precipitated from solution with ether as chloride which can be crystallised from warm 6 per cent. hydrochloric acid. Willstätter found the following very varying contents of cyanin in different flowers : blue cornflower, 0·75 ; deep violet-blue cornflower, 3·6 ; deep Bordeaux cornflower, 13-14 ; deep red dahlia, 20 ; Rosa gallica, 2 per cent. All are calculated on the dried petals.

In a similar manner oenin may be extracted from the pressed skins of black grapes with glacial acetic acid ; the pigment is precipitated

with ether, washed with ether, dissolved in water and recrystallised from warm picric acid solution as a picrate.

When two different anthocyanins occur together in an extract they are often very difficult to separate, and confusion has sometimes arisen by assigning a name to substances which are really mixtures of an anthocyanin and its methoxy derivative.

Willstätter and Zollinger found that anthocyanidins, anthocyanin monoglycosides and diglycosides could be differentiated by their distribution numbers between amyl alcohol and 0·5 per cent. aqueous hydrochloric acid; the concentration in these experiments being measured colorimetrically. With an increase in the number of hydroxyl groups in the molecule, the solubility in the aqueous layer increases. This method is of great value in characterising the natural compounds on isolation from the plant. The following are typical distribution numbers, the distribution number being the percentage of the total pigment taken up into the amyl alcohol:—

Diglucoside	Cyanin	1·8	per cent.
Rhamnoglucoside	Keracyanin	6·8	,,
Monoglucoside	Oenin	10·4	,,
,,	Chrysanthemin	19·0	,,
Monogalactoside	Idaein	10·0	,,
Anthocyanidin	Cyanidin	100·0	,,

Rhamnoglucosides are seen to be classed with the monoglucosides rather than with the diglucosides. Recent work with five different monoglucosides by Prof. R. Robinson, F. L. Levy and Miss Grove (private communication) has shown that the distribution number depends on the concentration of the pigment, but a distribution constant may be obtained on the assumption that the monoglucosides consist of double molecules in the aqueous layer and single molecules in the amyl alcohol.

The distribution ratio varies with the acid, and Willstätter and Schudel [4] have studied this variation in different solvents, using picrates, chloropicrates and dichloropicrates. The effect of the picric acid is to increase the solubility in organic solvents; for instance, anthocyanidin chlorides are insoluble in ether and the distribution ratio is very low, but their picrates can be completely extracted with ether from an aqueous solution. A difference in behaviour between the three typical anthocyanidins is noted in ethyl-amyl ether:—

	D.N.
Delphinidin picrate	2·5 per cent.
Cyanidin picrate	40 ,,
Pelargonidin picrate	100 ,,

Similar differential effects have been noted among the monoglucosides and diglucosides. Thus the monoglucoside picrates have a distribution number of 40-50 in ethyl acetate-water while diglucosides have a much lower value, 5-8 per cent.

By studying a large number of solvents it was found that carvone and also less effectively diethyl ketone could be employed for the separation of monoglucosides and diglucosides, since the distribution number of the former for carvone-water is about 80 per cent. and of the latter 0 per cent.

A quantitative separation and estimation of anthocyanidin, monoglucoside and diglucoside can be effected in the following way. First extract the anthocyanidin picrate by means of ether: follow this with carvone for the monoglucoside and by a mixture of amyl alcohol and acetophenone 2 : 1 for the diglucoside. The ether extract is diluted with light petroleum, and the other two extracts with ether; the several extracts are then washed with 0·5 per cent. aqueous hydrochloric acid, which removes the pigment, and after freeing from picric acid the solutions may be colorimetrically determined.

Certain anthocyanins which are diglycosides and contain two glucose molecules, such as pelargonin and mekocyanin, can be partially hydrolysed to monoglucosides by the use of concentrated hydrochloric acid; similarly antirrhinin, the glucorhamnoside, may be hydrolysed to the simple glucoside; this may mean that the two sugar molecules were originally present as a disaccharide, or alternatively were attached to two different hydroxyl groups, the attachment to one of which is much more easily broken than the other. The method of investigation by complete methylation and examination of the fission products of the methylated anthocyanin has been employed by Karrer,[5] but Robinson[6] and Robertson do not consider it reliable. Since both the diglycosides and the monoglycosides obtained from them by partial hydrolysis give identical colour reactions, it is therefore practically certain that the diglycosides contain disaccharides; there may conceivably exist diglycosides in which the sugar is attached at two different hydroxyl groups, but such have not been found. The problem of the constitution of these disaccharides then arises. Those yielding two glucose molecules may be any of the known sugars—maltose, cellobiose or gentiobiose, or an unknown isomer: they may be found to be gentiobiose, which occurs in the glycosides amygdalin and crocin and probably in lotusin and loroglossin. Similarly, the disaccharide yielding glucose and

rhamnose may be the rutinose of the flavone glycosides, but there is no material evidence as yet bearing on these points.

The biological significance of the bright anthocyanin colours in attracting insects for fertilisation to flower parts, and birds and other animals to fruits for the dispersal of seed, is obvious. Their physiological role is less understood. Anthocyanins occur in other parts of the plant beside the flower organs, particularly in spring and autumn when metabolic activity is at its highest; their production varies in an individual species with ecological factors, and they occur in different parts of different species at different seasons.

Injury to green parts, whether mechanical or by insects or fungi, causes anthocyanin production, as does drought, lowering of temperature or increased insolation at high altitudes: artificial sugar feeding also causes anthocyanin production.

These observations suggest that in seasons of high metabolic activity an excess of the starting products of synthesis are provided, and anthocyanin is produced. In spring and winter the anthocyanin probably serves a protective function as a light screen, just as flavones are supposed to do, but at other times its occurrence is unnecessary. The idea that the pigments play a part in the respiration cycle is no longer upheld.[7]

Anthocyanins occur in almost all species of plants, but the number of individuals appears to be limited, and many have a wide distribution.

The same pigment is often found to occur in widely divergent species, and a single species may contain several different pigments differing by a hydroxyl or a methoxyl group, but containing the same sugars attached in the same position.

The most well-known anthocyanins of settled constitution are described here: they owe their names to the plant in which they were first discovered; a great number were discovered by Willstätter and his collaborators, others have been found recently by Karrer and by other workers.

The following table gives the nature and positions of the sugar molecule in the anthocyanins so far as they are known. The few which contain parahydroxy benzoic or cinnamic acid are remarkable, but the significance of the occurrence of these acids is unknown:—

Pelargonidin Derivatives.

Callistephin	3-monoglucoside.
Pelargonenin	5-monoglucoside.
Pelargonin	3 : 5-diglucoside.
Punicin	5(?)-diglucoside.
Monardæin, Salvianin	3 : 5-diglucoside + parahydroxy cinnamic acid and malonic acid.

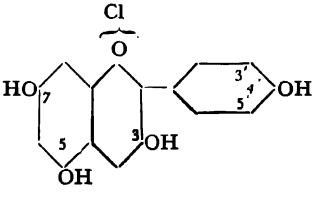
Cyanidin Derivatives.

Chrysanthemin (Asterin)	3-monoglucoside.
Idæin	3-galactoside.
Cyanin	3 : 5-diglucoside.
Mekocyanin	3-diglucoside.
Keracyanin, Antirrhinin, Prunicyanin	3-rhamnoglucoside.
Peonin	3′-methyl, 3 : 5-diglucoside.

Delphinidin Derivatives.

Vicin	Monorhamnoside + monoglucoside.
Gentianin	Monoglucoside + parahydroxy cinnamic acid.
Delphinin	Diglucoside + 2 parahydroxy benzoic acid.
Violanin	Rhamnoglucoside.
Petunin	3′-methyl, diglucoside.
Oenin (Primulin)	3′ : 5′-dimethyl, 3-monoglucoside.
Malvin	3′ : 5′-dimethyl, 3 : 5-diglucoside.
Hirsutin	3′ : 5′ : 7 trimethyl, 5-diglucoside.

Pelargonidin Derivatives :

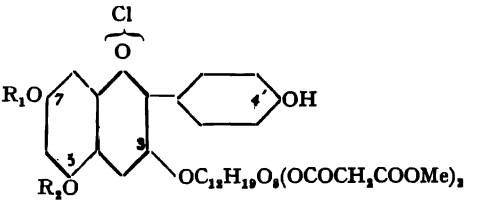


Pelargonin occurs in the Geranium, *Pelargonium zonale*, and in the scarlet dahlia, probably also in scarlet gladioli, red hyacinths, and many other plants. It is hydrolysed to one molecule of pelargonidin and two of glucose, attached in position 5, probably as a disaccharide.

Pelargonenin is obtained by careful hydrolysis of one molecule of glucose from pelargonin with cold concentrated hydrochloric acid ; it has not yet been found to occur naturally. Synthesis shows it to be pelargonidin 5-β-glucoside.

Callistephin, isolated from red carnations and the purple red aster, has proved to be pelargonidin 3-β-glucoside.

Monardæin[8, 9] from *Monarda didyma*, the Golden Balm, is identical with *Salvianin* from the pigment of scarlet *Salvia splendens*. Acid hydrolysis gives a large yield of malonic acid among the products. Alkaline hydrolysis yields parahydroxy cinnamic acid and monardin, identical except in optical rotation with pelargonin, hydrolysed with acids to two molecules of glucose and pelargonidin. The parahydroxy cinnamic acid is attached to the phenolic groups and not to the sugar, to which two molecules of the monomethyl ether of malonic acid are attached. The formula suggested by Karrer was



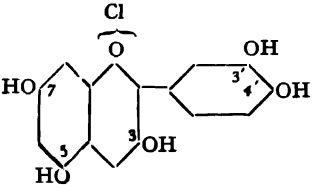
where R_1 or $R_2 = H$, or *p*-hydroxy cinnamoyl, but on Robinson's views the sugar is attached in the 5 position.

The malonic acid groups are methylated to account for the estimated methoxyl content of the compound.

Salvianin similarly is hydrolysed by alkalis to the diglycoside salvinin of Willstätter and Bolton, together with parahydroxy cinnamic acid and malonic acid. Salvia and Monarda are related, belonging to the sub-family Stachyoideæ.

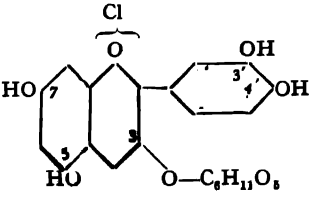
Punicin,[8] from pomegranate leaves, is identical except in optical rotation with monardin or pelargonin, and is hydrolysed to two molecules of glucose and pelargonidin.

Cyanidin Derivatives:



Cyanin, occurring in the cornflower, the rose and the deep red dahlia, is hydrolysed to cyanidin and two glucose molecules, probably attached as a 5-disaccharide.

Mekocyanin is obtained from the poppy, *Papaver rheas*, and is probably cyanidin 3-diglucoside. Careful hydrolysis yields a monoglucoside which is identical with *Chrysanthemin*, which occurs naturally in *Chrysanthemum indicum*, and is proved by synthesis to be cyanidin 3-glucoside:—



Asterin from the purple-red aster is identical with chrysanthemin,[10] so the name need no longer be used. An identical cyanidin monoglucoside is found in the blackberry [11] and the elderberry.[8]

Prunicyanin, from skins of the blackthorn, *Prunus spinosa*, yields cyanidin and rhamnose and glucose; it is considered to be a 3-saccharide.

Keracyanin, from the sweet cherry, *Prunus avium*, is also cyanidin gluco-rhamnoside, but gives different colour reactions to prunicyanin.

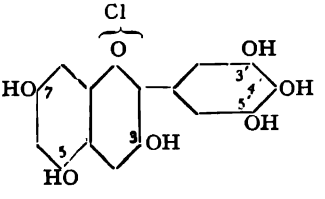
Antirrhinin,[12] from *Antirrhinum majus*, is said to be cyanidin 3-rhamnoglucoside: careful hydrolysis (Scott-Moncrieff) gives cyanidin 3-glucoside, identical with chrysanthemin.

Sambucin,[8] occurring in the skins of elderberry, *Sambucus nigra*, has been shown by Nolan [13] to consist partly of chrysanthemin, but also to contain a minor constituent which gives the colour reactions of chrysanthemin, but is in fact a bimolecular compound, which is named *Sambucicyanin*, made up of chrysanthemin and a cyanidin pentoglucoside. The pentoseglucoside from its colour reactions must, like chrysanthemin, be a 3-glucoside. This is the first record of the occurrence of a pentose in the anthocyanins.

Idæin is obtained from the cranberry, *Vaccinium vitis Idæa*, and hydrolyses to cyanidin and galactose; its constitution as a 3-galactoside has been confirmed by synthesis (private communication from Prof. Robinson).

Peonin, from Peony, is hydrolysed to two glucose molecules and peonidin, 3′-methyl cyanidin. It is probably a 5-saccharide.

Delphinidin Derivatives:



Delphinin, the pigment of larkspur, yields two glucose molecules, two of parahydroxy benzoic acid, which are supposed to be attached to the phenolic hydroxyl groups, and delphinidin.

Vicin,[8] obtained from the dark red vetch, according to Karrer, contains a mixture of a monoglucoside and a monorhamnoside of delphinidin.

Violanin, from the purple pansy and purple red viola, yields delphinidin, glucose, and rhamnose.

Gentianin,[8] from *Gentiana acaulis*, hydrolyses to one molecule each of glucose, parahydroxy cinnamic acid and delphinidin.

Petunin, from purple-blue petunia, may be a diglucoside of a delphinidin 3′-methyl ether: on the other hand, like myrtillin and some other pigments, it may be a mixture derived from delphinidin and malvidin.[5]

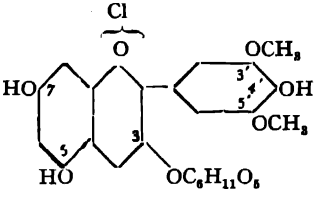
Malvin, from the violet flowers of the wild mallow, *Malva sylvestris*, and from various primulæ, yields malvidin and two molecules of glucose on hydrolysis. Malvidin is 3′ : 5′-*o*-dimethoxy delphinidin, and malvin probably its 5-diglucoside.

Althœin, from the black hollyhock, and

Ampelopsin, the pigment of the berries of *Ampelopsis quinquefolia*, are both monoglucosides which yield a mixture of malvidin and delphinidin.

Myrtillin, the pigment of the bilberry, is hydrolysed to glucose and galactose and a mixture of delphinidin and malvidin.

Oenin, the pigment of dark-blue grapes, is malvidin 3-glucoside. It is identical with primulin [14]:—



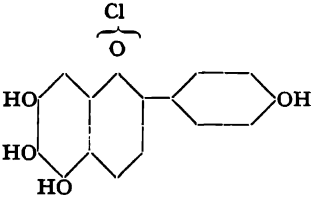
Anderson [15] has found that the European dark-blue grape, *Vitis vinifera*, contains oenin, while the American grapes, *Vitis labrusca*, *æstivalis* and *riparia*, contain a monoglucoside containing parahydroxycinnamic acid and a monomethyl ether of delphinidin. Seibel grapes, the hybrid obtained by crossing the two, contain oenin; the inherited tendency to produce oenin is therefore dominant. The monomethyl ether may well prove to be a mixture of oenin and delphinidin glycosides.

Hirsutin,[16] of *Primula hirsuta*, hydrolyses to two glucose molecules and hirsutidin, proved to be 7 : 3′ : 5′-trimethyl-delphinidin. It is probably a 5-saccharide.

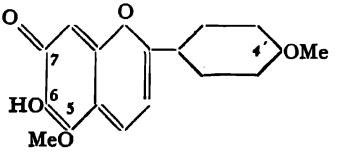
It has been shown that the colouring matter of Carajura is a flavylium derivative like the anthocyanins, except that it is related to the flavone scutellarein, rather than to a flavonol, possessing no 3-hydroxyl group. *Carajura* is a red pigment prepared by the Indians of the Rio Meta and the Orinoco from *Bignonia Chica*, for use as a flesh

paint. The leaves are extracted with water and the extract is heated with a ground bark called Aryane, which causes a precipitation of the colouring matter. The process is suggestive of the enzymic hydrolysis of a glycoside.

Chapman, A. G. Perkin and Robinson [17] have synthesised *carajuretin chloride*, which is produced by demethylation of *carajurin*, and shown it to be identical with " scutellareinidin chloride ":—



Carajurin itself has probably the structure of



an anhydroflavylium derivative.

Schudel,[4] working with Willstätter, has isolated the anthocyanin Betanin from beetroot, *Beta vulgaris*, and has found that it contains nitrogen; a similar anthocyanin occurs in species of Celosia; the members of this group are characterised by blue-toned red acid solutions and by the extreme instability of the anthocyanidins, but these interesting substances have apparently not been further investigated.

Mention may also be made of the yellow anthocyanin originally found by Willstätter in *Papaver alpinum*. The Iceland poppy, *Papaver nudicaule* and *Meconopsis cambrica*, also appear to contain similar pigments. (Private communication from Prof. Robinson and Miss Scott-Moncrieff.)

The investigation of the anthocyanins of various species and varieties of Primulæ [14, 16] has brought to light a number of interesting chemical and genetical relationships forming a point of departure for further work.

The flowers of *P. polyanthus* and the red, magenta, blue and slaty flowers of *P. sinensis* contain *malvidin 3-monoglucoside* (primulin); the flowers of *P. viscosa* and *integrifolia* contain *malvidin 5-diglucoside* (malvin). *P. hirsuta* contains a trimethyl delphinidin, *hirsutidin*,

which is 7-methyl malvidin, as a diglucoside. The leaves and stalks of ordinary varieties of *P. sinensis* contain a 3-monoglucoside of an anthocyanidin which possesses fewer methoxy groups than malvidin; the stalks and flowers of the " coral " variety of *P. sinensis* contain an orange-red pelargonidin derivative, which as yet is incompletely investigated.

The different coloured flowers of ordinary *P. sinensis* may be controlled by different *p*H conditions in which the pigment primulin occurs; a different factor is required to effect the considerable change from a delphinidin derivative to one of pelargonidin in the " coral " plants.

Different coloured varieties of *Pelargonium Zonale* have also been examined for their pigments. Willstätter isolated pelargonin from the scarlet flowers, while he found a certain violet-red variety contained cyanin with only traces of pelargonin. Recently, Miss Scott-Moncrieff [17a] has examined the pigments of the plants obtained on selfing the rose-pink variety " Constance," seventeen of which were like the parent and three of which were salmon-pinks, this colour being clearly recessive.

The rose-pinks contained cyanin, a slight trace of pelargonin and an appreciable amount of flavone; while the salmon-pinks contained only pelargonin and a trace of flavone.

Since both pigments have been shown by Robinson to be 5-diglucosides, they differ only by one hydroxyl group, and the Mendelian factor responsible for the colour change is the one that effects this alteration.

Willstätter found pelargonin in the blue-tinged rose flowers of *P. peltatum.*

The purple-red aster contains both pelargonidin and cyanidin as their 3-monoglucosides callistephin and asterin.

In the cornflower, Willstätter found the dark purple-red flowers to contain cyanin, while the light red to violet-red flowers contained pelargonin.

