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Chemical Plant Taxonomy

Edited by
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Preface

Systems of classification do not necessarily embody implications of relationship in their structure, but in fact, all those concerned with plants do employ such concepts to the greatest possible extent compatible with existing knowledge and practical utility. The ultimate natural system would be one based on an infallible knowledge of the genealogy, from one ancestral type, of every member included in it and, despite the impossibility of deriving such knowledge, this is the ideal towards which the more natural systems pretend. In this context, chemistry may have more to contribute than any morphological analysis, not only because of the relative evanescence of most plant tissues in geological deposits, but because the biochemistry of evolutionary processes can be deduced from existing forms.

Chemical plant taxonomy, then, although a very convenient designation for the activities which it encompasses, must not be taken to indicate that it is an attempt to classify plants solely on the basis of their chemical constituents. In fact its application has up to now usually added confirmatory evidence to agreed plant classification based on exomorphic and other characters. The use of chemical criteria, however, adds a powerful weapon to the armoury of the taxonomist. When properly applied it is undoubtedly more useful than much subjective morphology (e.g. leaf shape) although no more so than objective observations such as the numbers of a given organ. However, whereas the latter is but one parameter or "bit" of information, the total number of individual chemical compounds (including proteins and other polymers) which such an organ can contain may run into hundreds, each of which might be useful in the total description of the plant in question. Many of these compounds are so common that they have a small taxonomic value, but others, notably the so-called secondary plant products, are often restricted to certain taxa and may help to distinguish one group of plants from another. Often the variation in distribution can sharpen distinctions in indeterminate taxa, and may also disclose hitherto unsuspected relationships.

The vast explosion in the exploration of the distribution of natural products over the last 10 years is a result in part of a growing interest on the part of botanists in the chemistry and biochemistry of plants,

but more especially of the development of new analytical techniques in organic chemistry such as chromatography and ultra-violet, infra-red, nuclear magnetic resonance, and mass-spectroscopy. With the help of such methods a large number of individual compounds from any one plant can be identified unambiguously in a very short space of time.

The application of chemistry not only helps the taxonomist: the knowledge gained also stimulates the interest of the chemist and biochemist interested in biosynthetic processes. The occurrence of a given compound in one species and one of its congeners in a nearly related species often yields evidence of steps in biosynthesis which have previously been unsuspected.

This book is the first comprehensive attempt to survey the scope and usefulness of chemical plant taxonomy. Its form was determined by a Symposium held in Paris in October 1962 supported by The North Atlantic Treaty Organization. A perusal of any of the chapters will show that we are but at the beginning; that so much more needs to be done. The reasons for this are clear. With few exceptions those interested in chemical plant taxonomy are restricted either in their botanical or chemical field of activity. It is a formidable task to put together the whole of plant taxonomy and plant chemistry and emerge with an ultimate generalization. Nevertheless it is hoped that this volume will lay a secure foundation on which to build, or at any rate, begin to build a bridge between the two disciplines.

As Editor, I can only say how grateful I am to all the expert contributors who have so extensively displayed their knowledge of the subject, and especially to Dr. E. C. Bate-Smith, Prof. H. Erdtman and Prof. R. Hegnauer, whose work has stimulated so many others and to whom any praise for the usefulness of this book is due. Chapters 11, 12 and 14 are translations; they were originally submitted in French (11, 12) and German (14). I would also like to thank the staff of Academic Press for their valued co-operation.

T. SWAIN

April 1963

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CHAPTER 1

Methods of Classical Plant Taxonomy

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I. Introduction

It is difficult to give an adequate account of the methods of classical plant taxonomy in a limited space without neglecting certain fields which might legitimately be included under this title. Nor would it be particularly useful to dwell either on the factual historical background, which is in any case richly documented in botanical libraries, or on the details of the traditional taxonomist's procedure, which might be tedious and would certainly be difficult to illustrate. This chapter is therefore mainly concerned with the general practice of plant taxonomy, and with the historical background in so far as it is essential to explain that practice. Since, however, it is not possible to understand the methods of taxonomy without reference to principles or to the philosophical and general scientific background, I have thought it necessary to include such topics where appropriate.

II. Folk Taxonomy

Most text-books of plant taxonomy include a historical survey, which almost invariably begins with the botanical writings of the ancient

Greeks, especially Theophrastus and Dioscorides. Although such a starting-point is logical enough, for it is these classical writings which provided the basis for the mediaeval Herbals of Europe, it should be stressed that plant taxonomy, concerned with the recognition, naming, and classification of different *kinds* of plants, is an ancient and basic human activity arising from the very practical studies of food and medicine.

Students of linguistics and anthropology have provided fascinating examples of hierarchical plant taxonomies in the languages of primitive tribes. As an example I could take the case recently published by Conklin (1962) from the language of the Hanunóo tribe in the Philippines (Table I). In this example note, firstly, that the language provides the expected distinctions of "plant" (*v.* "animal") and "herb" (*v.* "woody plant"); secondly, that at approximately the level which we recognize as generic, there is the equivalent of a genus name "ladaq" (*Capsicum*); thirdly, that this element ("lada") operates as a generic name in the remaining classification; and, lastly, that the classification of what we would call "cultivars" of the Chilli pepper, *Capsicum annuum* L., is impressively detailed.

This example (and no doubt many others could be worked out) illuminates the background of our modern taxonomy in a way which seems to me both important and neglected. The taxonomy is called forth by the demands of practical situations. Words exist in the language to communicate gross distinctions between plant and animal, obvious distinctions between peppers and other kinds of plant, and quite subtle distinctions between agricultural "cultivars" of peppers. No clear line can be drawn between the act of naming and the act of classification, since every name implies a recognition of at least two groups (e.g. "peppers" *v.* "other plants"). The hierarchy of names arises as a response to the needs to distinguish more or less precisely for different purposes. Presumably all cultivated peppers need certain agricultural treatments; for such conversations "*lāda balaynun*" would suffice. When the relative merits of two crops are discussed, however, "cultivar names" are needed.