In the garden dahlia the deep, red-brown variety contained cyanin while the scarlet-red "Alt Heidelberg" variety contained pelargonin.

It is thus apparent that in many instances at least, colour varieties differ in containing different anthocyanins rather than in possessing the same anthocyanins in different *p*H environment. Indeed, on reflection it is evident that such *p*H changes as would be necessary to effect the colour shifts from red to blue and vice versa.

would have a profound effect on general metabolism, not only on the colour.

Reference has already been made on p. 36 to the very varying amounts of pigment found in different flowers, such as in the cornflower where increased pigment content throws the colour from blue to red.

Work has not been done to investigate to what degree anthoxanthins or carotenoids or other organic constituents of the flowers modify the shades due to the anthocyanin pigments themselves, nor is anything known of the possible effects which inorganic substances, such as iron salts, may produce.

A spectrographic method of examining plant extracts for pigments, used by Moir,[18] would seem of value, if developed, for tracing pigment distribution in botanical groups. He examined the absorption spectra of anthocyanidin salts and compared them with those of the dilute extracts from South African plants. Some caution in interpretation is necessary, since Schou [19] has shown that peonidin and malvidin have almost identical spectra. Pelargonidin glycosides were detected in Barberton daisy, brown nasturtium, scarlet salvia and *Chelone barbata*, and as part constituents in scarlet protea, bramble, eendagsbloem lily, and the red canna lily. Cyanidin glycosides occur in scarlet zinnia, pink convolvulus, deep red Ards rose, pink hydrangea, blue hydrangea, crimson verbena, *Lychnis coronaria*, scarlet geum, puce-coloured hollyhock, and the anthers of the agapanthus lily.

Delphinidin glycosides were found in blue agapanthus lily, azure plumbago, blue jacaranda, scarlet poinsettia, royal-blue convolvulus, and the common larkspur.

Petunidin glycosides were found in petunia, bramble, and habrothamnus. It should be mentioned, however, that the validity of this latter statement is impaired by the fact that the standard for comparison was only an extract of petunia flowers.

The structure of the anthocyanidins, with the position of their methoxy groups, has been settled, and also in many instances the point of attachment of the sugar to form anthocyanins, through the early work of Willstätter and latterly by the efforts of Karrer and particularly of Robinson.[20, 21] Examination of the decomposition products of alkali fusion, and comparison of their very characteristic colour reactions with those of the synthetic anthocyanidins of known structure, or derived by reduction of known flavones and flavonols, are the important methods of investigation. The colour reactions depend on certain combinations of hydroxyl groups being free and unprotected

by methoxy or sugar groups; the reactions with sodium carbonate determine the positions of the hydroxyl groups in the benzopyrylium nucleus, and the ferric chloride reactions those in the benzene nucleus; for the details of the argument reference must be made to the original work.

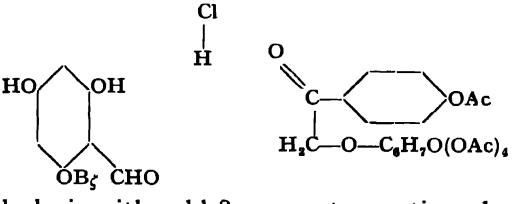
Thus "fisetinidin" gave almost the same colour reactions as cyanin, and "luteolinidin" as mekocyanin and chrysanthemin, suggesting that in the anthocyanins the sugar occupied the same hydroxyl groups as were missing in the synthetic derivatives.

To identify a synthetic derivative with a natural one, the above colour reactions are tested, and the behaviour of the two is compared in a range of buffer solutions of different *p*H: as they decompose on melting their identity cannot be simply checked by a mixed melting-point determination. Synthesis of the anthocyanins is the final proof of their structure; recently Robinson [21] has overcome the difficulties of preparing compounds which do not lose their sugar when condensed together, and of removing the protecting groups in the final stage.

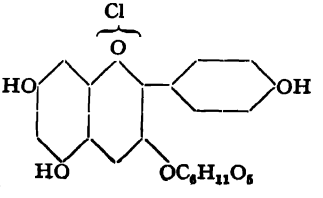
The discovery of the useful properties of *o*-benzoyl phloroglucinaldehyde has made the synthesis practicable, and Robinson and his collaborators are engaged in synthesising the actual glycosides. The synthesis of the anthocyanidins has been for some time an accomplished fact.

The first synthesis of a complete anthocyanin glycoside was that of *callistephin chloride.*

w-hydroxy-4-acetoxyacetophenone and acetobromoglucose were condensed with dry silver carbonate in benzene to give *w*-acetoglucosidoxy-4-acetophenone, which is treated in a mixture of ether and chloroform with *o*-benzoyl-phloroglucinaldehyde and dry hydrochloric acid:—

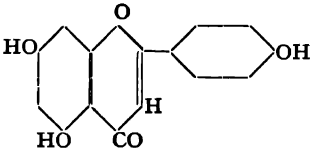


After hydrolysis with cold 8 per cent. caustic soda and acidifying, callistephin chloride was obtained:—

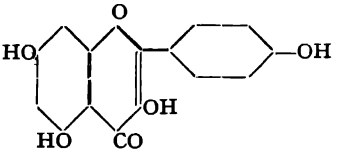


The progress of synthetic work makes it possible to synthesise all the possible types of anthocyanin glucosides. When this has been achieved, a new plant product will be easy to characterise by comparison of colour reactions with that of material of known structure. Distribution number experiments will then determine the nature of the sugar and whether the anthocyanin be a monoglycoside or a diglycoside.

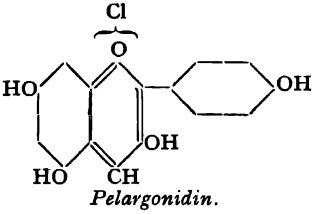
Comparison of the formulæ of flavones and flavonols, and other anthoxanthins with anthocyanidins, immediately suggests their possible interconversion or the derivation one from the other in the plant:—



Apigenin.



Kæmpferol.



Pelargonidin.

Kæmpferol, quercetin, isorhamnetin and myricetin possess the same carbon skeletons and have the same positions for their hydroxyl groups as pelargonidin, cyanidin, peonidin and delphinidin respectively: they differ only in having CO instead of =CH—.

Flavones, flavonols and flavanones may be reduced in the laboratory to the corresponding anthocyanidins by means of magnesium and hydrochloric acid or with sodium amalgam and hydrochloric acid.

Quercetin occurs as the yellow pigment of the rose, where the anthocyanin is cyanin, and in other flowers corresponding flavones and anthocyanins occur together, but, on the other hand, in the majority of cases the two types when occurring together are not simply related. A theory for the inheritance of anthocyanin colours has been developed by Mrs. Wheldale-Onslow,[22] in which anthocyanin colour is produced when there are present both chromogen, in the original theory a flavone, and an oxidase. There is good genetical evidence for the theory, but chemical evidence suggests reduction as the method of formation of anthocyanin; there is a possibility that an oxidation may intervene at one stage as a controlling factor. It is true that the

distribution of the pigments in flowers coincides with that of oxidases, but it is by no means certain whether a flavone is the parent chromogen of the anthocyanin.[23] In the antirrhinum, where the genetical factors have been most carefully worked out by Mrs. Wheldale-Onslow, the flavones are apigenin and luteolin and the anthocyanin was shown by Scott-Moncrieff to be a cyanidin glycoside.

In the pairs of flavonols and anthocyanins mentioned above, the parent flavonol glycosides are 3-saccharides, whereas the related anthocyanins are 5-saccharides.[24] This difference in position of the sugars in the two classes makes the relationships urged above less close. The derivation of anthocyanin from flavone is therefore not so simple and demands a hydrolysis and re-attachment of the sugar, or even often of a different sugar to a different hydroxyl group.

With the exception of the compound carajurin, all the anthocyanins so far known have but two hydroxyls on the benzopyrylium ring, those in positions 5 and 7, whereas in the flavones considerable variation in the number and position of the hydroxyl groups occurs, and it is surprising that no anthocyanidin has yet been isolated corresponding to these, though possibly such derivatives will be discovered.

The hypothesis that anthocyanins are derived in the plant from flavones is no longer tenable.

The question presents itself, do the sugars attach themselves to the finished anthocyanin or flavone after the plant has synthesised it, or are they attached to and so serve to protect the compounds which condense together to form the finished product, in the same way as the laboratory chemist uses his acetyl or methoxy groups ?

Robinson [25] has developed a general theory for the formation and origin of anthoxanthins and anthocyanins in the plant. He points out that their fundamental C_6–C_3–C_6 skeleton appears, with the centre portion more or less oxidised or reduced, in the series of structurally closely related compounds, the chalkones, flavonols, flavones, flavanones, anthocyanidins and also in catechin.

In the laboratory chalkones are convertible to flavones ; flavonols to anthocyanidins and anthocyanidins to catechins,[26] but by methods which the plant may not be able to reproduce. In the plant hexoses must form the raw material for the formation of the C_6 units and a triose for the C_3 unit.

Assembly is effected by aldol condensation first to a C_3–C_6 compound, which type in various states of oxidation is common in plant products. Such compounds have hydroxyl groups and methoxyl groups in the same position as those of the single aromatic nucleus

of the anthoxanthins and anthocyanins; this is illustrated by the relation of cinnamic acid to chrysin, anethole to kæmpferol, coumarin to datiscetin and caffeic acid or eugenol to cyanidin.

The process is completed by condensation of the other end of the C_3 unit with a further C_6 unit which usually assumes a phloroglucinol structure. Condensation with a molecule of formaldehyde would give the skeletons of the logwoods, brazilin and hæmatoxylin.

It is remarkable how many of these compounds are phloroglucinol derivatives, suggesting that this is the fundamental structure from which by loss or gain of a hydroxyl group the less common substances are derived.

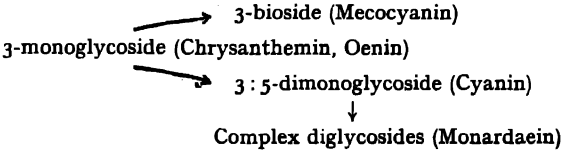
The theory leads to the conclusion that glycosides are formed by the sugars combining with the completed compounds, and play no part in their synthesis, which is supported by the known facts on the alternative positions which the sugars take up. The purpose of attachment of sugar is, therefore, most probably to render the compound more soluble and more easy of transport, as well as to render it more stable against oxidation in alkaline media.

The theory also provides some reconciliation of chemical and genetical evidence on the origin of anthocyanins, which may originate together with other members of the group from a common chromogen.

Addendum to page 38. G. M. and R. Robinson ("Nature," 5/9/31, p. 413) suggest that pelargonin, peonin, cyanin and malvin are not biosides but are dimonosides with separate glucose residues attached to positions 3 and 5.

Mecocyanin, prunicyanin and keracyanin ace biosides, the sugar being in position 3.

The anthocyanins are considered to be related as follows:—



CHAPTER IV.

GLYCOSIDES WITH PHYSIOLOGICAL ACTION.

Digitalis, Strophanthin, Saponin.

THE cardiac glycosides from Digitalis and Strophanthus species yield C_{23} and C_{24} aglucones which have been investigated in detail, in particular by Windaus and by Jacobs.

Jacobs has shown [1] that the aglucones of both groups, together with the less-known antiarigenin, scillarigenin and bufotalin, the poison from the tropical toad *Bufa agua*, all give similar reactions with Tollen's reagent and nitroprusside, and all contain an unsaturated lactone group. To this group, together with perhaps other common structural features, their pharmacological properties are owed, since hydrogenation converts them to practically non-toxic derivatives.

Similarity in other reactions makes it probable that the structural relationships between the two groups will be shown to be near in other respects as their investigation is proceeded with.

The digitalis and strophanthin glycosides are also remarkable in yielding the unusual deoxy-methyl-pentoses and form their only known source.

Digitalis Glycosides.

The leaves of the foxglove contain several closely related complex glycosides which form the active constituents of digitalis.

The group is unique in yielding the unusual sugars digitoxose and digitalose. Digitoxose has the formula $C_6H_{12}O_4$ and has been investigated by Kiliani [2] and finally shown by Micheel [3] to be a deoxy-methyl pentose of the following configuration :—

$$CH_3 \cdot \underset{OH}{\overset{H}{C}} \cdot \underset{OH}{\overset{H}{C}} \cdot \underset{OH}{\overset{H}{C}} \cdot CH_2 \cdot CHO$$

The configuration now assigned to digitoxose does not relate it simply to any of the naturally occurring hexoses or methyl pentoses. The problem of the method of its formation in the plant therefore possesses great interest. Theoretically, it may be considered as the deoxymethyl pentose related to the aldohexoses *d*-allose and *d*-altrose.

The sugar derived from digitalin and perhaps oleandrin is digitalose, $C_7H_{14}O_5$, to which Kiliani ascribes the structure of a methoxy methyl-pentose :—

$$CH_3 . CH(OH) \quad \begin{matrix} OH \\ C \\ H \end{matrix} . CH(OH) . CH(OCH_3) . CHO.$$

Only the leaves are officinal; they contain digitoxin, gitoxin, digoxin, gitalin. The seeds have a similar action, but they contain different glycosides. The commercial preparations of the seeds, the so-called *digitalinum germanicum*, have been separated into *digitalinum verum* and the glycosides digitalin, digitonin and gitonin.

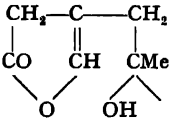
Cold water extracts from the leaves a mixture of glycosides, but hardly dissolves digitoxin, which is subsequently extracted by alcohol. Chloroform removes gitalin from the aqueous extract, leaving behind the impure digitalein fraction.

TABLE 4.

Glucoside.	Sugar.	Aglucone.	Aglucone formula.
Digitoxin	3-digitoxose +	digitoxigenin	$C_{23}H_{34}O_4$
Gitoxin	digitoxose +	gitoxigenin	$C_{23}H_{34}O_5$
Digoxin	3-digitoxose +	digoxigenin	$C_{23}H_{34}O_5$
Lanadigin	? digitoxose +	digoxigenin	
Digitalin	glucose + digitalose +	digitaligenin	$C_{23}H_{30}O_3$
Oleandrin	? digitalose +	digitaligenin	$C_{23}H_{30}O_3$
Gitalin	digitoxose +	anhydrogitaligenin	$C_{22}H_{34}O_5$
Digitonin	2-glucose + 2-galactose + 1-*d*-xylose +	digitogenin	$C_{26}H_{42}O_5$
Gitonin	3-galactose + pentose +	gitogenin	$C_{26}H_{42}O_5$

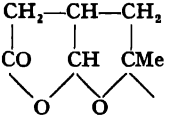
DIGITOXIN, the most active principle of the leaves, has the formula $C_{41}H_{64}O_{13}$. On hydrolysis it yields three molecules of digitoxose and digitoxigenin $C_{23}H_{34}O_4$. This is apparently a simply unsaturated, dihydroxy-lactone containing a system of four carbocyclic rings. One of the two hydroxyl groups is secondary and easily acetylated; the other is tertiary and is readily eliminated as water by combination with a neighbouring hydrogen atom.

The isomerisation with alkali hydroxide permits the identification of the following arrangements :—



There are apparently two digitoxins, one of which forms a hydrate. The commercial product made by Merck changed from one form to the other in 1895.

The alkali treatment is proving of importance in establishing the constitution of these aglucones : it results in the formation of *iso*-digitoxigenin :—



GITOXIN, $C_{41}H_{64}O_{14}$, obtained from Merck's by-products of the preparation of digitoxin by extraction with a boiling mixture of chloroform and methyl alcohol, is identical with Kraft's older anhydrogitalin. It is also present in the leaves of *D. lanata*.[4] On hydrolysis it yields digitoxose and gitoxigenin, $C_{23}H_{34}O_5$. This is an hydroxy derivative of digitoxigenin and is isomeric with periplogenin (*q.v.*).

From its behaviour on oxidation and isomerisation it is concluded that the second hydroxyl group (digitoxigenin has only one such) is not tertiary as originally supposed, but either secondary or primary, in which latter case the corresponding group of digitoxigenin would be methyl. It is formulated partially by Jacobs and Gastus.[5]

The leaves of *Digitalis lanata* have a physiological activity from three to four times that of the international standard leaf. The mixture of glycosides from the leaves contain a new glucoside digoxin.[6]

DIGOXIN is hydrolysed to 3 molecules of digitoxose and an aglucone digoxigenin, $C_{23}H_{34}O_5$, which contains three hydroxyl groups and a lactone group, which gives the red colour reaction with sodium nitroprusside typical of $\triangle^{\beta\gamma}$—unsaturated lactones.

It is probable that it has a common structure with gitoxigenin and digitoxigenin.

Four other glycosides have been isolated by Mannich, Mohs and Mauss [7] from the alcoholic extract of the dried leaves. The principal one, lanadigin, yields digitoxose, a small quantity of a non-reducing disaccharide and a genin probably identical with the digoxigenin of Smith.

DIGITALIN possesses in a high degree the physiological action of digitalis, decreasing the frequency and increasing the force of the beat of the heart; it yields digitaligenin, glucose and digitalose on hydrolysis.

Windaus considers digitaligenin to have the formula $C_{23}H_{30}O_3$.

GITALIN, $C_{28}H_{46}O_{10}$, an amorphous, neutral and very sparingly soluble glycoside possessing physiological activity, was also isolated by Kraft. By evaporation of the alcoholic solution he obtained the crystalline anhydrogitalin, $C_{28}H_{46}O_9$. Both gitalin and its anhydro derivative give the same products of hydrolysis, namely, digitoxose and anhydrogitaligenin, $C_{22}H_{34}O_5$.

Kiliani [8] states, on the other hand, that gitalin itself is a mixture of glycosides, separable by fractional solution in water and a mixture of organic solvents into fractions differing in physiological action, solubility and hydrolytic behaviour.

Digitalis Saponins.

DIGITONIN, which comprises one-half of the mixed glycosides of the seeds, belongs to the saponins: it dissolves sparingly in water, forming opalescent solutions which froth on agitation. It is hydrolysed to glucose (two molecules), galactose (two molecules), *d*-xylose and digitogenin. Characteristic is the formation of a crystalline precipitate with cholesterol.

Merck's preparation of digitonin is a mixture of glycosides; a constituent gitonin, $C_{49}H_{80}O_{23}$, has been isolated by Windaus, as an amorphous substance giving an additive compound with cholesterol.

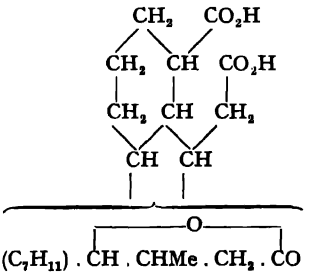
Kraft [9] has studied afresh the glycosides present in digitalis leaves and describes a member of the saponin class, digitosaponin, which is apparently identical with digitonin, but yields a pentose and digitosapogenin upon hydrolysis. Hydrolysis of glycosides such as digitonin is best effected with alcoholic hydrogen chloride when the main purpose is the isolation of the corresponding genin; the use of alcohol should be avoided if the liberated sugars are to be identified, since ethyl glycosides are formed to a considerable extent.