We should note two further points in this linguistic situation. Firstly, no explicit definitions of the terms are necessary for effective communication. Indeed, if we think about it, we all know that this is so in our everyday use of language—and if we doubt it, a moment's reflection will soon convince us of the truth. Definitions of terms become necessary in situations such as legal disputes (where, as in a recent English court case, success or failure turned on whether *Boletus edulis* "is" or "is not" a "mushroom"), or in situations where it becomes apparent that lack of implicit agreement on "what we are talking about" is vitiating an argument. It is tempting to pursue the implications for taxonomy of this

TABLE I

Hanunóo plant taxonomy (Conklin, 1962)

kāyuh (Plant)

qilamnun (Herbaceous plant)

lādaq (*Capsicum*)

lāda balaynun (*Capsicun annuum* L., chilli pepper)

lāda tirindukun-tigbayaq (*Capsicum frutescens* L.)

lāda balaynun mahārat (Houseyard chilli pepper)

lāda balaynun tagnānam

l.b.m. batūnis	*l.b.m. hapun*	*l.b.m. pasītih*	*l.b.m. pinasyak*	*l.b.m. qūtin-kutiq*	*l.b.m. tāhud-manuk* (Cockspur houseyard chilli pepper)	*l.b.t. mali-punkuk*	*l.b.t. pasītih*	*l.b.t. patuktuk*	*l.b.t. qarābaq*

view of language which has been developed in recent years (cf. Bambrough, 1961), but it lies outside the scope of this chapter. I must be content to emphasize that the *definition* of most common flowering plant genera, undertaken by Linnaeus, is similarly preceded by a long history of the *use* of these names.

The second point, of particular interest to chemical plant taxonomy, I feel, is that this primitive folk taxonomy cannot be said to be narrowly morphologically based. Indeed, it could be argued that it was a regrettable necessity which in the history of plant taxonomy forced us to base our modern classification on morphological characters. The importance of form arises from the impracticability of communicating through the written word or through illustration the description of different kinds of plants in anything other than visual terms. We stress morphology in plant taxonomy because our predecessors found it the easiest way to write and illustrate, and because by the time of Linnaeus we were so committed to it that any other way never occurred to us. I shall return to this point later, after a consideration of the taxonomy of lower plants.

I have spent what may seem to be a disproportionately long time on this single example, because I believe that it reveals to us the essential but often overlooked basis of all our taxonomic work. Classification of kinds of organisms, like all classifications, was and is a severely practical activity, and if we forget this, we find ourselves bogged down in sterile arguments.

III. Linnaean and Post-Linnaean Taxonomy

Bearing this in mind, let us look at the work of Linnaeus, who more than any other single figure made the framework of the "classical" plant taxonomy just over two centuries ago. It is tempting to think of Linnaeus as bringing order out of chaos; but it would be equally valid to stress that Linnaeus was no free agent, devising *de novo* an ideal system of classification and an ideal taxonomic procedure. On the contrary, he was severely limited (as it now appears to us) firstly by the existing classifications and nomenclature, which represented already a considerable body of work with a long history of its own; secondly by the selection of material on which that classification was based, which was mainly the higher plants of Europe; and thirdly by the philosophical and religious ideas of his time, which led him to formalize a system of *genera* and *species* derived from Aristotelian logic, and justified in terms of special creation.

Note that Linnaeus and all his "classical" predecessors from Theophrastus onwards were self-consciously making classifications, but paid relatively little attention to the question, "What is the classification for?".

Of course, the requirements of classical and mediaeval medicine in particular provided an easy justification for their taxonomic activity; and it is easy to see, in the shape of Angiosperm classifications which we are still using, that pre-Linnaean taxonomic activity had in many cases been exceptionally great where it was, for reasons of medicine or food, especially important. In this way it is, for example, possible to explain why the genera of Gramineae are on the whole smaller than those of Cyperaceae, or those of the Umbelliferae smaller than the Caryophyllaceae. The implications of this view of the shape and size of Angiosperm families and genera I have discussed elsewhere (Walters, 1961, 1962); this is not the place to enlarge upon them.

Linnaeus' answer to the question, "What is your classification for?", was simple enough. The classification revealed the Creator's plan, and the named "kinds" were created as such. This is clearly stated in "Philosophia Botanica" (1751).

> "Species numeramus, quot diversae formae in principio sunt creatae".
> "Genus omne est naturalis, in primordia tale creatum".

Both the genus and the species were to Linnaeus "natural", "real", distinctly created units. The genera he arranged in an artificial "Sexual System", making his higher groupings on the criteria of number of sexual parts in the flower. Both the fixed "binomial" of genus and species, and the artificial Sexual System, were practically successful. Here was now a workable system geared to a standard herbarium procedure, with an agreed terminology to describe morphological variation. On this basis, Floras were written with keys for identification, and the thousands of new plants from the newly explored Continents were described and classified. The herbarium method, the standardized description and terminology, the binomial, and even the use of Latin as an international scientific language have all survived intact from Linnaeus' work; only the "Sexual System" has been superseded by a "natural" system of Families.

Post-Linnaean taxonomy of the higher plants shows remarkably little change after the main modern families and genera were defined and described by de Jussieu (1789), A. P. de Candolle and others at the end of the eighteenth century, and in the first half of the nineteenth. It is fascinating to read de Candolle's excellent text-book (1819), and to find how much of it might be re-printed as a manual for taxonomists in the mid-twentieth century. He is, for example, quite realistic about the "creation" of genera. I like in particular his advice, in certain cases "to avoid useless nomenclatural changes, one should leave the genera as it has been customary to have them, and indicate divisions as simple sections".

De Candolle has clearly moved away from the Linnaean view; the species remain "fixed", although he acknowledges their variability and advocates cultivation techniques for testing the taxonomic importance of this variation; but the genera are what the taxonomist makes them.

IV. Darwin and "Evolutionary Taxonomy"

What of the impact of Darwin's ideas on taxonomy? It is commonly stated, or implied, that these were revolutionary. No taxonomist who has ever given a moment's thought to what he is doing in comparison to his predecessors can believe this. He knows that taxonomy in its broad outlines and in its more detailed practice has hardly been affected by evolutionary ideas. There is a curious dishonesty about this in much biological writing. Either the fact is denied, or it is glossed over, as if it is something to be ashamed of. A recent paper by Blackwelder (1962) takes to task the zoological taxonomists for not facing the fact squarely; it is refreshing and entertaining, and ought to be widely read. Of course it is true that the language used in much peripheral taxonomic writing has changed since Darwin. Where the pre-Darwinian botanists talk of "affinities", the Darwinian substitutes "evolutionary relationship"; where the pre-Darwinian might have drawn a diagram of the *Scala Naturae*, the Darwinian draws a "family tree". But shorn of these trimmings, a monograph by, say, Engler or Hallier from the second half of the nineteenth century is in no significant respect different in treatment from one by, say, Lindley or Bentham of pre-Darwinian date.

There is nothing in this situation of which the practising taxonomist need feel ashamed. It simply means that his activities are essential to the science as a whole, in that they provide a general framework of reference; it is neither practically possible nor theoretically desirable to alter the methods of classical taxonomy because we now have a fundamentally different picture of the origin of the diversity of the organic world from that of Linnaeus. The post-Darwinian mistake, of course, is to equate a natural classification naïvely with a phylogenetic one. This is a large subject, and one which lies somewhat outside the scope of this chapter; perhaps I should, however, make my own view clear. It is briefly this. Natural classifications, erected on the sum total of resemblances or differences in an indefinite number of characters, were made by taxonomists long before evolution was ever talked about. Such classifications can be made of any group of objects, whether living or not, which exhibit significant character correlations. They contrast with artificial classifications, usually erected for a specific purpose, which select a single criterion or group of criteria on which to base their groups. In this sense,

Linnaeus' "Sexual System" is artificial, and de Jussieu's "Natural Orders" are natural. The writers on logic have always made this clear (cf. J. S. Mill, 1843, quoted in Walters, 1962); it is a pity that so few taxonomists have shown much interest in logic or philosophy. For a recent statement, see Gilmour (1961).

What Darwin was concerned to show, was that the evolutionary process "explained" the previously mysterious "natural groups". In other words, the possibility of making a generally-agreed natural classification of organisms depends upon the process of evolution. This is emphatically *not* the same thing as saying that any and every natural classification must be phylogenetic. The relationship between the actual course of evolution and the patterns of organic variation to which it is the aim of taxonomy to provide a map is in itself a fascinating and complex study; the problem is only obscured and confused by a naïve equation of "natural" and "phylogenetic".

V. Relationship

The prestige of evolution as a new, invigorating idea in biology was undoubtedly responsible for the way in which this confusion has been written deeply into biological literature and into the thought-processes of biologists. Nowhere is this more evident than in the use of the overworked term "relationship". We all say: "X is related to Y". As the logician or mathematician would use the phrase, it is so general that it may tell us nothing useful without further qualification. A moment's reflection will convince us that *some* relationship could be said to exist between any and every pair of objects—if it is only that we are at that particular time thinking or speaking of them together. In the parlance of classical taxonomy we say "X is related to Y more closely than to Z" when we are impressed by the total resemblance, with respect to the characters we are using, between X and Y, and we are less impressed by the resemblance between X and Z. It is obvious that this, the taxonomist's "judgement of affinity", contains a large subjective element, and introduces the complex problem usually referred to as the "weighting of characters". Some characters, we say, are more important, more "fundamental" in taxonomy than others. Superficial resemblance is not to be confused with "real relationship". An oak tree (*Quercus*) may have large lobed deciduous leaves like other "unrelated" tree genera in North Temperate regions, or small simple evergreen leaves like other "unrelated" tree or shrub genera in the Mediterranean region. Both are *Quercus* species, closely "related" by their extremely similar flowers and fruit.

Such cases are easy, and have long been recognized in the classical taxonomy. We should, however, realize that a graded series of cases could be made, from ones in which "superficial resemblance" is recognized by all taxonomists to ones in which there is no consensus of opinion as to what is a "superficial" and what a "fundamental" similarity. Certain *a priori* principles have operated in classical taxonomy, and we still (perhaps only half-consciously) use these today; but we are rightly unsure of the theoretical basis for our choice. The problem is a severely practical one in cases where taxonomic structures must be devised *de novo*; it is less urgent where, as in Angiosperm taxonomy, the decisions were made for us centuries ago!

In this situation, it is at least encouraging to find that practising taxonomists are prepared to drag the problem out into the open and look at it, or even to submit their own taxonomic procedures to careful scrutiny. Two recent papers, one by zoological taxonomists (Cain and Harrison, 1958) and the other by a mycological taxonomist (Munk, 1962), might be read with interest by all their fellow-workers. The most obvious recommendation which I should want to make in the present situation is that in our published works we should cease to use the word "relationship" in the unqualified form at all, but studiously write "morphological resemblance", "genetic similarity" or whatever it is we are pointing out. It is emphatically *not* good enough to defend the use of "relationship" in taxonomic contexts because it means "evolutionary relationship". Quite apart from the fact that the context usually shows that the criterion is morphological resemblance, and that no data on phylogeny are available, the term "evolutionary relationship" itself will mean different things to different people (e.g. a geneticist and a palaeontologist).

VI. The Practice of Taxonomy

I should like to turn now to a consideration of the methods, in broad outline, which the practising taxonomist today has inherited from the past and is still using. This activity can be conveniently divided into three kinds: identification, revision and publication. (I am, of course, ignoring the many routine tasks concerned with the upkeep of large reference herbaria or museum collections, which need not concern us here.) His fellow-scientists and the general public will expect him to be able to identify plant material; that is, to tell them what name they can use to refer to the material or to find further information about it. His problem is to say what the specimen X is "the same" as—to *identify* it *in the existing classification*. His necessary equipment for this includes the following: Floras and Monographs, which are works of identification

and description written in technical language, originally and still basically in Latin; reference specimens preserved in herbaria or museum collections; and simple equipment for the study of detailed morphology and anatomy.

Now it could plausibly be argued that the identification of plant material should be organized with an apprenticeship system, like, for example, the training of garage mechanics. To some extent this is indeed what happens. The mysteries of the craft are transmitted from the experienced worker to the novice in institutions of remarkably standard pattern throughout the world. It always seems to me quite extraordinary that, notwithstanding the differences in language, history, and customs, what is happening inside a large herbarium is obviously the same kind of activity whether it is in Leningrad, Edinburgh, or, I expect, Peking.

The second kind of activity, namely revision of existing taxonomic work, or taxonomic research in groups where the existing information is obviously inadequate, cannot of course be sharply distinguished from the first. The attempt to identify a plant may quickly reveal the uselessness of the existing literature or collections. What happens then is unpredictable. Our taxonomist may be able to say what the genus is, but can only write "? sp" or "sp. nov.", and there the matter may rest. He may fail to identify the genus after exhaustive efforts, and create a new genus, but such situations are rare in higher plant taxonomy in the mid-twentieth century. He may even be stimulated to try his hand at revision of the group concerned.

If there is one thing on which most professional taxonomists would agree at the present time, it is surely that revisions or monographs on a sufficiently large scale are urgently needed, and yet are being produced at a dismally inadequate rate. There is no easy answer to this problem. My own view is that the difficulties are aggravated by the tendency of professional taxonomists to surround their activities with spurious "mystique". There is nothing to the game which a reasonably intelligent student interested in plants could not pick up with a few years' training and practice. "Taxonomists are born, not made" is a very dangerous slogan!