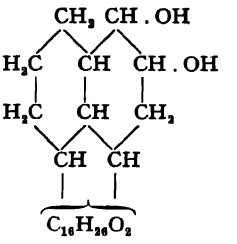
Digitogenin has the formula: $C_{26}H_{42}O_5$.

GITONIN,[10] a glycoside occurring in association with digitalin, gives, on acid hydrolysis, a dihydric alcohol, gitogenin, $C_{26}H_{42}O_4$, which is very similar in composition to digitogenin, $C_{26}H_{42}O_5$, obtained from digitonin in the same way. On further oxidation, gito-

genin yields gitogenic acid, $C_{26}H_{40}O_6$, which, if again oxidised with hot concentrated nitric acid, gives a substance having the constitution (I).

(I)





(II)

This on distillation with acetic anhydride gives a keto-lactone, which on further oxidation yields a dibasic acid, $C_{21}H_{30}O_6$. Gitogenin appears to have the formula (II). The same complicated four-ring system is present in gitogenin as in digitogenin, the only difference being the replacement of a CH_2 group in the former by a CH . OH group in the latter.

GITOGENIN is identical with the older digin and digitin.

TIGOGENIN.[11] The sapogenin prepared by the acid hydrolysis of crude gitonin when treated with light petroleum was separated into two fractions; the insoluble fraction consisted of gitogenin, whilst the soluble fraction contained a new sapogenin, designated *tigogenin*, $C_{26}H_{42}O_3$. A comparison of sarsapogenin, sarsapogenone, and sarsapogenoneoxime with tigogenin and its corresponding derivatives showed that these sapogenins were isomeric.

Hirohashi states that the youngest leaves of digitalis are the most active physiologically; they should be collected before inflorescence.

There is no difference in activity between red and white flowers. Cultivated digitalis is as active as the wild variety, and the first year's growth is as active as that of the second (Hatcher).

A careful study of the development of the glycosides in germinating and growing digitalis plants has been made by Straub. The amount of the glycosides was estimated by a pharmacological method, viz. by determining the number of lethal doses for a frog. The glycosides studied were digitalinum verum and digitalein, which are soluble in water, found in the seeds, and further digitoxin, which is insoluble in water but soluble in chloroform, and gitalin, soluble in both water and chloroform, found in the leaves. The glycosides of the seeds are not reserve materials but disappear during germination and are

stored in the leaves, in which organs they do not increase further in quantity.

The leaf glycosides are found in the earliest foliage leaves and continue to increase in quantity until they form 1 per cent. of the dried matter; it is supposed that they are only waste products of the metabolism of growth.

Oleander leaves contain two crystalline active glycosides similar to those in digitalis leaves. The whole of the active substances in oleander leaves are readily extracted by cold water: this solubility seems due to the large amount of a phenolic glycoside present in the leaves which is not a true tannin.

The chief product is Oleandrin-6, $C_{24}H_{34}O_7$, a glycoside which gives the reactions of digitalis strongly. Oleandrin-4 of Böhringer has the composition $C_{33}H_{46}O_8$.[12]

According to Windaus [13] Oleandrin of composition $C_{31}H_{48}O_9$ is decomposed by warm acids into digitaligenin and a non-crystallising sugar which is probably digitalose.

Strophanthus Glycosides.

TABLE 5.

HEART SPECIFIC GLYCOSIDES.

K-strophanthin-β	glucose + cymarose	strophanthidin
Cymarin	cymarose	,,
Ouabain	rhamnose	
Periplocin	glucose + cymarose	periplogenin
Periplocymarin	cymarose	periplogenin
Sarmentocymarin	glucose + cymarose	sarmentogenin
Convallamarin	glucose + galactose + methyl pentose	convallamaretin
Helleborein	glucose + arabinose	helleboretin
Antiarin	rhamnose	antiarigenin

The seeds of various strophanthus species of the *Apocynaceæ*, long used as arrow poisons, contain the active principle strophanthin which has a toxic action on the movements of the heart.

A number of different strophanthins have been described, but their formulæ are still uncertain. The work of Windaus and latterly Jacobs is bringing clarity into this field of enquiry. The sugar produced on hydrolysis has long been held on the evidence of Feist [14] to be a disaccharide strophanthobiose which on further hydrolysis was resolved into mannose and rhamnose. Later Windaus and Hermanns [15] showed that a sugar of the digitoxose type was character-

istic of the strophanthins. It has the formula $C_7H_{14}O_4$, is a methyl ether and has been named cymarose.

Feist's conclusion as to the presence of mannose in strophanthin, its only occurrence in a glycoside, must be regarded as erroneous.

The work of Jacobs and Hoffmann [16] has shown that crystalline *Kombé* strophanthin is a mixture containing the glycoside of strophanthobiose, and cymarin the simple cymarose glycoside of strophanthidin present in Canadian hemp and various species of *Apocynum*.

Cymarose is represented provisionally as

$$CH_3 \cdot CHOH \cdot CHOH \cdot CHOMe \cdot CH_2 \cdot CHO$$

and accordingly kombé strophanthin-β as

$$C_6H_{11}O_5 \cdot O \cdot C_7H_{12}O_2 \cdot O \cdot C_{23}H_{31}O_5.$$

The action of an enzyme present in the seeds of *S. courmonti* is of interest. It attacks the union between the sugars, forming glucose and cymarin. Emulsin, rhamnase, invertase are without action on the glycoside. Acids in the first place attack the junction with the aglucone. Strophanthobiase attacks other strophanthin glycosides—there is evidence that the preponderating glycoside in the mixture in S. Kombe seeds is a trioside with 2 molecules of glucose. A tetrasaccharide is also present.

In *Hispidus* strophanthin, the same aglucone is present, also cymarin; but the union with glucose is apparently different, as the cleavage between the sugars is not brought about by the same enzyme.

Ouabain, from *S. gratus*, is easily prepared crystalline so that it is used as a pure standard for the biological assay of digitalis and strophanthus. It is hydrolysed to rhamnose and a genin, $C_{24}H_{36}O_8$, which loses water and forms a resin.

Other strophanthins are (1) Periplocin,[17] of *Periploca græca* from the closely related family Asclepiadaceæ, hydrolysed by the enzyme strophanthobiase to periplocymarin, which has the composition $C_{30}H_{46}O_8$ resembling cymarin; this is further hydrolysed to periplogenin, $C_{23}H_{34}O_5$, which is an unsaturated lactone. (2) Sarmentocymarin [18] from *S. Sarmentosus*, also $C_{30}H_{46}O_8$, which on acid hydrolysis yields sarmentogenin, $C_{23}H_{34}O_5$.

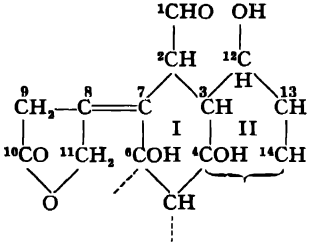
The two genins are isomeric with gitoxigenin and have the general characteristics of the strophanthidin series.

Jacobs [19] reports an enzymatic isomerisation of cymarin, forming a new isomeric aglucone devoid of the characteristic digitalis action.

According to Jacobs and Hoffmann [20] the saturation of the double linking associated with the lactone group in the cardiac glucosides

reduces the physiological activity to about one-twentieth, but does not abolish it—indicating it to be a property inherent to the molecule as a whole. The double bond does not determine the character of this action, but contributes to its intensity.

The inter-relationships as regards allocation of the three hydroxyl groups, the aldehyde group, and the unsaturated lactone group of the strophanthidin molecule have been deduced by methods presented in a series of studies by Jacobs in the strophanthidin and isostrophanthidin series.[21] These relationships are represented in the accompanying graphic formula in which we have adopted his numerical designations for the individual carbon atoms and rings:—



Brief mention may be made of certain other heart specific glycosides with aglucones of unknown constitution.

CONVALLAMARIN, from *Convallaria majalis*, $C_{23}H_{44}O_{12}$, yields a mixture of glucose, galactose and a methylpentose and convallamaretin when hydrolysed. It is accompanied by convallarin.

HELLEBOREIN,[22] present in the root and leaves of *H. niger*, $C_{21}H_{34}O_{10}$, yields glucose, arabinose, acetic acid and helleboretin, said to contain a terpene nucleus.

ANTIARIN,[23] $C_{27}H_{40}O_{10}$, the Javan arrow poison from *Antiaris toxicara*, contains two isomeric glycosides which are hydrolysed to the genin and rhamnose and to a syrup believed to contain a new methyl pentose, antiarose.

Others are adonin, movrin, said to yield fructose and arabinose.

Mention may also be made of the supposed glycosides of the squill (*Scilla maritima*), scillitin, scillain and scillarin.

Saponins.[24]

The saponins are a numerous, widely distributed class of glycosides found in a great variety of plants; they are known to be present in more than four-hundred plants belonging to about seventy different

orders, and of these about fifty have been studied and the saponins isolated.

Their most characteristic properties are the production of a soapy foam on mixing with water, and their toxicity, especially to cold-blooded animals such as frogs and fishes; these were recognised as characteristics of the plants containing them in very early times, for example, by the Greeks.

All the saponins have many characteristics in common. Physically they are white or cream-coloured powders, in most cases colloidal and only dialysable with great difficulty, although recently several crystalline members of the group have been discovered. To the latter class belong several of the digitonin glucosides, parillin, sarsasaponin and cyclamin.

They are soluble in water, giving clear solutions which froth strongly on agitation, form emulsions with oils or resins, prevent the deposition of finely divided precipitates, and occlude electrolytes and also many soluble dye-stuffs. The saponin of soapwort (*Saponaria officinalis*), for example, in its colloidal form gives a blue adsorption compound with iodine, although the crystalline constituent does not (Barger and Field). In general the saponins are insoluble in ether, benzene, chloroform, and cold ethyl alcohol, but freely soluble in hot alcohol.

They possess a very bitter, acrid taste, and the dust of the powdered saponins is very irritating and sternutatory. As already mentioned, they are strong poisons of fish, the action here being of a chemical nature; on the other hand, they possess hæmolytic action of a more physical type, occluding the red corpuscles of the blood. The more poisonous saponins are referred to as sapotoxins.

The saponins may be broadly divided, from a chemical standpoint, into neutral and acid saponins. Formerly they were classified according to their formulæ, the earliest members which were studied forming an homologous series of the general formula, $C_nH_{2n-8}O_{10}$ (sometimes termed, after its discoverer, Kobert's series). Subsequently other saponins were found to belong to a similar series, $C_nH_{2n-10}O_{18}$, whilst more recently other glycosides, the properties of which entitle them to be classified as saponins, have been isolated and do not fall in either homologous series.

On hydrolysis the saponins yield a variety of sugars (frequently several molecules of carbohydrate), and physiologically active substances termed sapogenins; the latter have not as a rule been thoroughly examined, but are often compounds of a polyhydroxylactone nature. The sugars found to exist in combination with the sapogenins vary,

glucose, galactose and arabinose being the more common, whilst more rarely other pentoses and fructose are obtained, also glucuronic acid.

The saponins are isolated from the root, leaves, seed, etc., of the plants by extraction with water and precipitation with neutral or basic lead acetate respectively, according as the saponin is acid or neutral. The precipitate is decomposed and the solution evaporated, the residue being extracted with chloroform and precipitated by ether.

All saponins form poisonous additive compounds with cholesterol.

Many sapogenins, such as those from guaiacum, saponarin, hederin and digitonin give terpene oils when distilled in hydrogen with zinc dust (van der Haar).

Ruzicka [25] has submitted the genins of a number of saponins to dehydrogenation by means of selenium, and obtains from the middle fraction of the products a trimethylnaphthalene identical with that previously prepared by him from such triterpenes as amyrin, lupeol and betulin. The name sapotalin is proposed for this naphthalene derivative.

The sapogenins tested were æscigenin, caryocarsapogenin, cyclamiretin, guaiac-sapogenin, glycyrrhetic acid, hederagenin, mimusops-sapogenin, quillaiasapogenin, sarsasapogenin, ursolic acid and sugar-beet sapogenin; only sarsasapogenin failed to yield sapotalin, but the lower boiling fractions gave methylheptenone.

The HEDERINS or saponins of the ivy (*Hedera helix*) have been investigated by van der Haar [26] and classified provisionally as α- and β-hederins, crystalline saponins insoluble in water; γ-hederin, amorphous glycosides insoluble in water; and Δ-hederin, the saponins soluble in water. At present only the α-hederin has been identified as an individual; it is a crystalline substance, $C_{42}H_{66}O_{11}$, hydrolysing to arabinose, rhamnose and α-hederagenin, $C_{31}H_{50}O_4$, a lactone, which yields a sesquiterpene on distillation with zinc dust. It is a dihydroxy acid possessing a primary and a secondary alcoholic group. Other hederins yield rhamnose, fructose and galactose and sapogenins, $C_{20}H_{32}O$, which likewise yield sesquiterpenes.

The same hederagenin is obtained from the shells of commercial soapnuts.

The saponins of *Aralia montana* yield arabinose, a methyl pentose, glucose, galactose, galacturonic acid, and sapogenins, e.g. araligenin, $C_{25}H_{49}(OH)COOH$, also related to the terpenes.

SAPORUBRIN, $(C_{18}H_{28}O_{10})_4$, is a sapotoxin found in the root of the soapwort, *Saponaria officinalis;* on hydrolysis it gives a series of products, shedding one molecule of sugar at a time, until finally the sapogenin, $C_{14}H_{22}O_2$, is obtained.

LEVANT SAPOTOXIN, $(C_{17}H_{26}O_{10}, H_2O)_2$, is very similar to saporubrin and occurs in the roots of *Gypsophila arrostii* or *G. paniculata;* it hydrolyses to four molecules of sugar (glucose and galactose) and a sapogenin, $C_{10}H_{16}O_2$. Its formula is being investigated by Jacobs.[27]

Gypsophila saponin [28] yields *l*-arabinose 12·2 per cent., rhamnose hydrate 22·2 per cent., glucose 13·2 per cent., galactose 17·3 per cent., and 21·8 per cent. of sapogenin.

CYCLAMIN, the saponin present in cyclamen tubers, $C_{63}H_{110}O_{33}$, breaks down on hydrolysis into glucose (3 mols.), *l*-arabinose (2 mols.), and an aglucone, $C_{35}H_{36}O_5$, which contains two hydroxyl and a carboxyl group.

The saponin from the seed kernels of *Mimusops elengi* yields *l*-arabinose (2 mols.), *l*-rhamnose (2 mols.), and glucose (1 mol.), together with the sapogenin, $C_{28}H_{40}(OH)_3CO_2H$. On distillation with zinc dust this gives a terpene hydrocarbon.

The saponins of sugar beet, of oleanol from olive leaves, and of caryophyllin from cloves are identical. They have the same structure, $OH.C_{30}H_{48}.CO_2H$, as ursolic acid.

Quillaic acid, $C_{19}H_{30}O_{10}$, and *Quillaia sapotoxin*, $C_{17}H_{26}O_{10}$, are respectively non-poisonous and poisonous constituents of the glucosides present in the bark of *Quellaja saponaria.* They are amorphous.

GITHAGIN from *Agrostemma sapotoxin*, $(C_{17}H_{26}O_{10})_2$, is a yellowish-white, highly poisonous amorphous glycoside found in the corn-cockle (*Lychnis* or *Agrostemma githago*); it is hydrolysed to four molecules of sugar and a sapogenin. *Githagenin*, $C_{25}H_{40}O(CO)(CH.OH)_2$, the aglucone has been studied by Wedekind,[29] who has prepared a number of oxidation products and derivatives.

The saponin of chestnut seeds, according to van der Haar [30] yields on hydrolysis glucose, a pentose, a methylpentose, galactose and glucuronic acid.

Sarsaparilla Glucosides.—Sarsaparilla, the dried root of *smilax* species, contains a mixture of saponins, amongst which are:—

PARILLIN, $C_{26}H_{44}O_{10}$, which hydrolyses to two sugars and parigenin, a phytosterolin, $C_{33}H_{56}O_6$, which gives glucose and a sitosterol on hydrolysis; sarsasaponin, $C_{44}H_{76}O_{20}$, $7H_2O$, crystals hydrolysing to three molecules of glucose and sarsasapogenin, $C_{26}H_{41}O_2(OH)$,

and smilacin or smilasaponin (von Schulz), which is not a homogeneous substance, according to Power and Salway.

Parillin,[31] from Honduras sarsaparilla, when obtained pure, is hydrolysed to glucose (2 mols.) and rhamnose (1 mol.) together with parigenin $C_{26}H_{42}O_3$.

Prunol and Urson, $C_{30}H_{48}(OH)CO_2H$, and probably also malol, are considered to be identical by van der Haar.[32] The name ursolic acid is proposed for them.

The *polysciasaponins* occurring in *Polyscias nodosa* have been studied by the same worker, who has separated them by fractional precipitation into at least two individual members, α-polysciasaponin, $C_{22}H_{36}O_{10}$, and Δ-polysciasaponin, $C_{25}H_{42}O_{10}$. Both are white amorphous powders, which hydrolyse to one molecule each of arabinose, glucose and a sapogenin $C_{26}H_{44}O_4$, a saturated lactone containing neither hydroxyl, methoxyl nor ethoxyl groups.

Other members of the group which may be cited are :—

CAULOSAPONIN, $C_{54}H_{88}O_{17}$, and CAULOPHYLLOSAPONIN, $C_{66}H_{104}O_{17}$, two crystalline saponins found by Power and Salway in *Caulophyllum thalictroides ;* JEGOSAPONIN, $C_{75}H_{80}O_{25}$, from *Styrax japonica*, in which it occurs as a crystalline calcium salt ; it gives on hydrolysis glucose, glucuronic and tiglic acids and a mixture of two sapogenins, $C_{33}H_{52}O_6$ and $C_{33}H_{52}O_7$; and two saponins, $C_{36}H_{56}O_{20}$ and $C_{37}H_{58}O_{20}$, from *Yucca angustifolia* and *Y. radiosa* respectively.

GLYCYRRHIZIN, the sweet principle of *Glycyrrhiza glabra* and other plants, is a polybasic carboxylic acid. On hydrolysis, 2 mols. of glucuronic acid are eliminated : the compound remaining, glycyrrhetic acid, $C_{31}H_{45}O_3(OH)_2 . COOH$, is considered by Tschirch to be a naphthalene derivative.

The roots of several species of the Convolvulaceæ family, growing in Mexico and South America, have long been used as purgatives ; they yield glycosidic resins of which the best known are Jalap resin from *Tubera Jalapæ*, known as Convolvulin, and Scammonium or Tampico-jalap from Orizaba root, *Ipomœa orizabensis*. Their investigation by Votoček led to the discovery of the rare methyl-pentose sugars rhodeose or *d*-fucose, and epirhamnose.

CONVOLVULIN, on dissolving in alcohol and reprecipitating with ether,[33] is separated into an ether soluble glycoside containing the sugar epirhamnose, and an ether insoluble rhamnoconvolvulic acid, $C_{52}H_{92}O_{32}$, $7H_2O$, which is hydrolysed to four molecules of glucose, two of rhamnose and convolvulinolic acid, proved by Votoček and

Prelog [34] to be 2 : 11-dihydroxypalmitic acid. They suggest that in the glycoside the hydroxyl groups each bear two glucose molecules and one molecule of rhamnose as a trisaccharide. This hydroxypalmitic acid is also found in turpethine, which contains the sugars rhodeose and glucose.