This brings me logically to publication as the third activity of our taxonomist. Taxonomy is, or ought to be, a practical activity. A large herbarium should be concerned, not only with routine identification, and with revision and research, but also with making available the information already theoretically in its possession. I say theoretically, because it is depressingly true in taxonomy, as in so many other branches of science and learning, that vast floods of information pour out in learned journals, in the reports of applied Institutes, in published theses, and it is

the ordering of this information which is the peculiar problem of our time. Here it seems to me that the taxonomists will have to put their houses in order in the next ten or twenty years. The leisurely pace of the herbarium suited to the Linnaean age simply will not do in the mid-twentieth century. However nostalgic we may feel when we look back to the apparently quietly ordered lives of our illustrious predecessors, we shall have to come to terms with reality and modernize our techniques and methods.

VII. Developments in Taxonomic Practice

This is a paper on classical taxonomy, and I cannot spend much time on pipe-dreams of the future. I should, however, like to make one or two suggestions. The first one concerns what the fashionable jargon now calls "data-processing". Taxonomy, as I see it, is data-processing for biology. It receives an enormous number of pieces of information—the specimen has five petals: it came from 3500 ft on Mt. Athos, etc.—then attempts to arrange them into a system, and to make them available again in published works in various combinations. Now the efficient mechanization of such activities is already carried through in all sorts of business and research organizations. There are no difficulties in devising a scheme to reorganize the traditional taxonomy along such, to us practising taxonomists, quite revolutionary lines. In fact, of course, it will be difficult to do; partly because we all obscurely resent being "pushed about by machines", and partly because of the sheer inertia of the existing system.

Perhaps the most hopeful sign in this situation is that a number of people are already working on what might be called pilot projects of the mechanization of taxonomy, and amongst these schemes we can undoubtedly expect to see something of the shape of things to come. I might refer, for example, to the International Plant Index scheme sponsored by the National Science Foundation and the New York Botanic Garden (see Gould, 1958, 1962). In England our own mechanized scheme for the collection of distribution data on the British Flora, which has recently appeared in Atlas form (Perring and Walters, 1962), has given valuable experience on a straightforward data-processing problem involving about 1,500,000 "pieces of information".

The second point relevant to the position of classical taxonomy today concerns the use of quantitative and biometric methods for the definition and description of taxa. It is often claimed that there is an important practical difference between pre-Darwinian and post-Darwinian taxonomy which arises from the abandonment of the idea of a

species as a fixed, created unit and its replacement by the idea of an interbreeding population varying in space and time. This difference, it is said, expresses itself in the use of ranges of measurements of particular characters in specific descriptions, or in more sophisticated statistical treatments of taxonomically significant variation. Such aspects are treated in the next chapter; it must suffice to say that the development of quantitative taxonomy can certainly be traced well into pre-Darwinian times, and to emphasize that none of the great taxonomists were ignorant of variation within species, nor did they obviously avoid describing it. Perhaps it would be more relevant to point to the very late development of statistics (which, like the cytogenetic studies it made possible, is an almost wholly twentieth-century growth), and to ask whether this was not the limiting factor to the growth of quantitative and biometric taxonomy.

It is certainly true that species descriptions in modern Floras do, on the whole, show a greater recognition of possible ranges of variation in measurable characters. It is also true that this tacit recognition of the variability permitted to a species fits uneasily in a system which retains, for nomenclatural purposes, the legal fiction that a species is represented by a single "type specimen". I do not find, however, that this situation makes practical difficulties; it is only dangerous if it is not recognized and understood. The important question is not: "Does *Bellis perennis* still mean the same thing to the taxonomist in the twentieth century as it did to Linnaeus?"—for to that question both "Yes" and "No" are correct answers—but rather "Does the taxonomic and nomenclatural framework we have inherited work reasonably well to provide a map of variation and a reference-system?" To this question in general I submit we can only answer "Yes", at least so far as the higher plants are concerned.

It is, however, legitimate to speculate on how far the statistical description of taxa might be taken. It is not too difficult, for example, to devise a numerical system of describing plants which could yield a formula unique for each recognized taxon, and there have been many suggestions along these lines (e.g. Rabel, 1940). What would be fantastically difficult would be to apply it consistently to the taxonomy of any sufficiently large group to make it possible to replace the existing language descriptions. Nor is it at all clear what useful purpose would be served by doing this. "Translation" back into words would still be needed for all ordinary users of the taxonomist's products. Yet "numerical taxonomy" will obviously attract active workers who are understandably critical of the existing structure and methods, and electronic computers now enable us to carry out operations of the kind needed in such studies within a reasonable time. (Sneath and Sokal (1962) outline some possible developments in this field.)

VIII. Taxonomy of Lower Plants

So far I have confined my attention to the flowering plants. This is justifiable, because it is obvious that classical plant taxonomy was almost wholly concerned with the dominant group of the Plant Kingdom. A few remarks on the taxonomy of the lower groups would, however, help to give a more balanced picture and at the same time provide further illustration of the essentially practical nature of taxonomy.

De Jussieu's "Genera Plantarum" (1789), which classifies the whole Plant Kingdom into 100 "natural orders", provides only five orders for the whole of the lower plants, viz. Fungi, Algae, Hepaticae, Musci and Filices. The taxonomy of the Fungi and Algae is, as might be expected, hardly recognizably developed. It is a nineteenth-century growth, into which a great deal of painstaking microscopic investigation was directed. The results can be conveniently seen, for example, in Berkeley's "Introduction to Cryptogamic Botany" (1857), in which the description of the morphology of many Algal and Fungal genera is combined with a recognizably modern framework of classification. The compound nature of the Lichens is not yet recognized, though they are well described and classified. Hofmeister's important work on the alternation of generations had just been published, and Berkeley discusses the homology of Coniferous pollen and *Selaginella* spores.

Berkeley devotes some pages to the defence of the study of lower plants, against those who "may be inclined to think that cryptogamic botanists are less honoured than is meet". He gives several reasons why the study is worth while. Firstly he says they are fascinating and often beautiful microscopic objects; but couples with this a stern warning to the investigator that "if beauty of form and singularity of structure be alone his object, his time may be passed agreeably enough, but in most cases, like ten thousand microscopists of the present day, he will be but a mere trifler, without any better aim than innocent amusement". One is tempted to say to this: "What better aim could there be?" It seems to be true that the fascination of microscopy as a hobby played a very important part in the development of lower plant studies; and it is still true that, in Bryology and Algology especially, amateurs often have a most detailed knowledge.

Berkeley's second reason for the study of cryptogams is perhaps less expected. It is that in their simple cellular structure the physiologist may study processes such as growth and reproduction far more easily than in the complex flowering plants. This has certainly proved to be the case in much modern plant physiology. The third one is even less expected; so many fossil plants are "related to the nobler Cryptogams" that their

study is peculiarly interesting. Reading this passage it is difficult to believe that it was written before the "Origin of Species". Finally, he produces the economic reason: that "so many of the diseases, both of plants and animals, arise from their presence". It is, of course, this enormous economic importance in the study of plant diseases in particular, which led to the great expansion of mycology and plant pathology in the century since Berkeley wrote, and which made detailed Fungal taxonomy a practical necessity.

One feature of the taxonomy of some lower plants which makes the subject of particular interest to chemical plant taxonomy is the relative importance of chemical and other non-morphological characters. The Lichens are especially interesting in this connection. Many Lichens have been used traditionally as a source of dyes, and were therefore perfectly well distinguished in the vernacular languages in Europe. They, and other relatively large Lichens resembling them in general form, were therefore classified into genera and species with little difficulty and no hesitation. Apparently some 800 species were described before the end of the eighteenth century. Their structure was, however, very imperfectly known, and still less understood. Similarly many of the higher Fungi, of such great importance for their edible or poisonous qualities, were known and described early; in the recognition of these many characters other than form were naturally employed. This is still true in modern taxonomic works on Fungi and Lichens. In these groups the complete dependence on the herbarium method was never established, for obvious reasons, and characters not preserved in the dried specimens were not automatically excluded by the rules of the game.

Independence of taxonomy from morphological distinctions is perhaps at its most extreme in the classification of the Bacteria and Viruses, where characters such as the reaction to chemical compounds or differential growth on particular media may be used. It is remarkable that even here the classical taxonomy and the Latin binomial have been imposed, and it is not surprising to find more serious questioning of the usefulness in such groups of a classical hierarchical taxonomy as used for Angiosperms.

A lesson which could be drawn from the survey of the taxonomy of the lower plants is that biochemical, physiological or other characters can obviously be used to make useful classifications, and that the interest of the scientist and the potential user of the taxonomy will inevitably determine their shape. The most satisfactory "natural" classification will reveal the maximum number of significant correlations between apparently unconnected characters. Thus we may take a familiar example which has embedded itself deeply into the classification of the Algae, the

differences in pigment responsible for the distinction of Green, Brown and Red Algae. These, appreciated as colour differences, showed a rough correlation with the type of reproductive structures, and most of the early nineteenth-century Algologists accepted these broad divisions. We can do no better with a century of further investigation.

IX. "Omega-taxonomy"

I feel that I must conclude this chapter with a brief reference to the concept of a single perfect natural classification—the "omega-taxonomy" of Turrill (1942). It is probably true to say that the majority of taxonomists envisage the gradual "improvement" of their natural classifications, by the inclusion of more and more data, towards a final goal. This goal is often equated with the "phylogenetic classification". I see no reason to suppose that our taxonomies will inevitably gradually improve in this way, and I cannot conceive of a single "omega-taxonomy" as a goal. The justification for including new data in taxonomy—be it chemical, cytological, genetical or of a type as yet unimagined—is that botanists as a whole will be interested in the correlations of characters so revealed and the generalizations which it becomes possible to make. Students of evolution are amongst these botanists, and the pattern of correlated variation will naturally have a special interest for them. Their interests are, however, sectional, like those of the developmental morphologist or the biochemical geneticist. The classical taxonomy still serves them all.

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CHAPTER 2

Species Concepts: Theoretical and Practical Aspects

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I. Introduction: The Word "Species"

"Species" belongs to that valuable but troublesome category of words that defy formal definition while discharging an important function in the communication of ideas. Such words have something of the quality of pronouns—on their own they are little more than neutral symbols: in an intelligible context they become suffused with a meaning which may be highly precise. It is to this property of the word itself that we owe no little part of the age-old controversy about the nature of species. "Species" have no "nature" other than we care to give them, and there can be no agreement between those who insist on employing the word in different senses. If different usages are adopted by intent, then the reason for disagreement is at least recognizable; but if, as usually happens, the word is allowed to take up meanings from several different private and undisclosed contexts, the genesis of the resultant chronic discord will be obscure even to the disputants. The greater part of the "species problem" is in fact one of definition—not of the word, but of the ways it may appropriately be used in the different contexts in which its use is deemed desirable.

It is the purpose of this chapter to discuss some of the contexts in

which the term species is used in biology. The focus will be principally upon the species as a taxonomic category, and the relevance to this classical usage of present-day knowledge concerning the nature and origin of variation in plant populations.

II. The Taxonomic Species and the Classificatory Process

The taxonomic system in current use has been inherited with no more than modest changes from Linnaeus. Its main characteristics, and in particular the hierarchical structure, were determined by philosophical ideas current during Linnaeus lifetime (Cain, 1958), and its stabilization of a nomenclature based upon generic and specific epithets represented a fitting into a philosophically respectable framework of a convenient naming method already in sporadic use in Europe at least from the time of Bauhin, more than a century before the publication of "Species Plantarum". Whatever may be said now in criticism of the Linnaean system, it is inconceivable that it should be displaced in the near future as the main reference framework for the higher groups of organisms; too much intellectual capital has been sunk in it, and in any case it may be doubted whether any other form of investment would yield better returns in the form of service to biology in general.

The function of the current version of the classical system is guarded by the "International Codes of Nomenclature". The important passage in the "Botanical Code" (1956) governing the use of the species category are Articles 2 and 23. Article 2 states that "Every plant is to be treated as belonging to a number of taxa of consecutively subordinate ranks, among which the rank of species (*species*) is basic", and Article 23 contains the sentence, "The name of a species is a binary combination consisting of the name of the genus followed by a single specific epithet".

The taxonomic species is thus the category to which all plants must be referred for the purposes of naming and classification. This is all the definition of it possible, or indeed required; and the nomenclature committees of successive International Congresses have wisely re-asserted the principle that it is no part of their duty to attempt any biological definition of this or any other taxonomic category. The strength of this particular species concept lies in the fact that it predicates no special kind of variational unit, since the absence of any rigid specification permits Linnaean binomial nomenclature to be extended to all groups. Or perhaps it might be more logical to say that because it is generally agreed that the binomial system *should* be extended to all groups, the necessary corollary is that the species category to which it is applied cannot be defined exclusively in terms of the variation pattern in any one group.