Jalapin (Scammonin, orizabin) yields on hydrolysis the sugars glucose, rhamnose and rhodeose [35] and *d*-jalaponic [36] acid, which Davies and Adams [37] have shown by synthesis to be 11-hydroxy hexadecanoic acid, as Asahina [38] first suggested.

Related to the convolvulin glycosides is Pharbitinic acid,[39] from *Pharbitis Nil*, which is hydrolysed to rhamnose, glucose and ipurolic acid, probably 2 : 11 di-hydroxymyristic acid :—

$$CH_3 . (CH_2)_8 . CH(OH) . (CH_2)_7 . CH(OH) . CH_2 . COOH.$$

CHAPTER V.

OTHER NATURAL GLYCOSIDES.

Mustard Oils, Cyanophoric Glycosides, Nucleosides, Indican, Pentosides.

The Mustard Oil Glucosides.

A number of plants belonging to the Cruciferæ yield glucosides containing sulphur. These give rise to mustard oils when hydrolysed by the enzyme myrosin which accompanies them in the plant. The best-known representatives of this class are sinigrin and sinalbin, found in the seeds of the black and white mustard. When the seed of black mustard is bruised and moistened, the odour of allylisothiocyanate is easily recognised. The myrosin and the glucoside are contained in separate cells in the seed and do not interact until brought together by the solvent.

TABLE 6.

	Formulæ.	Aglucone.
Sinigrin . .	$C_{10}H_{16}O_9NS_2K$	Allyl-isothiocyanate
Gluconapin .	$C_{11}H_{18}O_9NS_2K$	Crotonyl-isothiocyanate
Glucocheirolin .	$C_{11}H_{20}O_{11}NS_2K$	Aliphatic sulphone isothiocyanate
Glucoerysolin .	$C_{12}H_{22}O_{11}NS_2K$	Homologue of above
Glucotropæolin	$C_{14}H_{18}O_9NS_2K$	Benzyl-isothiocyanate
Gluconasturtiin	$C_{15}H_{20}O_9NS_2K$	Phenylethyl-isothiocyanate
Sinalbin . .	$C_{30}H_{42}O_{15}N_2S_2$	*p*-hydroxybenzylisothiocyanate, choline and sinapinic acid

The recognition of an ethereal oil as the active principle of black mustard dates from 1730 (Boerhave). Bussy was the first to isolate the glucoside, which he termed potassium myronate, and the accompanying enzyme, myrosin. Will and Körner gave the name sinigrin to the glucoside, and showed that it is hydrolysed to allylisothiocyanate, glucose and potassium hydrogen sulphate :—

$$C_{10}H_{16}O_9NS_2K + H_2O = C_3H_5 . NCS + C_6H_{12}O_6 + KHSO_4.$$

Sinigrin was subsequently investigated in detail by Gadamer,[1] who proposed the formula

$$C_3H_5N : C\begin{cases} O \cdot SO_3K \\ S \cdot C_6H_{11}O_5 \end{cases}$$

It is hydrolysed neither by emulsin nor by yeast extract nor any known enzyme other than myrosin. As hydrolysis proceeds, the increasing quantity of acid potassium sulphate formed renders the ferment less active and ultimately stops its action.

Guignard [2] has investigated the localisation of myrosin in the plant. It occurs in special cells with finely granular contents which are free from starch, chlorophyll, fatty matter and aleurone grains.

The extraction of myrosin has been further studied by Heiduschka and Pyriki [3] who, on examining a number of *cruciferæ* find all to contain myrosin, but that in many instances allyl thiocarbimide is present instead of sinigrin.

Details of the preparation of sinigrin have lately been given by Hérissey and Boivin.[4]

When potassium methoxide is added to sinigrin, potassium sulphate separates at once, and on adding ammoniacal silver nitrate the silver salt of thioglucose is obtained, proving that the glucose molecule is attached to the sulphur atom in the glucoside :—

$$C_3H_5 \cdot N : C \cdot (OSO_3K) \cdot S \cdot C_6H_{11}O_5 \longrightarrow C_3H_5 \cdot N : C(O\,Me) \cdot S \cdot C_6H_{11}O_5 \longrightarrow HS \cdot C_6H_{11}O_5.$$

According to Schneider and M. Becker [5] the scission of sinigrin by aqueous silver nitrate, in the presence of silver carbonate to remove the free nitric acid formed, in accordance with the equation

$$C_3H_5N : C(O \cdot SO_3K) \cdot S \cdot C_6H_{11}O_5 + 2AgNO_3 + H_2O = C_3H_5N : C(O \cdot SO_3Ag) \cdot SAg + C_6H_{12}O_6 + HNO_3 + KNO_3$$

yields a sugar solution which has a higher dextrorotation (mutarotation ultimately the equilibrium value) than that required for the total liberated β-glucose, showing that the product of hydrolysis is α-glucose. Conversely, enzymic hydrolysis of the same glucoside gives a small lævorotation, converted by addition of a few drops of ammonia into a dextrorotation of ten times this value (corresponding with 55 per cent. hydrolysis). If sinigrin is a β-glucoside the silver nitrate scission must involve a Walden inversion about the terminal carbon atom of glucose, a view confirmed [6] by Schneider, Fischer and Specht, who find that silver nitrate liberates α-glucose from β-thioglucose.

Wrede[7] and others have shown that the thioglucose obtained from sinigrin is not identical with that prepared synthetically, as shown by the fact that it is lævorotatory $[a]_D - 50°$ whereas the synthetic product has $[a]_D + 1{\cdot}6°$. The pentacetyl derivatives are also different. It is suggested[8] that these are α- and β-forms which are presumably more stable than the corresponding forms of glucose.

GLUCONAPIN, which is present to a small extent in the seed of rape, *Brassica rapus*, yields glucose and crotonyl mustard oil, $CH_3 . CH : CH . CH_2 . N : C : S$, on hydrolysis. It and probably the next higher homologue are constituents of several cruciferæ. Isobutyl mustard oil is the prime constituent of the ethereal oil of *Cochlearia officinalis*, but it has not been identified as a glucoside.

GLUCOCHEIROLIN, $C_{11}H_{20}O_{11}NS_3K$, H_2O, occurring in wallflower seeds, has been studied by Schneider[9] and found to be a derivative of an aliphatic sulphone. Glucheirolin is

$$CH_3 . SO_2 . CH_2 . CH_2 . CH_2 . N : C(O . SO_3K) . S . C_6H_{11}O_5.$$

It is hydrolysed to glucose and cheirolin by myrosin.

ERYSOLIN, $CH_3 . SO_2 . CH_2 . CH_2 . CH_2 . CH_2 . N : C : S$, the next higher homologue of cheirolin, is obtained from the seeds of the bright orange wallflower, *Erysimum perowskianum*, where it is undoubtedly present as a glucoside.

SINALBIN, the glucoside of white mustard seed, is considerably more complex. It is likewise hydrolysed by myrosin, which accompanies it in the seeds, to glucose, sinalbin mustard oil (*p*-hydroxybenzyl-isothiocyanate) and acid sinapin sulphate :—

$$C_{30}H_{42}O_{16}N_2S_2 + H_2O = C_6H_{12}O_6 + C_7H_7O . NCS + C_{16}H_{24}O_5N . HSO_4.$$

Barium hydroxide converts acid sinapin sulphate into choline and sinapinic acid :—

$$HO . CH_2CH_2N(CH_3)_3OH \quad \text{and} \quad C_6H_2(OH)(OMe)_2 . CH : CH . CO_2H.$$

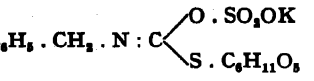
It is of interest that the alcohol corresponding with this acid is syringenin, a constituent of the glucoside syringin.

The glucoside thus has the structure

$$C_6H_4(OH) . CH_2 . N : C\begin{matrix} O . SO_2 . C_{16}H_{24}O_6N \\ S . C_6H_{11}O_5 \end{matrix}$$

The ethereal oils of a number of cresses are mustard oils, originally present as glucosides, though these have only seldom been isolated, as the myrosin present immediately hydrolyses them. Thus benzyl

mustard oil is present in *Tropæolum majus* and in *Lepidium sativum* as glucotropæolin:—



The oil of *Nasturtium officinale* contains the next higher homologue, phenylethyl mustard oil, undoubtedly as the glucoside Gluconasturtiin.

A mustard oil containing a pentose has been described by Angelico [10] under the name potassium atractilate. It is the poisonous principle of the root of *Atractylis gummifera*, and on hydrolysis yields a pentose, valeric acid, sulphuric acid and an unidentified aglucone.

Cyanophoric Glycosides.

Hydrocyanic acid has frequently been isolated from plants, and though it may be present otherwise its formation is ascribed almost invariably to the decomposition of a glycoside. Besides amygdalin, an ever-growing number of other glycosides have been isolated, which yield hydrogen cyanide when hydrolysed; they are conveniently grouped together under the term cyanophoric glycosides. The distribution of hydrogen cyanide is proving much wider than was at one time imagined; its production has been observed in many plants of economic importance. A useful list [1] of plants which yield prussic acid has been compiled by Greshoff and also by Rosenthaler, who enumerates 360 varieties in 148 species and 41 families, particular reference being made to the distribution of the acid in the various parts of the plant. Generally the presence of alkaloids and terpenes appears to be incompatible with the presence of hydrocyanic acid.

TABLE 7.

Glycoside.	Formula.	Sugar.	Aglucone.
Amygdalin . . .	$C_{20}H_{27}O_{11}N$	Gentiobiose	Benzaldehyde + HCN, i.e. *d*-mandelonitrile
Prunasin	$C_{14}H_{17}O_6N$	Glucose	*d*-mandelonitrile
Prulaurasin . . .	$C_{14}H_{17}O_6N$	,,	*r*-mandelonitrile
Sambunigrin . . .	$C_{14}H_{17}O_6N$	,,	*l*-mandelonitrile
Dhurrin	$C_{14}H_{17}O_7N$	,,	*p*-hydroxymandelonitrile
Gynocardin . . .	$C_{13}H_{19}O_9N$	,,	Unknown
Linamarin . . .	$C_{10}H_{17}O_6N$	,,	Acetone cyanhydrin
Lotusin	$C_{32}H_{31}O_{11}N$	Gentiobiose	Lotoflavin
Vicianin	$C_{19}H_{25}O_{10}N$	Vicianose	Mandelonitrile.

Amygdalin.

$$C_6H_5 . CH(CN)—O—C_6H_{10}O_4—O—C_6H_{11}O_5$$
$$\beta \qquad\qquad \beta$$

mandelonitrile — glucose — glucose

mandelonitrile-β-glucoside. Gentiobiose.

Amygdalin is perhaps the best known and at the same time the most interesting of the glycosides; it has formed the subject of repeated and fruitful investigation ever since its discovery one hundred years ago. It yields benzaldehyde, hydrogen cyanide and two molecules of glucose on hydrolysis. It is found in large quantities in bitter almonds and in the kernels of apricots, peaches, plums and most fruits belonging to the Rosaceæ. It is the effective principle of the so-called essence of bitter almonds, and is widely used as a flavouring material. Like most glycosides it is a colourless, crystalline, bitter substance, soluble in water.

The presence of hydrogen cyanide in the aqueous distillate of bitter almonds was observed at the very beginning of the nineteenth century by Bohm; the crystalline glycoside was first obtained by Robiquet and Boutron Charlard in 1830.

In 1837 Liebig and Wöhler[2] found that amygdalin was hydrolysed by a certain nitrogenous substance, also existing in the almond, to which they gave the name emulsin, in accordance with the equation

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}O_{11}N} + 2H_2O = \underset{\text{Benzaldehyde.}}{C_7H_6O} + \underset{\text{Hydrogen cyanide.}}{HCN} + 2\underset{\text{Glucose.}}{C_6H_{12}O_6}$$

They proved it to be a glycoside of benzaldehyde cyanhydrin.

Ludwig[3] in 1856 pointed out that hot mineral acids hydrolyse amygdalin, giving rise to the same products as emulsin does. Schiff[4] was the first to suggest that the two glucose molecules were united as a biose:—

$$C_6H_5 . CH(CN) . O—C_6H_{10}O_4 . O . C_6H_{11}O_5,$$

and this view became generally accepted when it was shown by Fischer[5] that amygdalin may be resolved by an enzyme, contained in yeast extract, into a molecule of glucose and one of a new glucoside which he termed mandelonitrile glucoside:—

$$C_6H_5 . CH(CN) . O—C_6H_{11}O_5.$$

Fischer came to the guarded conclusion that amygdalin was a derivative either of maltose or of a closely related diglucose. However,

neither in its behaviour towards enzymes nor in its chemical properties does amygdalin behave as a maltoside. Ultimately, after a considerable number of investigations, the sugar has been proved by Haworth and Wylan [6] to be identical with gentiobiose, formerly one of the rarest of the natural sugars, which is a constituent of gentianose of the gentian root. Accordingly, amygdalin is mandelonitrile β-glucose-6-β-glucoside, a conclusion in harmony with Hudson's [7] optical calculations. Finally, Campbell [8] and Haworth have effected its synthesis from acetobromogentiobiose and ethyl-*dl*-mandelate, similar syntheses having been achieved by Zemplen and Kunz [9] and by Kuhn.[10] Gentiobiose has also been identified as the sugar in α-crocin, the glycoside of saffron.[11] The first product of acid hydrolysis of amygdalin is *d*-mandelonitrile glucoside; strong acids give *l*-mandelic acid.

The action of enzymes on amygdalin is peculiar owing to the fact that both yeast extract and emulsin are mixtures and that they contain a common constituent.

Yeast extract hydrolyses amygdalin to glucose and mandelonitrile glucoside by means of an enzyme named amygdalase, which differs from maltase and is more stable towards heat. Almond extract, that is emulsin, contains prunase (β-glucosidase) in addition to amygdalase [12, 13] and hydrolyses the glucoside completely, the amygdalase acting if anything the faster. Consequently no biose sugar is detectable at any time, whereas it is possible to isolate mandelonitrile glucoside. It is probable that the two actions follow one another and that prunase is unable to act until the molecule has first been simplified by the action of amygdalase. This is taken as proof that the second molecule of glucose in some way shields the prunasin part of the molecule from attack.

The second enzyme in emulsin has been found in the leaves of many plants, where it occurs without amygdalase. Since it was first found in the leaves of the common cherry laurel it has been named prunase and the mandelonitrile glucoside on which it acts is termed prunasin.

In addition to *d*-mandelonitrile glucoside obtained from amygdalin three other glucosides having the same composition are known. These are: *prulaurasin*, first described in the amorphous state under the name laurocerasin, and since obtained crystalline from the cherry laurel by Hérissey; *sambunigrin*, separated by Bourquelot and Danjou [14] from the leaves of the common elder (*Sambucus nigra*); and *prunasin*, found by Hérissey [15] in the young branches of *Cerasus*

Padus and by Power and Moore,[16] who obtained it from wild cherry bark (*Prunus serotina*). These substances are all mandelonitrile glucosides; their properties are set out in the following table :—

TABLE 8.

	M.-pt.	$[\alpha]_D$.
Prunasin = dextro-mandelonitrile glucoside* . . .	147°-150°	− 26·9°
Prulaurasin = racemic mandelonitrile glucoside . .	120°-122°	− 52·7°
Sambunigrin = lævo-mandelonitrile glucoside . . .	151°-152°	− 76·3°

PRULAURASIN is, in fact, a racemic mixture of the two stereoisomeric *d*- and *l*-mandelonitrile *β*-glucosides, and is analogous to isoamygdalin, the racemic form of amygdalin, which was first prepared by the action of alkali on amygdalin by Walker [17] and subsequently studied by Dakin [18]; it yields inactive mandelic acid when hydrolysed by acids; indeed, prulaurasin can be obtained by acting on isoamygdalin with yeast extract—amygdalase (Hérissey). *Sambunigrin* is the *β*-glucoside of *l*-mandelonitrile glucoside, and derived from a still unknown isomeride of amygdalin. *Prulaurasin* is obtained from either of the other two optical isomerides, when their aqueous solutions are rendered slightly alkaline. It is remarkable that both forms of mandelonitrile glucoside should be present in a plant, and quite likely that the racemic mixture was formed in the process of extraction.

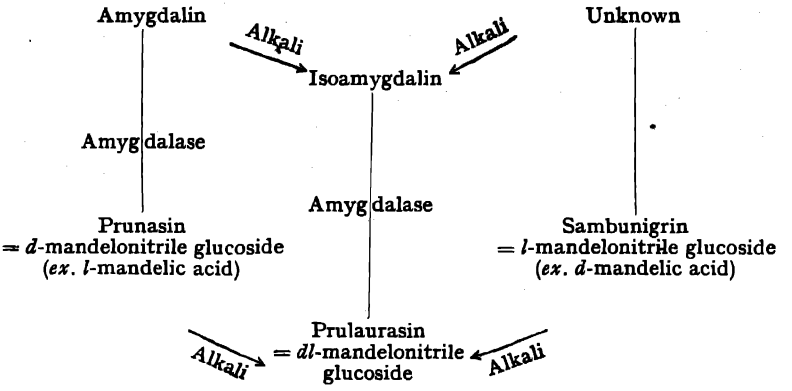
The true relationship of these glucosides was first established by Caldwell and Courtauld,[19] and their conclusions have been entirely confirmed by Bourquelot and Hérissey.

The kernels of the cherry laurel contain as much as 4 per cent. of amygdalin: this plant, like most others, stores a more elaborate product in its seeds than is present in the leaves.

The inter-relationship of these compounds is indicated in the accompanying scheme.[20] Possibly the unknown isomeride of amygdalin will also be found in the plant.

The synthesis of the mandelonitrile glucosides was successfully carried out by Fischer and Bergmann,[21] who obtained the acetylated glucoside of ethyl mandelate in racemic form by treatment of the synthetic ester with acetobromoglucose and silver oxide in the conventional manner. A methyl alcoholic solution of ammonia trans-

*According to the existing nomenclature *l*-mandelic acid forms *d*-mandelonitrile.



formed the ester into the corresponding glucosidic amide, and this was resolved into its pure optically-active forms by fractional crystallisation. The individual forms were converted by the action of phosphorus oxychloride into the tetra-acetyl derivatives of *d*- and *l*-mandelonitrile glucosides, which were, of course, identical with the tetra-acetates of prunasin and sambunigrin respectively.

On removal of the acetyl groups by hydrolysis racemisation sets in, and the product was found to be *r*-mandelonitrile glucoside, or prulaurasin. The synthetic racemic glucoside was resolved by fractional crystallisation into *d*-mandelonitrile glucoside * (prunasin) and *l*-mandelonitrile glucoside (sambunigrin).

DHURRIN, first isolated by Dunstan and Henry [22] from the leaves and stems of the great millet, is a para-hydroxy-mandelonitrile glucoside, and therefore closely related to the three mandelonitrile glucosides just described. Like them it is hydrolysed by emulsin.