These considerations suggest that in the taxonomic context the question, "What is a species?", is scarcely relevant, since it cannot be answered in any generally useful manner. We can, however, substitute another which certainly is relevant: namely, "What kinds of variational unit are most conveniently named as taxonomic species?". Provided that we can say what properties we require of taxonomic species, this question is obviously of a form permitting an answer, even if in any particular group under treatment that answer should turn out to be "None".

What, then, are the properties required of a taxonomic species? This question can scarcely be considered without some reference to the philosophy of classification in general. The essence of the Linnaean method is the establishment of a hierarchy of classes, those of each rank grouped to form those of the rank above, the basic one being the species, to which the individual organisms are referred. This classificatory process, like all others, has the primary function of permitting inductive generalizations about the classes created; in simpler terms, it is a method of ordering otherwise chaotic data and establishing regularities for the purpose of facilitating human thought (Gilmour, 1941, 1951).

The basis of class formation must, of course, be the assessment of similarities and differences between the objects classified. There is nothing in hierarchical classification as such which demands that the criteria of likeness should be of any particular kind, morphological or otherwise; nor is there any implicit requirement for the numbers of criteria adopted, few or many. It has, however, been traditional for nomenclatural taxonomy mainly to adopt morphological characteristics, although these could be drawn from any phase of the life-cycle; and it has long been accepted that the best taxonomic practice requires the utilization of as many features of an organism as should prove to be feasible. The distinction between so-called "artificial" and "natural" classification lies in the way criteria are employed. The description *artificial* is applicable to any classification based upon resort to one or a few criteria as discriminants which happen to provide a ready means of subdivision (as in the construction of keys). *Natural* classification in the general theory of taxonomy is, by contrast, based upon overall resemblance, or, we may say, upon the maximum correlation of attributes. Whereas an artificial group is definable by particular characteristics possessed in common by all its members, a natural assemblage need not owe its unity to the possession by *all* members by any common character, but to the fact that any pair show more characteristics in common than would either with one from another such assemblage (for further discussion of these points see Bather, 1927 and Heslop-Harrison, 1962).

Although it can be shown that Linnaeus himself did not employ the

designation "natural" in the above sense (Cain, 1958), it is natural assemblages of this kind which he and subsequent systematists have tended to form when grouping by overall likeness. And it is important to note that this is true at all levels of the Linnaean hierarchy, including that of the species. Traditionally, the taxonomic species has been composed of individuals placed together because of overall likeness. A degree of morphological homogeneity is therefore the first requirement; any individual of a species must have more characteristics in common with any other than would either with an individual of a different species.

The second requirement is related but not identical: namely that a species should be distinct as a group from others. Linnaean species were mostly of this kind, at least as they were known to him; and a search for discontinuities by whereby the "boundaries" of a species may be marked is a long-accepted part of taxonomic practice. Distinction in this sense is not to be confused with "degree of difference". The emphasis is upon the constancy of the discontinuity, and not upon its magnitude.

The third requirement is that a taxonomic species should have some degree of persistence in time. The "fixity" of species in this sense was part of the Linnaean dogma, and it is obvious that only if the variational units named as species do have some continuity in time can Linnaean-style classification have any lasting validity. This requirement had been appreciated by pre-Linnaean systematists, especially Ray (1686); and de Candolle (1819) in the post-Linnaean, pre-Darwinian period recognized some of its implications—for example, that species diagnoses must accommodate all phases of the life-history and allow for fluctuating environmentally induced variation.

It may be useful to reiterate at this point that the properties just set out—overall resemblance of the constituent individuals; distinction from other groups of the same kind; and persistence in time—are those demanded of variational units which would completely and unequivocally fulfil the requirements for a species in Linnaean-style classification. The question, as we have seen, is whether such units are to be found among organisms in nature. It is important to note that it was largely the demands of hierarchical classification itself that established this set of properties for the taxonomic species; it was not that a prior understanding of the nature of organic variation led to the design of a suitable classificatory system. Yet the history of taxonomic practice shows that workers have again and again irrationally assumed that assemblages possessing these attributes *must* be present in all groups. Now it is entirely possible to refute such an assumption without at the same time asserting that there are not, in some groups, variational units which do, more or less, reveal these properties. Darwin has been criticized for his famous

statement in "The Origin of Species" that "I look at the term species as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other" on the grounds that there are groups in which species do have an "objective" reality; but the context of the quotation reveals that he was in fact referring to the practical question of the use of the species category in systematics ("Origin", Edn. 6, 1872), and it is arguable that what he regarded as arbitrary was the imposition of species subdivisions in groups where the variation pattern did not submit readily to such treatment.

That the requirements of Linnaean-style classification have generated a "species concept" is scarcely to be doubted; it is here that we can discern the origin of the *morphological species* discussed by Mayr (1942) and others. Linnaean methods have met with reasonable success over two centuries, and this itself suggests that a good deal of biological variation can be fitted into a framework founded upon this kind of species concept. For the great systematists of the nineteenth century, the recipe for success lay in a synthetic approach to the business of species-making (see, for example, the essay of Bentham, 1874). Their work has lasted because they maintained a balanced view of the relationship of "similarity" and "difference" in application to organisms, avoiding excessive subdivision. Further, we may see in the work of such men as Wettstein a growth of the idea that distributional data are significant in the assessment of taxonomic relationship, and with it a shift of emphasis from individuals to populations.

The morphological concept of the species thus became refined in the hands of skilled systematists by the addition of mitigating considerations. The Linnaean method lends itself to debasement, however, as well as refinement. An aspect may be seen in the philosophy of typology. The idea of a "type" is itself confused, and some clarification is therefore necessary. The *nomenclatural type* of a taxonomic species is the individual specimen of it to which the binomial must remain anchored; it is a practical device intended to assist in stabilizing nomenclature and does not concern us here. In general usage, "type" can assume a different meaning. In relation to a species, the *typical individual* might be that which an author supposed to be modal, carrying all the characters regarded as essential in his species diagnosis. In its naïve form, this kind of typological thinking promotes a serious abuse of the Linnaean method, since it leads to the remorseless pressing of the search for difference, and so ultimately to the meticulous description of individuals as the basis of species diagnosis. The inevitable outcome is the endless multiplication of binomials as finer and finer differential characteristics are located.

At a higher level of sophistication, typology leads to the concept of the

archetype of a taxon (Danser, 1950), a hypothetical reconstruction of the organism in which every characteristic is represented in its supposed most primitive state. Whatever merit this conception of a ground-plan may have in relation to the higher classificatory categories (see discussion by Lam, 1959) it would seem to have little or no contribution to make to the rationalizing of taxonomy at a sub-generic level.

III. The Taxonomic Species Category in Application

A. Biological Qualities of "Good" Taxonomic Species

Although in classical taxonomic practice morphological affinity formed the principal reason for grouping individuals together in species, a distinctive flavour was early imparted to plant and animal taxonomy by the principle enunciated by John Ray that species should "breed true". Living, reproducing, and dying are distinctive biological properties, and irrespective of how much we may wish to assimilate the taxonomy of organisms into the general theory of taxonomy, we are faced with the need to set it somewhat apart since its subjects are not static and inanimate. During the early years of last century, through the development of ideas about variation derived both from formal systematic studies and field observation, other considerations were added to that of Ray, and a conception of the biological properties of the "good" Linnaean species among sexual organisms gradually took form—a conception which, it may be noted, owed nothing at all to the theory of evolution. In his "Introduction to Botany" of 1832, Lindley wrote: "A species is an assemblage of individuals agreeing with each other in all essential characters of vegetation and fructification capable of reproduction by seed without change, breeding freely together and producing perfect seed from which progeny can be reared. Such are the true limits of species. . . ."

Lindley is here making the discovery that in sexual groups the entities classified by systematists as species have biological qualities other than the morphological resemblance of the constituent individuals, and, at the same time, is hinting in the use of the word "true" that these properties are to be considered in some sense definitive. Mayr (1957) has listed early zoological authors who formulated similar ideas.

The time sequence in the growth of appreciation of the biological nature of classificatory species in sexual groups is significant. Mayr has pointed out that virtually all the early systematists seem to have looked upon the species as an aggregate of individuals, unconnected except by descent. Yet, by applying the principle of grouping by likeness in the framework of the Linnaean taxonomic system, they successfully defined entities, the biological unity of which was only later fully to be appre-

ciated. That this should have happened is perhaps not too surprising; after all, as several authors have commented, when primitive tribes name the organisms in their environments, they are almost as successful in defining species limits as a trained taxonomist, and they certainly work primarily by assessment of likeness. But what merits attention is that the method brought to perfection by Linnaeus, a method with roots in Aristotelean philosophy, met with reasonable success in many sexual groups only because suitable variational units happened to exist in such groups. The success of the method might almost be said to have been fortuitous, were it not for the fact that had early trials been unsuccessful, other systems would have been evolved.

B. The "Biological" Species

Increasing understanding of the causes of the patterns of variation found in sexual groups, aided in the last few decades by a transfusion of ideas from population genetics, has led to the formulation of a theoretical basis for the so-called *biological species*. This concept has been developed most fully by Mayr (1940, 1942, 1948, 1949, 1955, 1957), whose writings on the topic merit attention from all biologists. Mayr's statement (1940) may be taken as typical of the kind of species definition to which this concept leads: "Species are groups of actually or potentially interbreeding populations which are reproductively isolated from other such groups."

We owe to Mayr himself the important observation that all species concepts of this kind are dualistic. They incorporate, either obviously or in some concealed manner, criteria of two different kinds, and in consequence are neither as simple to comprehend as they may at first appear, nor necessarily as useful as a guide for practice.

The dualism in the above statement is obvious; on the one hand there is the criterion of reproductive isolation, and on the other the idea of collectivity, inherent in the phrase, "groups of actually or potentially interbreeding populations". Reproductive isolation is, of course, the factor which determines that, in any one locality, populations of different biological species shall maintain their identity; in the genetical jargon, that their "gene pools" shall not run together. It hardly needs emphasis that what matters in this respect is the situation in nature, not what can be brought about in the laboratory or experimental garden. It is not therefore to be supposed that experimental tests for interfertility can provide an unequivocal means of species delimitation. The revelation of an innate barrier to miscegenation would certainly mean that a species "boundary" had been located, but the experimental demonstration of

interfertility would by no means prove that two groups shared a common gene pool in their natural environment when the possible barriers to gene exchange between wild populations are so many and so subtle (Heslop-Harrison, 1955).

The idea of collectivity contained in biological species definitions brings its own difficulties, and yet it is indispensable if the concept is not to be reduced to what Mayr has called the *non-dimensional species*—one which can be recognized in one place and at one time simply because it coexists with others but remains independent from them (Mayr, 1955). The principal difficulty lies in the idea of *potential* interfertility between remote populations. Once this is introduced, the question arises as to how it is to be assessed. Obviously all the objections to the experimental gauging of the interfertility of sympatric populations apply also when allopatric populations are concerned—and more so, since at least when populations are sympatric there is the hope that ecological study might reveal the factors which restrict gene-flow between them.

Acknowledging the impracticability of employing experimental tests, Mayr (1942) suggests that "the conspecificity of allopatric forms . . . which depends upon their potential capacity for interbreeding, can be decided only by inference, based upon a careful analysis of the morphological differences of the compared forms". Like the species of classical taxonomy, the biological species is thus a classificatory unit, an assemblage based upon correlation of attributes, not one to be defined or circumscribed by any single objective test.

We may reasonably enquire what has been the value of the development of ideas about the biological species if in the last analysis what emerges is a taxonomic concept not much different in kind from that of Linnaeus, and based like his upon an exercise of human judgment. The answer is that by approaching the matter this way we can see some biological justification for applying Linnaean-style classification in sexual groups, and we can hope to understand something of the evolutionary and genetical situations which nomenclatural species in these groups represent; we can, in fact, make still more inductive generalization about species than we could assuming them to be only congeries of similar objects. There is, moreover, the important consequence that an understanding of the kind of biological unit we are trying to grasp for classification is bound to make for better taxonomic practice. This aspect is so important as to merit separate discussion.