GYNOCARDIN, isolated by Power [23] from the oleaginous seeds of *Gynocardia odorata*, yields prussic acid, glucose and a diketone, formula $C_6H_8O_4$, on hydrolysis. It is accompanied in the seeds by an enzyme, gynocardase, which also decomposes amygdalin.

LINAMARIN or PHASEOLUNATIN, $C_6H_{11}O_5 . O . CMe_2 . CN$, was first isolated by Jorissen and Hairs [24] from young flax plants and subsequently by Dunstan and Henry from *Phaseolus lunatus*. The latter authors considered it to be acetonecyanhydrin-α-glucoside, but it has since been shown to be a derivative of β-glucose. Hydrogen cyanide and acetone have been obtained from a number of plants on hydrolysis, and possibly linamarin is widely distributed. It is the

* Fischer uses the inverse notation, deriving the glucosides from *d*- and *l*-mandelic acids and not from their nitriles.

glucoside in the seeds of the rubber tree, *Hevea brasiliensis*. The glucoside is accompanied in plants by a specific enzyme linase which has been fully investigated by Armstrong and Eyre. *Phaseolus lunatus* contains two enzymes—an emulsin which, however, according to Dunstan, is without action on phaseolunatin and an enzyme which hydrolyses both phaseolunatin and amygdalin, forming mandelonitrile glucoside in the latter case, and is obviously amygdalase.

Linamarin has been synthesised by Fischer and Anger[28] from acetobromoglucose, thus confirming its structure as a glucoside. The acetobromoglucose condenses with ethylhydroxyisobutyrate to ethyl-tetracetyl-glucosido-α-hydroxyisobutyrate,

$$(OAc)_4 . C_6H_7O . O . CMe_2 . CO_2Et,$$

which is extremely slowly hydrolysed by emulsin. Ammonia converts it into α-hydroxybutyramideglucoside,

$$C_6H_{11}O_5 . O . CMe_2 . CO . NH_2,$$

of which the tetracetate on treatment with phosphoryl chloride yields tetracetyl-linamarin.

LOTUSIN,[25] discovered by Dunstan and Henry in *Lotus arabicus*, is of interest for two reasons. Like amygdalin it gives rise to two molecules of glucose on hydrolysis and therefore probably contains a disaccharide. The other products of hydrolysis are prussic acid and lotoflavin—an isomeride of fisetin. In the alkaline hydrolysis one of the glucose residues is obtained as heptagluconic acid, indicating that the cyanogen radicle is associated with the sugar residue, but the exact structure of lotusin remains in doubt. Lotusin is not hydrolysed by almond emulsin, but it is resolved by an enzyme (lotase) which accompanies it, but as this also decomposes amygdalin and salicin it probably contains emulsin.

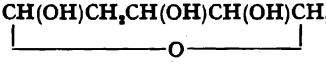
VICIANIN has been found only in the seeds of a wild vetch (*Vicia angustifolia*). It is decomposed by an enzyme (vicianase) present in certain vetches, into hydrogen cyanide, benzaldehyde and a disaccharide, $C_{11}H_{20}O_{10}$, vicianose, which is hydrolysed further by the emulsin of almonds into glucose and *l*-arabinose (Bertrand).[26] Accordingly, vicianin represents amygdalin, in which one molecule of glucose is replaced by arabinose. Vicianose has been synthesised by Helferich and Bredereck[27] and shown thereby to be *d*-glucose 6-β-*l*-arabinoside.

Nucleosides.

Glycosides with aglucones containing nitrogen belonging to the purine or pyrimidine series occur both in plants and animals. In these the sugar is coupled to the amino or imino groups which are equivalent to the hydroxyl of other aglucones. These nucleosides occur free or are obtained from the nucleotides in which they are combined with phosphoric acid, by neutral hydrolysis under pressure. The animal and plant nucleic acids, the acid constituents of the nucleoproteins, are assemblages of four different nucleotides containing different bases.[1]

In plants the nucleotides consist of a molecule of phosphoric acid combined with *d-ribosides* of *guanine*, *adenine*, *cytosine* or *uracil*, though there is a possibility that uracil may be derived by secondary decomposition of cytosine.

Animal nucleic acid, though more resistant, may be decomposed in the same way into phosphoric acid and its component nucleosides, which are derived from the same bases, except that *thymine* replaces uracil. The sugar is, however, different and breaks down to lævulinic acid and formic acid and was originally thought to be a hexose, but has recently been shown by Levene [2] to be *d*-2-deoxy-ribose,



which was obtained from guanine nucleoside.

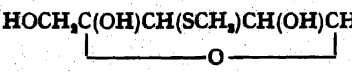
Animal nucleic acid, therefore, contains in a reduced state the same sugar as occurs in plant nucleic acid.

The simple nucleotides, *inosinic* and *guanylic* acids, are extranuclear constituents of the glandular tissue of animals, in which *hypoxanthine* and *guanine* respectively are combined with phosphoric acid and *d*-ribose.

Similarly *adenine*, *cytosine* and *uracil* *d*-ribosides are obtained from the β-nucleo protein of the pancreas.[3]

These observations show that *d*-ribose is not solely found in plant nucleosides.

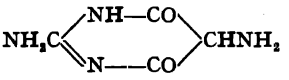
An adenine " hexoside " [4] was found in yeast, of which the sugar contains sulphur and is identified by Levene as a *thioketopentose*, whose structure is not yet settled in detail:—



Adenosin, *cytidin*, *uridin* and *guanosin* are the names given to the nucleosides of adenine, cytosine, uracil and guanine respectively.

Guanosin is identical with the compound *Vernin*, which is widely distributed in plants, originally discovered by Schulze in the seeds of *Lupinus luteus* and recognised by him and Castoro as a pentoside.

Vicin, a nucleoside present in many species of vetch, is hydrolysed to glucose and divicin, which is given the structure by Levene [5]:—



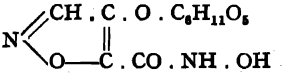
It is accompanied by convicin, which is hydrolysed to alloxantin.

A number of synthetic nucleosides have been made.

Levene and Jacobs [6] identified the sugar obtained from plant nucleic acids as *d*-ribose and used it for the synthesis of the hexose sugars *d*-allose and *d*-altrose. Robinson (p. 4) has, however, suggested that it is in reality *d*-xylose and undergoes inversion during hydrolysis, involving the splitting off of phosphoric acid. Levene does not accept this conclusion, so that the question is to some extent an open one, though the fairly common occurrence of *d*-xylose in glycosides and its obvious derivation from glucose make it rational to accept Robinson's hypothesis.

Amino sugar glucosides, that is derivatives of glucosamine, have not been discovered in natural products, though they have been prepared by synthesis. It is possible to speculate as to the glycosidic nature of the glucoproteins such as Willstätter and Stoll [7] have shown to be present in highly purified enzymes. Peroxydase, for example, contains 8·5 per cent. of nitrogen and yields 30 per cent. pentose and an equimolecular quantity of a hexose.

HIPTAGIN, $C_{10}H_{14}O_9N_2$, the glucoside of the root bark of *Hiptage madablota*, is resolved by acids into glucose and an isoxazole [1] with a hydroxylamine residue. This is the first naturally occurring hydroxylamine compound: with dilute alkali it forms even in the cold ammonia and hydrogen cyanide. It is probably formed in the plant by reduction of nitrates to hydroxylamine and condensation of this base with aldehydes and ketones. It is formulated



Indican.

Plants which yield indigo do not contain the colouring matter as such, but in the form of a glucoside indican, which is readily extracted

from the leaf by means of acetone. Indican yields glucose and indoxyl on hydrolysis; the colourless indoxyl undergoes further oxidation to indigotin, the blue colouring matter :—

$$\underset{\text{Indican.}}{C_{14}H_{17}O_6N} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Indoxyl.}}{C_8H_7ON}$$

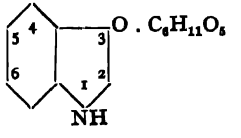
$$2\underset{\text{Indoxyl.}}{C_8H_7ON} + O_2 = 2H_2O + \underset{\text{Indigotin.}}{C_{16}H_{10}O_2N_2}.$$

Indigotin is readily obtained on hydrolysing indican with dilute acids containing a little ferric chloride as an oxygen carrier, but the yield under these conditions is not quantitative. In the plant an oxydase plays an important part in the formation of indigotin.

Indican is hydrolysed by a specific enzyme, indemulsin, which is present in the leaves of the indigo plant. Emulsin [1] also slowly hydrolyses indican, but its action is far less intense than that of the *Indigofera* enzyme preparations. The yield of indigotin in this case is also below the theoretical, especially when hydrolysis is slow : this is due to the great instability of indoxyl and in part also to the occlusion of indoxyl by the enzyme. It may be improved by adding a small quantity of sulphuric acid to the mixture at the commencement of the reaction.

Indican was first isolated by Schunk in 1855 from *Polygonum tinctorium*, but it was not until 1898 that its correct formula was indicated by Marchlewski and Radcliffe.[2] Hazewinkel [3] supplied the proof that it is an indoxyl glucoside. Much work on it was subsequently done by Perkin [4] and others. Its synthesis has been effected by Robertson,[5] by the interaction of methyl-3-hydroxyindole-2-carboxylate and acetobromoglucose.

Indican is thus definitely 3-β-glucosidoxyindole :—



6-Bromoindican has been synthesised by Robertson and Waters,[6] starting from methyl-4-bromoanthranilate. It closely resembles indican itself in properties; the 6-bromoindoxyl liberated on hydrolysis is readily oxidised to 6 : 6′-dibromoindigotin, the renowned Tyrian purple of the ancient Romans, which is formed by the action of light on an unknown precursor present in the glands of certain marine molluscs. It does not occur in plants.

" Thioindican " has been synthesised by Craik and Macbeth [7] by the same general method.

Pentosides.

The glycosides so far dealt with have fallen into well-defined groups, according to the nature of their aglucones. It is not proposed even to list here the numerous plant glycosides of which the structure of the aglucone and often even of the sugar constituent is unknown. Reference must be made, however, to certain interesting pentosides and methylpentosides in addition to those which have already been mentioned in earlier chapters.

Glycosides containing primeverose = 6 glucose-β-*d*-xyloside :—

This disaccharide is proving to be widely distributed in nature. It has been obtained :—

i. From the glycosides primeverin and primulaverin isolated from *Primula officinalis* by Goris and Vischniac.
ii. From monotropitin,[8] identical with gaultherin (p. 14).
iii. From genticaulin,[9] the glycoside of the gentian.
iv. From rhamnicosin, a glycoside, $C_{26}H_{30}O_{15}$, present in *Rhamnus* species: it is the source of china green.[10] The aglucone $C_{15}H_{12}O_6$ is probably a penta-hydroxymethylanthranol.

The glycosides are hydrolysed by an accompanying enzyme to which the names, betulase, gaultherase and primeverase have been given. It is perhaps best called primeverase to indicate that it hydrolyses glycosides of the disaccharide.[11]

The sugar reduces Fehling's solution and forms an osazone, thus differing from the non-reducing sugar present in dibenzoyl glucoxylose (*q.v.*). Proof of its structure is afforded by its synthesis effected by Helferich and Rauch[12] from acetobromoxylose and β-*d*-glucose 1 : 2 : 3 : 4-tetra-acetate in chloroform solution.

Glycosides containing vicianose=6-glucose-β-*l*-arabinoside. These are vicianin, violutin, gein (*q.v.*). The synthesis has been effected by Helferich and Bredereck.[13]

OROBRANCHIN, from *O. rapum*, yields among its products of acid hydrolysis glucose, rhamnose and caffeic acid.

SOLANINE,[14] $C_{44}H_{71}O_{15}N$, contains a glucose-galactose-rhamnose trisaccharide, the glucose being joined to the aglucone solanidine, which has the composition $C_{26}H_{41}ON$.

CHINOVIN. The bark of most species of Cinchona contains one or both isomeric forms of this glycoside. The bark is extracted with milk of lime and the glucoside precipitated by means of hydrochloric acid. On hydrolysis by means of alcoholic acid the ethyl glycoside

of a sugar is obtained, identified by Fischer and Liebermann [15] as a methyl pentose and named chinovose (quinovose). Quite recently Freudenberg [16] has proved chinovose to be *d*-epirhamnose. The aglucone, chinovaic acid, $C_{32}H_{48}O_6$ (?), has not been fully investigated.

Derivatives of α-glucose other than maltose and starch are rare in plants and the few observations as to their occurrence require full confirmation.

Phillyrin, present in *Forsythia suspensa* and *Olea fragrans*, is hydrolysed, according to Kolle and Hjerlow,[17] by α-glucosidase, by rhamnase, and by acids to glucose and phillygenin, $C_{33}H_{35}O_{10}$.

Steviosin, the very sweet principle of the leaves of *Stevia Rebaudiana*, is said by Bridel and Lavieille to be hydrolysed to α-glucose.

Colin and Guéguen [18] state that *Rhodymenia palmata* contains an α-monogalactoside of glycerol in which the secondary alcohol group is concerned in the attachment of the sugar. Unaffected by emulsin, it is hydrolysed by bottom yeast extract. A compound of α-galactose is said to be the typical sugar of seaweeds (Florideæ).

CHAPTER VI.

THE SYNTHETIC GLYCOSIDES.

A NUMBER of the natural glycosides have been prepared synthetically, and by the same methods the glycosides corresponding to a variety of substances can be obtained. The first synthesis of the natural glycosides was by means of the crude acetochloroglucose first prepared by Colley [1] in 1870 by the action of acetyl chloride on glucose. Michael [2] coupled this with the potassium salt of phenols, preparing in this manner phenol glucoside, helicin, salicin and methyl arbutin; Drouin [3] by the same method obtained the glucosides of thymol and α-naphthol. Fischer obtained the alkyl glucosides from acetochloroglucose, but the α- and β-methyl glucosides, of importance in the structural chemistry of the sugars, and their higher homologues, are more usually prepared in other ways.

Following the discovery of the crystalline α- and β-acetochloroglucoses, attempts were made to extend and improve Michael's synthetical method, but were at first only successful in making β-compounds.[4] Ordinary dextrorotatory tetra-acetyl glucosidyl chloride and bromide are to be regarded, through Hudson's work,[5] as belonging to the α-series; they give in alcoholic solution in presence of silver carbonate β-glucosides, presumably by a Walden inversion.

Pure tetra-acetyl β-glucosidyl chloride had been prepared, but a satisfactory process for preparing α-glucosides from it could not be developed, owing to the ease with which it changes to the α-chloride and thus gives β-glucosides. Most of the β-glucosides synthesised have been prepared by the condensation of the aglucone with acetobromoglucose in presence of silver oxide.

The best method of preparing acetobromoglucose is that given by Fischer.[6] Powdered anhydrous crystalline glucose, dissolved in about five times its weight of acetic anhydride, is boiled with half its weight of sodium acetate for two or three hours. The product is poured into a large volume of ice water and the crude β-glucose pentacetate is freed from acetic anhydride as much as is possible by

pulverisation under water and then crystallised from 96 per cent. alcohol, when it is obtained in 74 per cent. yield.

One part of the pentacetate is left with two parts of the commercial solution of hydrobromic acid in acetic acid for two hours at the ordinary temperature. Four parts of chloroform are added and the mixture shaken with twice its weight of ice water; the chloroform extract is run off and the residue again shaken with a little chloroform, after which the united chloroform solutions are washed with water, dried over calcium chloride, and the chloroform removed under a vacuum. The oily residue is triturated with light petroleum until crystallisation sets in and subsequently the collected crystals are rapidly recrystallised from a little amyl alcohol, washed with light petroleum, and stored in a vacuum over soda-lime.

Levene and Raymond [7] state that better yields are obtained with less trouble if the crude bromacetyl product is taken up in toluene and the solution concentrated under reduced pressure at 40° to 50° until the residue is syrupy. Fresh toluene is added and removed again, this process being thrice repeated. The product then usually crystallises in the flask and after treatment with charcoal can be recrystallised from warm ether.

α-glucosides were first obtained by a modification of the glucoside synthesis, introduced by Fischer,[6] which consists in warming acetobromoglucose with alcohols and phenols in presence of quinoline.

In this process, in addition to the usual inversion of the α-bromide to give a β-glucoside, α-glucoside is formed directly and the mixture of α- and β-glucosides formed can be separated in the instance of phenyl-glucoside by a crystallisation from carbon tetrachloride. This synthesis of α-glucosides was important as they had not been previously available.

Hickinbottom has recently shown [8] how to obtain α-glucosides from β-glucosidyl chlorides. He found that the 2 : trichloro-acetyl-3 : 4 : 6-triacetyl-β-glucosidyl chloride and 3 : 4 : 6-triacetyl-β-glucosidyl chloride which he used were converted in alcoholic solution into the isomeric α-derivatives, at the same time in presence of silver oxide α-glucoside formation took place. To obtain a maximum yield of α-glucoside, therefore, isomerisation is reduced to a minimum by limiting the concentration of alcohol and using an indifferent solvent such as benzene as a diluent. α-phenyl glucoside was the only glucoside, apart from the alkyl glucosides, which was made, and the application of this potentially valuable method to the production of α-glucosides of more complicated aglucones is awaited.

When only the β-glucoside of phenolic compounds is required the Fischer method above gives somewhat poor yields. Robertson and Waters [9] state that if silver oxide is present in the quinoline method β-glucosides are formed exclusively from phenols, though the method is useless for alcohols. Pure dry materials must be used, and after adding silver oxide with stirring to the mixture of acetobromoglucose and the aglucone, it is kept in a desiccator for an hour and then poured into water. The tetra-acetyl derivative precipitated is recrystallised in the instance of phenyl glucoside from alcohol and subsequently hydrolysed by saturating in methyl alcohol at 0° with ammonia and leaving for a number of hours ; removal of the ammonia and solvent in a vacuum leaves a crystallisable residue.

Synthetic glycosides have also been derived from triacetylbromoglucosamine by Irvine [10] and his co-workers, whilst an important group of syntheses are those effected by the biological method of Bourquelot, by the use of enzymes.

The experimental methods available make it theoretically possible to synthesise any desired glycoside, especially since Fischer's discovery how to make the α-glucosides. A variety of materials are thus available for the more exact study of the selective action of enzymes, the resistance of glycosides to acid hydrolysis and the effect of structure and the nature of the aglucone on the optical and other properties.

Interesting β-glucosides are those of menthol and borneol ; they were the first synthetical terpene glucosides made by the Fischer method and are closely allied to the terpene glycuronic acid derivatives. Similar synthetic glucosides are those of geraniol and cyclohexanol [11] (Fischer and Helferich), and of citronellol, camphene, di-hydrocarveol, fenchyl alcohol, terpineol, cis-terpene, sabinol and santenol [12] (Hämäläinien).

Salway [13] has synthesised the similar ceryl and myricyl glucosides, and those of sitosterol and cholesterol ; and an investigation by Power and Salway [13a] showed that a number of natural compounds previously assumed to be phytosterols were really glucosides (phytosterolins). Amongst these were ipuranol, from olive bark (*Ipomœa purpurea*), etc., citrullol, found in colocynth and *Euonymus atropurpureum*, bryanol in bryony root, and cluytianol from *Taraxacum* root, trifolianol from *T. pratense* and *incarnatum*, calabrol, cucurbitol, etc. Most of these are glucosides of sitosterol, $C_{27}H_{46}O$, or one of its isomerides, or they are mixtures of it with the glucoside of stigmasterol, $C_{30}H_{50}O$.

Mauthner [14] has synthesised glucovanillic acid, glucopara-hydroxybenzoic acid, and other phenolcarboxylic acid glucosides, employing their methyl esters in the condensation with acetobromoglucose. He has also prepared some of the glucohydroxy aceto- and benzophenones, the para-hydroxyacetophenone derivative being found naturally in pine needles and known as picein [15] (Tanret).

The glucosides of phloroglucinol, resorcinol, and 2 : 4 : 6-tribromophenol were obtained by shaking an ethereal solution of acetobromoglucose with an alkaline solution of the phenols; the first mentioned is identical with the glucoside obtained from phloridzin by Gremer and Seuffert and is capable of inducing diabetes (Fischer and Strauss).[16]

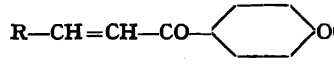
Glucosides with long side-chains have been prepared by Bargellini [17] by condensation of helicin and similar glucosides with different hydroxy ketones, for example, with para-hydroxyacetophenone, when the compound

$$C_6H_{11}O_5 . O . C_6H_4 . CH : CH . CO . C_6H_4 . OH$$

is formed.

These glucosides are stated not to be hydrolysed by acids and also not to be resolved by emulsin, and therefore said to belong to the α-series. Since helicin is a β-glucoside this conclusion cannot be accepted, and it is more probable that the long side-chain profoundly alters the properties of the glucoside.

Bargellini [18] has also synthesised chalkone glycosides by condensation of the natural para-hydroxyacetophenone glucoside picein, with various aldehydes benzaldehyde, anisaldehyde, vanillin, piperonaldehyde and cinnamaldehyde all yield their respective glucosides.



In his work on the synthesis of anthocyanins, the most complicated natural glycosides yet synthesised, Robinson [19] and his collaborators have made several synthetic glucosides which are derivatives of phloroglucinaldehyde or alternatively of acetophenone. A successful anthocyanin synthesis demands the use of a general synthetic method, which does not depend on demethylation, which will allow assembly of components without eliminating their glucose residue, and further will allow hydrolysis of the protecting acetyl and benzoyl groups from the final product without affecting the glucose. The use of monobenzoyl phloroglucinaldehyde gave a compound of suitable reactivity fulfilling these requirements.

The glucoside components were made by adding dry silver carbonate to a solution of acetobromoglucose and the appropriate hydroxy compound in dry benzene solution at 35°; after stirring for thirty-five minutes and refluxing for fifteen minutes, the glucoside is precipitated from solution with petroleum and subsequently crystallised.

By the use of suitable components anthocyanin glucosides having the sugar attached to the positions 3, 5, 7 and 4′ respectively, of which the first two types are also known in natural anthocyanins, have been synthesised.

Synthetic glucosides have been used to investigate the effect of coupling a substance with a sugar on its physiological activity. Acetobromoglucose reacts with the silver salts of organic acids to form glucosides which are really acetylated glucose esters of the acids. The acetyl radicles cannot be eliminated without at the same time detaching the sugar group so that the influence of the glucose molecule on the physiological activity of the acids cannot in this instance be observed.

Sabalitschka and Schweitzer [20] have prepared glucosides of various parahydroxy benzoic acids and compared their bactericidal activities against *Staphylococcus pyogenes aureus.*

Taking the standard activity of phenol as 1, these glucosides, all hydrolysable by emulsin, have the following values :—

Phenol	1
Parahydroxy benzoic acid	3·6
,, ,, ,, glucoside	3·0
Methyl ester of *p*-OH-benzoic acid	2·6
,, ,, ,, glucoside	2·7
3-chloro-4-OH-benzoic acid	37·0
,, ,, ,, glucoside	4·3

The activity of the chloroacid is much lowered by glucoside formation.

Glaser and Widwerk [21] examined the three nitrophenol glucosides for disinfectant properties. The nitrophenols themselves have a bactericidal power intermediate between the cresols and phenol, but their glucosides have but slight toxicity. The utility of the animal organism's pairing of the same substances with glucuronic acid is made evident.

Karrer and Hurwitz [22] have synthesised a β-glycerol glucoside of known constitution, in which the sugar residue is attached through a primary alcohol group, by combining acetobromoglucose with isopropylidene glycerol and subsequent careful hydrolysis. The product is hydrolysed by emulsin and is identical with that obtained by Bourquelot and others from glycerol and glucose by means of emulsin.

The β-glycosides of cetyl alcohol, glycollic acid and glycol were made by Fischer.

Irvine and Hynd [10] have prepared α-aminohelicin and α-aminosalicin by condensing salicylic aldehyde and saligenin, in presence of morphine, with triacetyl-bromo-glucosamine: α-aminomorphine glucoside appears as a by-product. An aminomethyl-glucoside, different from that obtained by the action of ammonia on bromo methyl-glucoside (Fischer and Zach), was prepared by the same workers from triacetyl-bromoglucosamine and methyl alcohol. Mannich also has made a glucoside of morphine.[23]

The α-phenyl glucoside behaves normally in that it is hydrolysed by maltase but not by emulsin; acids, however, hydrolyse it nearly twice as quickly as the β-isomeride.

The α- and β-menthyl-glucosides [24] have been obtained by the Fischer method. The former is very sparingly soluble in water and easily isolated, as much as 50 per cent. being obtained from aceto-bromoglucose. It is thus the easiest synthetic cyclic α-glucoside to prepare and will be of interest for physiological studies. The α- and β-menthylglucosides behave normally towards maltase and emulsin, the β-isomeride being somewhat more rapidly hydrolysed by acids.

Tetracetylgalactosidyl bromide has been used by Robertson to prepare galactosides of phenolic and terpene aglucones.[25]

α-mannosides and α-rhamnosides have been prepared by interaction of the anhydro compounds obtained from glucal and rhamnal with alcohols. Menthol maltoside was made by E. and H. Fischer.[26]

Ryan has prepared arabinose and xylose glycosides.[27]

Great interest attaches to the synthesis of the glucosides containing hydrogen cyanide. Fischer [28] has synthesised the natural glucosides derived from *d*- and *l*-mandelic acid and also linamarin, and the method can doubtless be extended to give synthetic material for the study of the many interesting chemical and physiological problems presented by these glucosides.

An example of such compounds is glyconitrile-*d*-glucoside,

$$C_6H_{11}O_5 \cdot O \cdot CH_2 \cdot CN, \quad [\alpha]_D -46°$$

which is hydrolysed by emulsin with difficulty. The corresponding glyconitrile-*d*-celloside $[\alpha]_D -29°$ is hydrolysed by emulsin with comparative ease, giving hydrocyanic acid and glucose.

Purine and Pyrimidine Glycosides, or Synthetic Nucleosides.

The synthetic purine glycosides, also prepared by Fischer,[29] may prove of medicinal as well as of scientific interest; they were obtained by the action of compounds of the type of acetobromoglucose upon the silver derivatives of the purines; glucosides, galactosides, and rhamnosides of adenine, guanine, hypoxanthine, theobromine and theophylline having thus been made. By condensation with phosphoric acid these compounds would be expected to produce synthetic nucleotides, and this was in fact attained when theophylline glucoside was treated with phosphorus oxychloride in pyridine solution, the product being a hydrated theophyllineglucosidophosphoric acid :—

$$(C_7H_7O_2N_4-C_6H_9O_5-HPO_2),\ 2H_2O$$

Purine residue. Glucose residue. Phosphoric acid residue.

The method of production of the purine glucosides has been patented by Bayer and Co.

Of equal interest are the pyrimidine glucosides synthesised by Hahn and Laves.[30] The mononucleotides, units of the nucleic acids, consist of a molecule each of phosphoric acid, a carbohydrate and a purine or pyrimidine, hence the interest in the glucosides of the latter.

Pyrimidine glucosides were prepared by condensation of the silver salts of pyrimidines with acetobromoglucose and deacetylation giving a number of glucosidic derivatives.

Synthetic Sulphur Glucosides.

Glucose reacts with ethylmercaptan [31] to form glucose ethylmercaptal—

$$CH_2(OH) . (CH . OH)_4 . CH\begin{matrix}SEt\\SEt\end{matrix}$$

which is not a true glucoside in itself, but on treatment with mercuric chloride (1 mol.) forms ethylthioglucoside. This tastes bitter, does not reduce Fehling solution and is hydrolysed by acids, but is stable to alkalis. Excess of mercuric chloride removes the second mercaptan residue; if this reaction is carried out in alcoholic solution an alkylglucoside is obtained.

Boiling potassium disulphide solution converts acetobromoglucose into the octacetate of dithiodiglucose (Wrede).[37] On reduction with zinc dust and acetic anhydride the pentacetate of β-thioglucose is obtained; diazomethane converts it into the tetracetate of β-methylthioglucoside.

Potel,[32] by condensing mercaptans with sugars in strong hydrochloric acid solution, has made their glycosides:—

$$C_6H_{11}O_5S—R.$$

Glucose *n*-propyl, *n*-butyl, *n*-heptyl mercaptans and the corresponding galactose derivatives, which are said to form more readily, were synthesised. Acids hydrolyse the glycosides back to hexose and mercaptan.

By the interaction of acetobromoglucose and the potassium salt of thiophenol, β-thiophenol glucoside,[33] $C_6H_5S \cdot C_6H_{11}O_5$, has been obtained. This is not hydrolysed by emulsin and is very resistant towards hydrolysis by dilute acids. Analogous compounds have been made by Schneider [34] and co-workers from the silver salts of thiourethanes, the products having the general formula

$$R \cdot N : C(OC_2H_5) \cdot S \cdot C_6H_{11}O_5.$$

The products are amorphous and the acetyl free glucosides very easily undergo further hydrolysis to urethanes, $R \cdot NH \cdot CO_2Et$, and thioglucose, which is readily isolated in the form of its silver salt. Decomposition also takes place in another way to form thiourethanes and glucose. This latter decomposition is met with in the case of the natural mustard oil glucosides, and phenylthiourethaneglucoside occupies an intermediate position between these and the aliphatic thiourethanes.

Myrosin is without influence on the synthetic thioglucosides.

Enzyme Synthesis of Glucosides.

The subject of the synthesis of glucosides by means of enzymes belongs properly to Haldane's monograph on enzyme action, and therefore only certain limited aspects of the question will be considered here.

Whereas in dilute aqueous solution the hydrolysis of say β-methylglucoside by emulsin is complete, hydrolysis is retarded by the presence of increasing amounts of methyl alcohol, until in presence of a certain proportion of alcohol the enzyme is able to synthesise glucoside from glucose and the alcohol. Definite proof that in this simple case the same glucoside is synthesised as is hydrolysed is afforded by its isolation in a pure state. Hence the reaction

$$\text{glucoside} + \text{water} \rightleftharpoons \text{glucose} + \text{methyl alcohol}$$

is reversible, and Bourquelot,[38] to whom the development of this subject is primarily due, has proved that the ordinary physico-chemical laws governing such reversible reactions apply here also. For example, the rates of hydrolysis and synthesis are the same and the same equilibrium is reached from both directions. The temperature limits of these reactions and the proportions of the various alcohols which can be used without destroying the activity of the enzyme have been determined. Yeast extract, i.e. maltase, effects the synthesis of α-methylglucoside.

The reaction has been extended successfully to other alcohols, the enzyme being allowed to act on sugars dissolved in alcohols containing varying amounts of water or acetone. In this way crystalline glycol, glycerol, geranyl and cinnamyl-β-glucosides and alkyl and benzyl galactosides have been obtained by means of emulsin; some of these had not been induced to crystallise when made by other methods. These synthetic glucosides are hydrolysed by emulsin. Latterly many other alcohol glucosides have been prepared in like manner, for example, those of the terpene alcohols; these, it will be remembered, are transformed into glucuronates in the animal system.

In these syntheses the enzymes are not in true solution but are acting as colloids in virtue of their surface. The work more particularly of Armstrong has shown that enzymes are very active hydrolytically, even when quite insoluble in the medium employed. Thus finely ground leaf material, prepared by protracted autolysis and frequent washing with water, and therefore divested of all soluble matter, was very active towards salicin and other glucosides.

Similarly emulsin is capable of synthesising and hydrolysing β-glucosides in a neutral liquid such as acetone in which it is completely insoluble.

As showing the applicability of the Bourquelot synthesis α-methyl-mannoside [35] is obtained by the action of an enzyme-seminase, present in germinated seeds of lucerne, on a solution of mannose in 10 per cent. methyl alcohol. This particular enzyme is the only one which at all actively attacks α-methyl-mannoside, with the usual exception of *Aspergillus niger*.

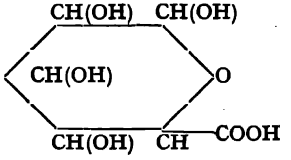
An interesting synthesis of salicin and other glucosides is that studied by Ciamician and Ravenna.[36] When plants—well-grown maize plants were chosen—are inoculated with glucosides or their aromatic products of hydrolysis, a reversible change takes place, resulting in a chemical equilibrium. Salicin is in part hydrolysed, saligenin in part transformed into salicin, the final ratio in the full-

grown plant of combined to free saligenin being 1 : 2. On taking a large number of plants it was possible to isolate the salicin synthesised in this manner. Confirmation of this work appears desirable. It is suggested that the absorption by both adult and germinating plants of certain aromatic compounds leads to the formation of the corresponding glucosides in the plants. Thus maize and beans, when treated with weak saligenin solutions, form salicin.

CHAPTER VII.

THE URONIC ACIDS.

THE general term uronic acid, first suggested by Schryver, is now given to those oxidation products of the sugars in which the primary alcohol or side-chain carbon group is oxidised to carboxyl, the pyran ring remaining intact.

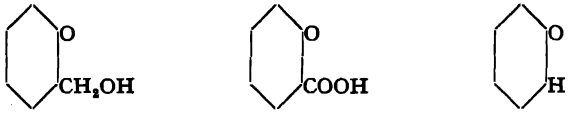


Two members of the group, glucuronic and galacturonic [1] acids are widely distributed in plants. Glucuronic acid is found in the urine, combined with a variety of substances, forming compounds of glucosidic nature. Normally glucose is rapidly oxidised in the animal organism to carbon dioxide and water. When certain substances such as chloral or camphor, which are oxidised in the body only with difficulty, are brought into the system, the organism has the power of combining them with glucose to form glucosides. In these compounds one end of the glucose molecule is shielded from attack, but oxidation takes place at the other extremity of the molecule, and a glucuronic acid derivative is formed. They are excreted in the urine. The faculty of removing injurious substances from circulation in combination with glucose seems to be common to both the animal and the vegetable kingdom, and the glycosides in the plant may be compared to the glucuronic acid derivatives in the animal. The glucuronates behave like glucosides, and form glucuronic acid when hydrolysed by mineral acids.

Uronic acids can be formed from any polysaccharides in which the side chain carbinol group is free and the potential reducing group linked. This change is easily effected in practice by oxidation with bromine in alkaline or neutral solutions. Thus lactose finally gives galacturonic acid and sucrose yields glucuronic acid.[2] Bergmann

and Wolff [3] prepare both α- and β-menthylglucuronic acids by oxidation with bromine in pyridine solution of the corresponding menthyl glucosides.

When heated with hydrochloric acid the uronic acids are decomposed quantitatively to carbon dioxide and furfural and they can be estimated in this manner. In contact with certain bacteria, glucuronic acid loses carbon dioxide and passes into xylose:—



d-glucose → *d*-glucuronic acid → *d*-xylose.

It is very probable that in the plant xylose is formed from glucose by decarboxylation in this manner, an hypothesis which is in harmony with the frequent occurrence of the two sugars together, with their association as a disaccharide in glycosides and, as in the saponins, their association also with glucuronic acid. The shoot juices of the bamboo contain crystalline xylose and glucuronic acid.

It is equally probable that *l*-arabinose is derived via galacturonic acid from galactose by decarboxylation and here again the two sugars are generally associated together in plants, as for example in pectins and gums.

Glucuronic acid, $C_6H_{10}O_7$, m.p. 154°, $[\alpha]_D$ + 36·2°, exists like the parent sugar in α- and β-modifications. The initial rotatory power is $[\alpha]_D$ + 3·6°.

In boiling aqueous solutions it is transformed into the corresponding lactone, which crystallises well. The paired acids, like the glucosides, are lævorotatory. Glucuronic acid is usually prepared by hydrolysis of glucuronates.[4, 5, 6, 7] Formerly that most employed was euxanthic acid, a substance obtained in India from the urine of cows which have been fed with mango leaves. Euxanthic acid is very readily hydrolysed by dilute acids and breaks down into euxanthone, i.e., 2 : 8-dihydroxyxanthone and glucuronic acid. Since aniline dyes have almost entirely displaced euxanthic acid from the market the latter has become very scarce. A convenient source of glucuronic acid has been found in the menthol compound obtained in the urine of rabbits after administration of menthol. The urine is extracted with ether and ammonia added, when the ammonium salt separates.[8, 9]

d-Glucuronic acid may also conveniently be prepared from gum-arabic, about 50 grams being obtained from 1 kilogram of gum.[10]

Methyl glucuronate is obtained on oxidising α-methyl glucoside with hydrogen peroxide and a trace of ferric salt.

A vast number of substances when introduced into the organism are excreted in the urine as " paired " glucuronic acid compounds. Almost every organic group yields an example. Two interesting flavone derivatives, baicalin and scutellarin (p. 25), contain glucuronic acid instead of glucose.

Galacturonic acid, m.p. 159° $[\alpha]_D = +53{\cdot}6°$, after 24 hours, is conveniently made from commercial lemon pectin.[11]

Ehrlich and Schubert[12] have prepared it in α- and β-forms from sugar beet. The α variety obtained from hot aqueous or dilute alcoholic solutions has m.p. 156° to 159° and $[\alpha]_D = +107°$, falling to $+55{\cdot}6°$ for the anhydrous substance. It gives a violet colour with magenta sulphurous acid and is considered to have an aldehydic open chain structure. The β variety obtained on boiling the α-form with a large excess of absolute alcohol and rapid evaporation of the solvent has m.p. 160° $[\alpha]_D = +27°$ rising to $+55{\cdot}3°$. It gives no colour with magenta and is considered to have the normal pyran ring structure.

According to W. Palladin and W. Lewtschenko,[13] the ethereal extract of a plant dissolved in aqueous hydrochloric acid gives a bluish-violet coloration with naphthoresorcinol when glucuronic acid is present. The reaction is given by fresh leaves of *Taraxacum officinale*, by the germinating seeds of beans and barley, by the etiolated leaves of the bean plant, and by *Aspergillus niger*. A series of other plants did not give the reaction.

Polyuronic Acids.

The alginic acid of seaweeds[14] is a polyuronic acid in which the presence of *d*-mannuronic acid residues is inferred. Mannose is a constituent of the hemicellulose of white oak and of the cellulose of coniferous woods, where it may well be present as mannuronic acid.

Orange pectin[15] contains arabinogalacturonic acid, whereas apple pectin contains digalacturonic acid.[16]

Another aldobiuronic acid has been isolated by Anderson and Crowder[17] from flax seed mucilage. It is composed of *l*-rhamnose and *d*-galacturonic acid. Cholla gum is composed of galactose, *l*-arabinose and a *l*-rhamnose galacturonic acid complex.[18] No doubt further compounds of this type will be discovered.

Apparently the specific substances of certain types of bacillus are either themselves polysaccharides or intimately associated with such

compounds. These polysaccharides on acid hydrolysis yield aldobiuronic acids. Thus the pneumococcus bacteria of type III contains glucuronic acid linked with glucose in a disaccharide.[19] What is said to be an isomeride of this disaccharide is present in the Friedländer Type A bacillus.[20] It is suggested provisionally that the isomerism is due to the point of attachment of the glucose half of the molecule.

A product of similar specific activity has been derived by partial hydrolysis from gum-arabic.[21] By the methylation method it has been shown by Challinor, Haworth and Hirst [22] to have the structure of a 6-galactopyranose glucuronic acid.

d-mannose and *d*-arabinose have been obtained from tubercle bacilli by Maxim [23] and by Renfrew [24] as well as by Anderson.[25] The occurrence of *d*-arabinose in these sources is worthy of note, since *l*-arabinose is the usual variety found in plants. Inositol is also present.

Pectins.

Substances accompanying cellulose in plant cell-walls may be divided into three classes: (i) lignins, (ii) hemicelluloses, (iii) pectins. Products belonging to the two latter classes are formed by conjugation of glucuronic and galacturonic acids with sugars. They belong to a distinct chemical group, for which the name "polyuronides" is suggested.

Pectins have a much greater uronic acid content than hemicelluloses, while uronic acids are absent from lignins. Lignified tissues contain lignins and hemicelluloses in relatively large amounts, with only traces of pectins. Non-lignified tissues, on the other hand, contain relatively large amounts of pectins, small amounts of hemicelluloses, and no lignin.

Pectins undergo decarboxylation on treatment with weak alkaline solutions, even at room temperature, yielding, among other products, hemicelluloses which still contain uronic groups. These resist decarboxylation on treatment with alkalis, and resemble in all respects hemicellulose isolated directly from timbers. These results indicate that decarboxylation takes place when plant tissues lignify.

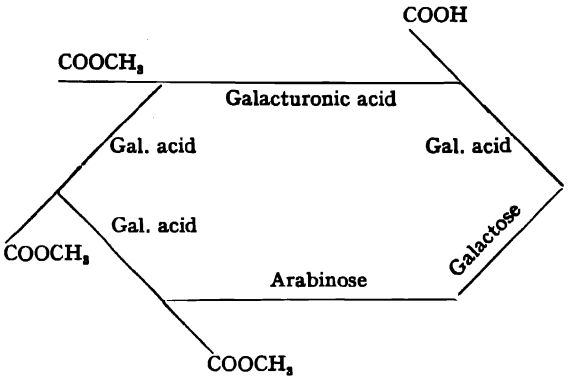
There are three pectic substances, viz.:—

(*a*) Protopectin, the middle lamella of the cell walls of unripe fruits and other parts of plants, which is insoluble and gives rise to the other forms. It is a combination pectin and cellulose, the two substances being liberated in equal proportions upon hydrolysis.

(*b*) Pectin, which occurs in ripe fruits and is soluble.

(*c*) Pectic acid present in over-ripe fruits, which is a product of the hydrolysis of pectin.

The work of several investigators, in particular Ehrlich and Schubert,[26] also Nanji, Paton and Ling,[27] whose paper gives references to the earlier work, have established that pectin is a complex, $C_{35}H_{50}O_{33}$, in which four contiguous galacturonic acid units are combined with arabinose and galactose; three of the acid groups are methylated, the fourth carboxyl group is free.



The synthesis of pectin in the plant is supposed to result from the condensation of galactose to a hexagalactan which is then oxidised and esterified.

The pectic acids, $C_{41}H_{60}O_{36}$, derived from the sugar beet and from such fruits as oranges, red currants, strawberries, etc., are similarly constituted, being built up from 4 uronic acid, 1 galactose, 1 arabinose, 2 methyl alcohol and 2 acetic acid units.[28]

From apple pectin three tetragalacturonic acids are obtained on treatment of the pectic acid with 2·5 per cent. hydrochloric acid. They probably differ in regard to the formation of internal lactones. The tetra-araban obtained from the hydropectin of beet is probably formed from the tetragalacturonic acid by loss of the four free carbonyl groups [29] as a result of enzymic processes.

According to Gabel and Kaprianov,[30] the amount of pectic acid in a number of different kinds of Russian tobacco varied from 13 to 20 per cent. of the dry weight. In the unfermented tobacco, the acid is present as the dimethylester; in the fermented as the monomethylester.

During fermentation [31] the pectin methyl-alcohol diminishes.

Von Fellenberg regards pectin as the precursor of lignin derived

from it by loss of oxygen and water. In growing twigs increase in lignin is accompanied by a decrease in pectin. Acids of the galacturonic type were obtained by Dorée and Barton Wright [32] from the stone cell material of the pear.

According to Schmidt,[33] the cell membrane of plants is made up of cellulose, hemi-cellulose and incrustation. O'Dwyer [34] has isolated two types of hemicellulose from beechwood. One of them yields galactose, galacturonic acid and arabinose on hydrolysis and the other gives glucuronic acid and xylose.

Glucuronic acid occurs in the hydrolytic products of the hemicelluloses of a number of widely different plants. Storey and Martin [35] claim to have established the presence of uronic acids in a number of soils.

CHAPTER VIII.

THE FUNCTION OF GLYCOSIDES IN PLANTS.

OPINIONS will always be divided as to the real significance of glycosides in the economy of the plant. Probably they are of use in a number of ways and no one explanation will cover the functions of all the members of the group. It must be remembered that the detailed investigation of the constituents of plants is still only in its infancy; in the main it has been confined to the detection and isolation of those useful in medicine or attracting attention as colouring matters. It is already obvious, however, that individual species have their characteristic glycosides and that the distribution of sugars other than glucose is very wide.

In most, if not in all cases, the glycosides are accompanied by appropriate enzymes which are able to hydrolyse the glycoside. Enzyme and glycoside do not exist in the same cells, as normally there is no hydrolysis. They are brought together should the cellular structure be damaged or in some instances during germination.

In the cherry-laurel, according to Guignard, "emulsin" exists in the endodermis; in the almond, it is found in the axis of the embryo in the pericycle which lies immediately under the endodermis, and in the cotyledons in both the endodermis and the pericycle. Bourquelot, who prepared both glycoside and enzyme from the stems of *Monotropa*, showed they are not present in the same cells.

The earliest investigations of this nature are due to Marshall Ward and Dunlop.[1] The fruits of the Persian berry (*Rhamnus infectorius*) contain a glycoside known as xanthorhamnin, which, when hydrolysed, yields rhamnetin and a trisaccharide, rhamninose, which further hydrolysis converts into galactose and rhamnose. The seeds contain an enzyme, termed rhamnase, capable of hydrolysing the glycoside; this is confined to the raphe of the seed, which is composed of parenchymatous cells containing a brilliant oily-looking colourless substance. When the pulp or an extract of the pericarp of the fruit is digested with an extract of the seeds a copious yellow precipitate of rhamnetin is formed.

Generally speaking, there are two types of enzymes in plants represented firstly by the typical β-glucosidase, usually called emulsin in the literature, or more recently prunase, which is widely distributed and hydrolyses all β-glucosides; by rhamnase hydrolysing derivatives of rhamnose; also by myrosin which breaks down the link between sulphur and sugar; and, secondly, by a large and increasing number of specific enzymes which in the main are limited in their action to splitting off the aglucones from the poly-saccharide glycosides which they accompany in the plant, or alternatively to splitting off one sugar molecule, leaving a simpler glycoside.

The following list indicates some only of the enzymes in question: in nearly all instances their further study is desirable:—

TABLE 9.

GLUCOSIDOCLASTIC ENZYMES.

Enzyme.	Hydrolyses.	Products.
Class I.—General in action.		
Emulsin, i.e. Prunase	Many natural glucosides Synthetic β-glucosides	β-glucose + aglucone
Rhamnase	Rhamnosides	Rhamnose + aglucones
Myrosin	Mustard oil glucosides	Glucose + sulphur aglucone
Class II.—Specific in action.		
Amygdalase	Amygdalin	Glucose + prunasin
Gease	Gein	Vicianose + eugenol
Vicianase	Vicianin	Vicianose + benzaldehyde cyanhydrin
Erythrozyme	Madder	Glucose + hydroxyanthraquinones
Primeverase	Gaultherin Spiræin Primeverin and several glycosides	Primeverose + aglucones
Linase	Linamarin	Glucose + acetone cyanhydrin
Lotase	Lotusin	Disaccharide + lotoflavin + HCN
Indemulsin	Indigo	Glucose + indoxyl
Strophanthobiase	Strophanthins	Glucose + cymarin

Emulsin from almonds, which is a mixture of enzymes, hydrolyses nearly every β-glucoside. Those instances in which a substance, thought to be a β-glucoside, is not hydrolysed require a special explanation; thus gaultherin is in reality a primeveroside and not a glucoside. Emulsin also hydrolyses β-galactosides.

The enzyme relationships of the polysaccharide glycosides are still obscure. β-glucosidase, so widely present, hydrolyses β-glucoside

junctions either of sugar to sugar as in amygdalin or of sugar to aglucone as in salicin. Such an enzyme as that in *Rhamnus utilis*,[2] which hydrolyses rutin to rutinose, robinin to robinose, xanthorhamnin to rhamninose, primeverin to primeverose, helicin and amygdalin, must surely be a complex mixture containing, like emulsin, both β-glucosidase and β-galactosidase.

In very many cases glycosides function as reserve materials, and when required by the plant they are hydrolysed by the accompanying enzyme and pass into circulation. It would appear that the glycoside stored in the seed is often of a more complex character than that present in the leaves of the same plant, containing more than one sugar or two molecules of the same sugar in its molecule, whereas the leaf glycoside is a simple one. The special enzyme required to hydrolyse it is present only in the seed and absent from the leaf. Thus the seeds of Prunus species contain amygdalin together with the enzymes, amygdalase and prunase, required for its complete hydrolysis; the leaves contain mandelonitrile glucosides and prunase but not amygdalase.

The use of the enzyme rhamnase present in *Rhamnus frangula* which splits the aglucone off rhamnosides appears likely to facilitate enquiry into cases where the rhamnose portion of a disaccharide sugar acts as the link attaching it to the non-sugar section. Examples of its use are afforded by the discovery of rhamninose and robinose.

As evidence accumulates it is a striking fact that in addition to glucose, both galactose, arabinose and xylose, several methyl pentoses and even the uronic acids are all constituents of glycosides either as simple components or in polysaccharides. Two interesting flavone derivatives contain glucuronic acid instead of glucose.

Galactose, except in the saponins and rhamnose trisaccharides, is relatively rare.

Fructose is recorded as present in a few saponins, but further proof of its identity is required. The difficulty of purifying these compounds is so great that they may easily be contaminated by other sugars.

The methyl pentoses are *l*-rhamnose, *d*-fucose, synonymous with *d*-rhodeose, and *d*-epirhamnose, the last having been so far only identified twice. Unusual sugars are digitoxose, cymarose and digitalose, and one with a branched carbon chain, apiose, the only other sugar of this type being hamamelose from a tannin. It may well be that further search when methods are available will show that some of these are of more general occurrence. Both *d*- and *l*-forms

of arabinose are found—the former only in barbaloin. The occurrence of *d*-fucose is striking; its antipode, *l*-fucose, is common in seaweeds in the polymeric form, fucosan, as a component of the cell wall.

It is attractive to speculate how these many sugars originate and why mere combination with glucose was not sufficient for the plant.

When two molecules of glucose are present, there is no evidence so far that they are united to different hydroxyls of an aglucone to form a diglucoside, though such compounds of dihydroxyanthraquinone have been synthesised. It is always assumed they are present as a disaccharide; for example, two molecules of glucose are united to form gentiobiose which is present in amygdalin and in α-crocin, and it may be surmised that when its identification is made easier, this sugar will be found to be much more widely distributed.

Glucose and xylose occur in a disaccharide primeverose; glucose and arabinose in vicianose; glucose and rhamnose in rutinose. In all these the glucose group is free to unite with the aglucone, so that the glycosides are first of all β-glucosides; in primeverose and vicianose it is the terminal side-chain hydroxyl of glucose on carbon 6 which is attached to the second sugar; this may also be true in rutinose.

Though our knowledge of the trisaccharides is vague, it is probable that here also glucose is often attached to the aglucone.

It is of interest to note how in the attachment of sugar to aglucone and of sugar to sugar the β-type of linkage prevails, whereas in those syntheses in the plant which lead to the formation of starch, dextrin, and maltose, the α-type of union exists. The production of glycosides on the one hand and of starch on the other in the plant seem to be kept strictly apart.

Other di- and polysaccharides present in plants in glycosidic combination are listed in the following table, which indicates the present state of knowledge in regard to them:—

TABLE 10.

Disaccharides present in Glycosides:—

Glucoapiose	= Glucose + apiose.
Primeverose	= 6-glucose-β-*d*-xyloside.
Vicianose	= 6-glucose-β-*l*-arabinoside.
Rutinose	= Glucose-*l*-rhamnoside.
Gentiobiose	= 6-glucose-β-glucoside.
Strophanthobiose	= Cymarose-glucoside.

Trisaccharides present in Glycosides:—

Robinose	= Galactose-rhamnose-rhamnoside
Rhamninose	= Isomeric with above.
Solanose	= Glucose-galactose-rhamnose.
Scammose	= Glucose-rhamnose-rhodeose.

The most important function of glycosides would appear to be their action in keeping dormant and unchanged substances of great importance in the metabolism of the plant until the precise moment when they are required. The greater number of the aglucones enumerated in Chapters II.–V. are substances which in their free state are liable to undergo oxidation, polymerisation or other change : combination with the sugar renders them inert and stable. The evidence that all the sensitive plant colouring matters are bound up with and protected by a number of sugar molecules is all in favour of this hypothesis.

Since any particular glycoside is only hydrolysed by its specific enzyme, the supply of these materials for whatever purpose they are required is regulated by a very sensitive control. The glycoside-enzyme systems are to be regarded as constituting a controlling mechanism for plant metabolism.

In the animal organism similar substances are converted into paired uronic acid derivatives and for the same purpose.

Glucosides may also serve as a method of putting harmful waste products out of action : thus phenolic residues are rendered soluble by combination with glucose and are transferred osmotically to other portions of the plant.

Bunge has pointed out that very many of the non-sugar constituents of glycosides are antiseptic and therefore bactericidal in character. In the seeds of plants the reserve stores of food-stuffs form an excellent medium for the development of micro-organisms which would rapidly spread but for the protective action of the glycoside. In the almond, directly the seed is penetrated, the amygdalin is hydrolysed and all bacterial action prevented. The universal presence of glycosides in the bark of plants may be similarly explained : they ensure an antiseptic treatment of all wounds in the integument.

Glycosides sometimes function as reserve materials which are hydrolysed and pass into circulation when required.

The amount of glycoside present varies considerably in different species of the same plant, and varies also according to the time of year. It also differs in the male and female plant of the same species. Unfortunately, the material at present available for the discussion of this question is very scanty. Jowett and Potter, who investigated the bark from thirty-three samples of willow and poplar, found considerable variation in the occurrence of salicin. In April the bark from the female tree contained about three times as much salicin as that from the male; three months later the conditions were reversed.

It is suggested that salicin acts as a reserve material; it is stored away in the winter for use in the coming spring, when it is hydrolysed by the accompanying ferment, both saligenin and glucose being used by the plant. The plant is enabled to store in the form of a glucoside a material which it can neither tolerate in quantity nor produce at short notice when required. Owing to their special functions the reserve is drawn upon to an unequal extent by the male and female trees. Taxicatin,[3] the glucoside of the leaves and young shoots of the yew (*Taxus baccata*), occurs in greatest quantity in the plant during the autumn and winter; apparently it is utilised in the spring when the young shoots begin to assimilate. The cyanophoric glycoside in the leaves of *Sambucus nigra*, according to Guignard, seems to fulfil a different function, as its amount diminishes only slightly with age, and at the end of the vegetative period the glycoside does not migrate to the stems but remains in the leaves till they drop off.

The variations in the composition of the root of the gentian during a year's growth have been studied by Bridel.[4] The gentian root contains a glycoside, gentiopicrin, and the carbohydrates glucose, fructose, sucrose, and gentianose, the last of which is hydrolysed by invertase. The amount of carbohydrate hydrolysed by invertase increases from a minimum (1·2 per cent.) early in June to a maximum (7·8 per cent.) in August and then remains constant. The amount of glycoside (2 per cent.) does not vary much; it increases a little in June and July. In May and June gentianose is largely replaced by gentiobiose. The sucrose increases from 1 per cent. in July to 4 per cent. or more in November: it is absent when growth commences in the spring.

According to Cavazza the amount of tannin in the leaves of forest trees reaches a maximum in September, whereas the amount in the twigs shows maxima in July and December, and varies inversely as that in the leaves.

A comparison of the amount of glucoside and enzyme in flax seeds grown under conditions of drought and high temperature with those grown under damp and low temperature conditions has been made by Collins and Blair. Under the latter conditions the total amount of hydrogen cyanide produced falls 20 per cent., but the activity of the enzyme increases by a like amount. This is the general effect of growing linseed in this country, whereas seeds of oriental origin are rich in total hydrogen cyanide.

A large class of substances which are capable of acting as promoters or hormones and giving a very delicate but directed stimulus to plant metabolism are constituents of glycosides.

Anæsthetics such as chloroform or ether are well known to have a remarkable action on plants in stimulating growth. Of significance in this connection is Guignard's observation that exposure of living plants to the action of anæsthetics brings about interaction between the glycoside and the corresponding enzyme. Mustard oil is formed in the leaves of certain cruciferæ, hydrogen cyanide in laurel leaves and other cyanophoric plants, when submitted to the action of chloroform.

The investigations of H. E. and E. F. Armstrong [5] have shown that a variety of substances, having the property in common that they have but little affinity for water, are able to penetrate the walls of certain plant cells. As a consequence variations in equilibrium are set up within the cell, and changes are induced which involve alteration of the concentration and the liberation of hydrolytic enzymes.

The general name hormone has been applied by them to substances which are active in this manner: the group includes not only carbon dioxide but materials such as hydrogen cyanide, hydrocarbons, alcohols, phenols, ethers, esters, aldehydes, mustard oils, etc., all of which are normal products of hydrolysis of the plant glycosides. The hormones include most of the substances which Overton, Löb., Czapek, and others have classed as solvents of lipoids.

The result of the liberation of enzymes within the cell will be hydrolysis of complex carbohydrates, glycosides, proteins, etc., and the materials so formed will be active in still further stimulating change. If unchecked, change will proceed until autolysis is complete: in practice the intervention of oxydases is made manifest by the appearance of brown and other pigments.

It will be seen that the plant cell carries its own hormones or activators in an inactive form bound up as glycosides. If for any reason during the twenty-four hour period a little of the glycoside becomes hydrolysed, the hormone will be liberated and a very delicate stimulus given to the cell to begin down-grade changes such as normally take place at night.

Glycosides and Animal Nutrition.

The recognition of the potent effect of the constituents of glycosides in acting as stimuli and starters of active metabolism may be of importance in studying the nutrition of animals. It is well known that the herbage of one pasture may have the power of fattening an animal, whereas similar grass on an adjoining field, though equally readily consumed by the animal, fails to bring it into condition for the market.

This is especially the case in Romney Marsh, where one field will fatten six or eight or more sheep to the acre, whereas an adjoining field will do little more than keep the sheep in a growing condition. Hall and Russell, who investigated this difference in 1912, found that the floral type in the two fields was constant but that a leafy habit of growth obtained in the fattening field and a stemmy habit in the poorer fields. The ordinary methods of chemical analysis failed to reveal any difference either in the herbage or the soils. Since this date much evidence has accumulated in favour of the importance of quality as well as of quantity in animal feeding and the subject is one of the greatest importance to agriculturists.

Subtle differences between the grasses of these two fields have hitherto defied detection, but it is not impossible that the presence or absence of certain glycosides or similar constituents may have some bearing on the difference.

Glycosides are likely to play a very important part as "test materials" in the solution of this and many problems of plant chemistry. Their non-sugar constituents can frequently be detected with great accuracy and delicacy and even localised *in situ* in the tissue, and they also can be estimated quantitatively. In this respect the glycosides which yield hydrogen cyanide on hydrolysis are of particular value, more especially as many hundreds of qualitative tests can be made in relatively short time.

In testing for cyanide it is most convenient to make use of stout glass tubes, about $3\frac{1}{2}$ inches long and $\frac{1}{2}$ inch wide, provided with good corks. The leaf material having been pushed into the tube, two or three drops of chloroform or toluene are added and a slip of moist picrate paper is inserted; the tube is then corked up. It is conveniently incubated in a waistcoat breast-pocket or in the trousers pocket. When cyanide is present the paper reddens perceptibly within half-an-hour, as a rule; to make certain, the test should be prolonged over 24 hours. To prepare the picrate paper, slips of filter paper about three-eighths of an inch wide are dipped into a solution of 5 grm. picric acid and 50 grms. sodium carbonate in 1 litre of water; after draining the paper, it is hung from a pin to dry until it is only just perceptibly moist; it is then cut up into $\frac{3}{4}$-inch lengths and stored in a closed tube. It is well to keep a piece of the paper in each of the stock of tubes carried, so as to make sure that hydrogen cyanide has not been stored up in the cork.

H. E. and E. F. Armstrong [6] have made observations on the behaviour of *Lotus corniculatus* collected during several years both over

Great Britain and a greater part of Europe. Whereas *L. corniculatus* usually contains a cyanogenetic glycoside and the corresponding enzyme, it is established that a botanically indistinguishable form exists from which the glycoside is absent.

Lotus uliginosus, which some botanists regard as a distinct species, is free both from the glycoside and the correlated enzyme: it grows, as a rule, in damp situations and is distinguished by its rank growth and coarse tubular stem. The normal form of *L. corniculatus* contains both glycoside and enzyme; a widely distributed second form is rich in enzyme but lacks the glycoside, and a third rare form lacks both glycoside and enzyme.

Lotus ranks rather as a weed than as a fodder plant and is a minor constituent of most pastures, but it is of great interest that white clover, *Trifolium repens*, shows similar differences. Two varieties are recognised by seedsmen—the cultivated and wild—of which the latter is often said to be the more valuable. The wild variety contains a cyanogenetic glycoside and the correlated enzyme, whereas the cultivated lacks glycoside and has very little enzyme.

Guérin,[7] who has examined twelve varieties of the genus Lotus, finds they all contain the hydrocyanic acid glycoside, the content of which varies widely in plants of the same species grown at different altitudes. *L. edulis* shows the highest content (in early July), *L. corniculatus* the lowest. He further finds[8] that throughout the *Leguminosæ* it may be localised in various entirely different organs. In the vetches it occurs only in the seeds; in lotus it is absent in the seeds but appears after germination in the leaf-like cotyledons and is present in the leaf stalk, the root, and the flower. In *Tetragonolobus*, *Doryncium* and *Bonjeania* it is absent from the seeds and from the cotyledons as also from the leaf stalks. In *Phaseolus lunatus*, on the other hand, it is present in both seeds and leaf stalks.

Glycosides possessing a bitter taste or having poisonous properties serve to protect such important organisms as the seeds or fruits of plants against animals. In some instances the plant is only poisonous at certain stages of its growth. Thus an Egyptian plant, *Lotus Arabicus*,[9] is poisonous in the early stages, but becomes a useful forage when allowed to mature: it contains the glycoside lotusin, which yields hydrogen cyanide when hydrolysed.

Treub regards glycosides containing acetone cyanhydrin as primary materials for protein synthesis. The occurrence of acetone in the seeds of the rubber tree is perhaps significant in view of the possible conversion of it into isoprene—the mother substance of natural rubber.

CHAPTER IX.

THE UTILISATION OF CARBOHYDRATES IN PLANTS.

THE possibility that β-glucose is the first product in the vital synthesis of carbohydrates and the mechanism of its transformation into the other naturally occurring sugars has been discussed in the earlier chapters, as well as the reactions which might lead to the formation from sugar of various aglucones. It remains to give brief consideration to certain other aspects of the utilisation of carbohydrates in the vegetable world.

Carbohydrates and the Enzyme Balance.

In dealing with carbohydrate metabolism in plants there is abundant evidence that a very delicate balance exists between the various enzymatic processes which take place simultaneously. It is obvious that the introduction from without of agencies which will affect this balance will have a more or less profound influence in altering the changes which take place.

One of the most delicate means of regulating the balance is that afforded by change of temperature. A rise or fall in the temperature does not influence all enzyme reactions alike—for example, some are retarded by cold much more than others.

A typical example is that afforded by the potato tuber during storage (Müller-Thurgau [1, 2]). Three changes take place simultaneously: starch is being transformed into sugar, sugar into starch, and also by the process of respiration into carbon dioxide. A decrease in the temperature hinders all three reactions, but it has least effect on the formation of sugar from starch. Accordingly, when the potato is stored at 0° sugar is formed till the amount increases to 3 per cent. At — 1° all enzyme action ceases. At + 3° there is still formation of sugar, but the enzymes acting to destroy it tend to keep the amount down to 0·5 per cent. At + 6° the rate of formation of sugar from starch and that of the reverse change are equal; above this temperature the formation of starch predominates. In consequence no sugar is stored and any sugar previously present is destroyed.

The effect of a further rise in temperature on the enzyme balance has not been worked out in such detail, but there is no doubt that the influence is equally profound. This conception of the regulation of metabolism affords an explanation of the sudden development of plant growth due to a warm day in spring when the rise in temperature favours synthetic changes ; or of the injury caused to hot-house plants by exposing them to a temperature colder than that to which they are accustomed, whereby an abnormal preponderance of hydrolytic activity is favoured which, if unduly prolonged, may lead to the disintegration of the protoplasmic structure and death of the plant.

In plants which are killed by frost it is supposed that as a result of the removal of the water as ice the concentration of the cell fluid becomes such that the soluble proteins are precipitated from solution. This salting out of the protein is prevented by the presence of non-electrolytes such as sugar ; Lidforss,[2] to whom this explanation is due, has shown that the leaves of winter plants are free from starch but contain much sugar. The warm days of early spring bring about the regeneration of starch and partial disappearance of sugar ; in consequence the cell is but ill-protected against the effects of a subsequent sudden snap of frost.

By keeping cut leaves with their stalks in sugar solutions for a few days, and so getting them to take up sugar, they are rendered cold resistant.

This subject has become of practical economic importance in connection with refrigeration, and it is receiving particular attention at the Cambridge Low Temperature Station.

Apparently the capacity to withstand freezing temperatures is a genetic factor, as is also the capacity in fresh fruits to tolerate for long periods low temperatures above the freezing-point.

It may be regarded as established that sugar exercises a protective action against frost injury, but whether by preventing the precipitation and dissolving action of concentrated salt solutions upon elements in the colloidal matrix of the protoplasm or by increasing the water-holding power of the system as a whole, is not clear. It must be remembered that the sugar cane and the sugar beet are notably susceptible to cold though they contain great quantities of sugar : evidently other factors besides sugar are concerned in frost tolerance. A protein such as egg yolk is protected by the addition of sugar from the irreversible effects of exposure to freezing below — 10° C.

There is some evidence that the abnormal production of galactose in plants is associated with frost. Thus Lippmann [3] records the

appearance of galactose as a crystalline efflorescence on ivy berries following a sharp frost, the first after a late dry autumn, also of crystals of fructose on some half-ripe tomatoes which had been exposed to a sudden frost after an unusually warm autumn. The disturbance of growth in the sugar beet caused by frost leads to the production of more raffinose than usual: whether in both cases this is due to the breakdown of galactans or to conversion of glucose into galactose it is impossible to say.

Newton and Brown [4] have followed the changes during the autumn and winter of wheat plants of varying degrees of hardiness by analysing both the pressed juice and the entire tissues. The most important adaption to winter is the reduction of water content which is a feature of the hardiest varieties which also showed the greatest increase in sugar concentration. There was no indication either here or in conifer leaves that pentosans played any part in frost resistance.

The Ripening of Fleshy Fruits.

In the first stages after fertilisation the changes in the young fruit resemble those in the leaf: a variety of acids, tannins, and sometimes starch then accumulates, and ultimately, as the fruit becomes ripe, carbohydrates and fruit ethers or aromatic substances are formed, and the bitter, acid or astringent taste disappears together with the starch.

The inter-relationship of the materials concerned and the enzymes which effect their transformation, possesses numerous points of interest —the scope of the present work limits discussion here mainly to the carbohydrates. A distinction has been drawn between three types of fruit (Gerber [5]) which in the preliminary stages are rich either in acids, tannins or starch: the subsequent changes differ somewhat in each type.

The three important fruits of commerce are the apple, the banana, and the orange; we shall seek to give an outline of the ripening and the changes during storage of each of them.

In the apple,[6] which when it has become an article of commerce is already in its senescence, the *protein* content reaches a maximum very early: it decreases during growth and remains practically constant during storage. The *acid* content also reaches a maximum at an early date, decreases during growth and continues to decrease during storage. The *starch* content is a maximum about the middle of the growth period and has all disappeared very soon after gathering, being transformed into sugars. The *cane sugar* is at a maximum about the time of maturity and decreases after gathering by transformation

into *hexose* sugars, the percentage of which increases during storage, especially if there is much loss of water by evaporation. The cell walls built up of *cellulose* and *pectins* are gradually resolved after maturity into their simpler soluble compounds, the process causing the softening of the fruit.

The middle lamella which cements together adjoining cell walls consists largely of protopectin, which is considered to be a combination between pectin and cellulose, these two substances being liberated in uniform proportions when it is hydrolysed. On ripening, the first change is the disruption of the union between pectin and cellulose followed by the saponification of the pectin—a galacturonic acid derivative (*q.v.*) when the tissue structure completely disintegrates.

According to Kelhofer,[7] the percentage of sugar is highest in the flesh, the acidity increases towards the centre, the tannin from the centre outwards. The distribution is the same in ripe as in unripe apples, but during ripening the amount of acid greatly diminishes.

The skin of the apple is impervious to water but is perforated by minute openings which connect with the meshwork of air channels which enables the living apple to breathe. The respiratory activity of the mature apple is only about one-tenth of that of the young fruit, at maturity it rises suddenly again to about double its rate before the change, it then decreases until the tissues die. It is influenced both by race and nutrition.

During bulk storage, the heat produced by respiration must be eliminated. On the voyage from Australia the heat generated by the apples is three or four times as great as that leaking through the insulated walls.

An elaborate investigation into the effects of climate upon quality has recently been carried out in the United States.[8] The fruit from a hundred different varieties of apples all growing on the same farm has been analysed over a period of six years. Very complete records were kept of sunshine, rainfall and temperature during the growing period of these years. In 1923 the total sugar content of the crop was the highest recorded. This year had the most sunshine.

In 1924 the total sugar content was the lowest recorded. This year had the lowest mean summer temperature, and only moderate sunshine.

The 1923 crop was of exceptional high quality as regards colour, size, and flavour, and stored well. That of 1924 season was markedly inferior in appearance, colour and quality, and broke down prematurely in storage.

The experiments afford clear proof of the potency of sunshine.

In the apple [9] at the opening of the fruit buds, the flowers are low in reducing, total, and acid hydrolysable sugars, but all increase as the flowers progress towards full bloom. The nitrogen content decreases up to full bloom.

As a typical starchy fruit the banana [9a, b] may be considered. During ripening there is an evolution of carbon dioxide and a considerable conversion of starch into sugar. Thus Prinsen-Geerligs [10] found during six days the amount of starch decreased from 31 to 9 per cent., the cane sugar rose from 0·8 to 13·6 per cent., and the invert sugar from 0·25 to 8·3 per cent. The presence of oxygen is necessary for ripening; in an atmosphere of nitrogen the starch remains intact.

A careful study of the enzymes present in extracts of bananas gathered at different stages of ripening has been made by Tallarico.[11] The catalytic enzyme which decomposes hydrogen peroxide is very active in the green fruit but weakens as it ripens. Diastase is only active in the green fruit or at the beginning of ripening, it then disappears. Invertase is absent during the green stage; the amount very rapidly increases during ripening and then gradually disappears. A proteoclastic enzyme is evident during ripening and then likewise vanishes. Maltase is not present at any period.

During ripening the skin of the banana changes from green to yellow, deep brown and finally black; the fruit is then fully ripe. This change is due to an oxydase acting on some aromatic substances liberated from a glycoside. The black colour is quickly produced when a yellow banana skin is disintegrated by mincing or when the entire skin is exposed to the vapour of some excitant. Under natural conditions the stimulus which leads to blackening is given from within the fruit by the liberation of the characteristic ester of the banana. In the case of most fruits, it would seem that the final appearance which is associated with ripeness is conditioned by stimulus from within rather than by any environmental influence.

According to Griebel [12] during the ripening of bananas the tannin-rich cell contents of the latex tubes present in the pith become coagulated, and the tannins pass from a soluble to an insoluble condition. Subsequently the latex tubes break down forming the so-called inclusion cells. These cells are found to contain acetaldehyde in an easily liberated condition. Fruit in which the normal process of ripening has been suppressed by frost is tasteless and odourless, the formation of sugar from starch and the production of amyl acetate

having been inhibited ; at the same time the fruit contains tannin in a soluble condition and only traces of acetaldehyde are present.

Acetaldehyde is considered to be a normal constituent of the " inclusion " cells in the mesocarp of fruits, and to be responsible for the coagulation and consequent disappearance of the rough taste of the tannins during the process of ripening.

Vinson [13] has found that invertase is present in the date throughout the green stages but remains in an insoluble *endo* form : during ripening it becomes readily soluble, changing to the *ecto* form. The change coincides very closely in point of time with the conversion of the soluble tannins into an insoluble form. The unripe date contains much cane sugar ; in the ripe fruit this is converted into invert sugar. Influences, such as have been considered under the name of hormones, which destroy the structure of the protoplasm, liberate the endo-enzyme, provided always that the dates have reached a certain stage of development.

The acids in fruits are chiefly malic, tartaric and citric. Gerber [5] considers that during ripening they are in part converted into sugar and in part oxidised to carbon dioxide. Temperature has an important influence on the rate of oxidation. Experiments with fungi (*Sterigmatocytis*) have shown that whereas at 12° glucose is attacked preferentially to tartaric acid, at 20° the rate of attack is equal, at 37° the tartaric acid is least resistant. Malic acid is oxidised more easily than glucose at all temperatures : fruits containing it, such as apples, can ripen, therefore, in colder climates than those containing tartaric acid, like grapes. Citric acid is still more resistant to attack, and fruits, such as oranges and lemons, require warmer climates in order to ripen.

In oranges [14] citric and malic acids are present ; during ripening the quantity at first increases but then becomes much smaller. Sucrose diminishes in amount, glucose and fructose increase.

During the ripening of sloes [15] the amount of fructose increases whilst that of glucose decreases, together with the acids and tannin ; the loss is in part due to respiration. The same authors have studied the changes in medlars and quinces during ripening.

The ripening of grapes has been studied by Copeman [16] in S. Africa. The sugar content increases rapidly during ripening but only slowly after maturity ; the acid content decreases. There was a connection between the nitrogen content and the texture but not the ripening.

In the water melon according to Ivanov,[17] glucose is formed in the early stages of ripening and is gradually transformed into fructose.

When the latter begins to preponderate the two sugars combine forming sucrose.

It is well known that cultivation increases the sugar content of fruits. Thus cultivation of the wild form of water melon leads to the appearance of sucrose.

In the ripening of cereals the object is to store starch instead of converting it into sugar. The enzymes act synthetically, and there is a gradual accumulation of carbohydrate within the endosperm tissue. The slowly matured, plump grains contain a higher proportion of starch than the small and rapidly ripened grains.

In the sweet potato, *Ipomœa batatas*, the conversion of starch into sugar is apparently connected with the cessation of the activity of the leaves, as until the stem is cut off or the tubers harvested very little sugar is formed. The transformation results first in the production of reducing sugars from starch which are then converted into sucrose. It is of interest that the change, although slower at low temperatures, ultimately goes much further, so that much more sugar is formed.

The genesis of the carbohydrates in wheat has been studied by Colin and Belval.[18] Prior to the formation of the ear the stem of the wheat plant contains only those carbohydrates coming to it from the leaf, and the ratio of reducing sugars to sucrose is much greater in it than in the leaf. From the month of June, however, as soon as the corn has shot, a change occurs. After the sugars have been extracted by alcohol from the stem a residue remains which, on hydrolysis with acid, gives a large amount of lævulose, and at the time of ripening the carbohydrates of the stem consist of sucrose and lævulosans.

Immature wheat grains contain 6 per cent. of fructosan, 1·5 per cent. of sucrose and less than 1 per cent. of a reducing sugar, these constituting all the (soluble) reserve carbohydrates (starch, 5-6 per cent.). As maturity is approached, starch is formed in increasing quantities (50-60 per cent.), the soluble carbohydrates lose their importance and their relative proportions change. The rotatory powers before and after inversion steadily increase, and the dextrose : lævulose ratio gradually approaches unity. At maturity only 0·15 per cent. of free reducing sugar, 0·40 per cent. of crystallisable sugar, and roughly the same amount of lævulosan are present.

Chemical examination of maize plants of various ages shows that the amount of pentosans in the plant increases with the total dry matter, being approximately one-sixth of the dry matter. In the early stages of growth, the production of pentosans in the plant-tissue

is greater than that of dry matter; its formation from starch, etc., is suggested. The percentage of pentosan in particular organs of the plant increases with the development of that organ. Only traces of methyl pentosans were found, but pentoses were found in small but regular quantities throughout the plant's growth. The pentosans in green corn tissue are destroyed by *Bacillus flavigina*, and also by *B. coli communis*.

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