C. "Biological" Species and Taxonomic Practice

The major change which a biological view of species requires is a shift of emphasis from individuals to populations. We have seen above that

"population thinking" has long been a feature of the most successful taxonomic practice, and there is no doubt that the better systematists have always had a sense of the essential unity of local breeding populations, although frequently it has been little more than subconscious. The fusion of Mendelian and Darwinian ideas in population genetics has produced a picture of the panmictic population as an evolutionary unit now subscribed to by the majority of geneticists (for general surveys, see Huxley, 1942, and Dobzhansky, 1951). In a recent paper I have described the situation as follows (Heslop-Harrison, 1960b). "Among sexually reproducing organisms, a breeding population or *gamodeme* may be defined as an assemblage of individuals so situated in space and time, and so similar to each other in morphology and physiology, that they are all capable of interbreeding within the limits imposed by sex differences, incompatibility barriers and the like. Such a population contains the means of generating hereditary variation, of conserving it, of recombining it in almost limitless ways, of concealing it, of exposing it when appropriate, and of losing it. Its members must necessarily have arisen from a common ancestry; they are potentially capable of combining their heredity in future progeny, and in the present are isolated to a greater or lesser degree from those of other gamodemes. A species, in the common biological sense of the term, may usually be taken to consist of a mosaic of such populations, classified together primarily because of morphological similarities."

If we are to have nomenclatural species conforming to this pattern, it is apparent that we must temper in practice the three requirements—of internal homogeneity based upon morphological resemblance; of distinctness from others; and of fixity—to accommodate what we now know of the dynamics of gamodemes. An important implication is that the species category can never be the appropriate one for the classification of paramorphs generated in the normal course of gene segregation and recombination, irrespective of how distinctive they may appear. A species diagnosis must allow for all the biotypes likely to be encountered within the gamodemes subsumed; "splitting" types of species-making which cut across breeding units stand utterly condemned.

A further implication is that distributional studies are inseparable from comparative morphological studies if "good" taxonomy is to result. It has been said that distribution is not a taxonomic character: but the species in the above sense is a space-time concept, and its properties cannot be investigated with casually acquired individuals of unknown provenance. With such materials the variation within gamodemes is not open to assessment, nor is there evidence upon which gamodemes may themselves be collated. Invariably when decisions about species limits

are involved, population samples are required, together with information about whether the populations are disjunct, continuous, or overlapping.

D. Infraspecific Variation

The application of the species category in sexual groups in the manner just discussed means that a great deal of variation will be "infraspecific" in that it is not taken into account in defining species limits. Furthermore, the amount of submerged variation will vary from species to species depending upon the kind of evolutionary situation each represents. If it should be the genetical or evolutionary situation within species which is under consideration, a formal taxonomic treatment complete with nomenclature may be quite superfluous. If classification is required at all in these circumstances it may be best based exclusively on the most apposite criteria, producing a special-purpose system of the type discussed in a later section. On the other hand, there may be reasons for attempting a formal taxonomic treatment of infraspecific variation, and the question arises as to the principles upon which this might be based.

There are only two levels on which taxonomic treatment of infraspecific variation might be required, (1) to deal with variation with gamodemes, and (2) to accommodate variation between them.

In the first case, which involves the cleavage of breeding units, the most likely reason for requiring subdivision is that some particular character or combination of characters is regarded as sufficiently distinctive, or sufficiently important for some practical purpose, for the individuals expressing it to be graced with a name, even if it be evident that the differentiae are either dependent upon one or a few gene loci and so susceptible to obliteration in some progeny by recombination, or due to environmentally imposed modification and similarly without permanence in inheritance. The lowest ranks of taxa sanctioned by the "International Code of Botanical Nomenclature" (1956), *varietas*, *subvarietas*, *forma* and *subforma*, are available as categories for the classification of this kind of infraspecific unit if formal naming is deemed necessary. Which of these is employed in any particular situation is largely a matter of taste, since rational distinctions are scarcely possible.

In the second situation we are dealing with variation which by inference must always be geographical, since gamodemes of the same species must be allopatric, even if their spatial separation—occasioned perhaps by slightly different ecological tolerances—is slight. Microgeographical differentiation associated with adaptation under the pressure of differential selective factors may well be significant in evolutionary studies, but it is rare indeed that any useful purpose is served by

describing, naming and classifying strictly local populations within the framework of formal taxonomy. On the other hand, *regional* variation is often of a kind which attracts taxonomic attention.

We reach here one of the crucial issues in the taxonomic treatment of variation at and around the level of the Linnaean species. Species are assemblages of vicarious populations, brought together on the basis of assessment of similarities and differences. Obviously it is possible to expunge all geographical "infraspecific" variation by the simple expedient of setting the level of character correlation required between conspecific populations at a high level; what emerges then is a mosaic of vicarious, highly homogeneous, species. Conversely, geographically heterogeneous species can be created by setting wide limits to the species diagnosis.

Regrettably there is no unequivocal principle upon which a decision between these possible paths can be based, and taxonomic judgment and taste remain the main arbiters. Where there is gradual or intergrading variation between remote populations and this has been known to a systematist, they have usually been treated as conspecific. Where there is an abrupt dislocation, or where an impressive geographical barrier like the Atlantic Ocean intervenes—or sometimes simply where a herbarium-bound systematist has been unaware of intermediates—the populations have been named as different species. Nevertheless, something may be said about the biological aspect of this situation. The morphological affinities of vicarious forms and their status in their respective biomes taken together may persuade us to the view that they are indeed "local representatives of the same species", when taxonomic practice will be adjusted appropriately. The judgment here is not *simply* upon degree of morphological difference—a question of the form, "Could these really co-exist sympatrically?" is being asked, or, in Mayr's words, the potentiality for interbreeding is being assessed.

The consequence of a liberal attitude to species definition is that many become geographically heterogeneous. The *polytypic species* is in fact a common and important biological phenomenon, the significance of which has perhaps in the past been less appreciated by plant than by higher-animal systematists. It is not the purpose here to discuss its general evolutionary implications, but it may be noted that the divergence of different segments need not necessarily be dependent on differential selection, although this will commonly be so. Diversification may result from fortuitous gene fixation, or from the establishment of divergent colonies by founder individuals which deviate in their genetical constitution from the mode of the original parental population. Where the regional variation of a polytypic species is conspicuous, taxonomic

recognition will certainly be demanded. This is the occasion, *par excellence*, for the use of the category of *subspecies*—in a sense which has, indeed, become classical in higher animal taxonomy.

E. The Refractory Cases

The Linnaean system has been reasonably successful in containing some of the variation of sexual groups, and the growth of understanding of the biological nature of taxonomic species shows why this should have been. But its satisfactory application has not proved universally possible, and there can be little doubt that as more is discovered about the variation of groups which currently seem reasonably well accommodated within the framework of the orthodox taxonomic system, many will be found to have been fitted only by the most brutal of procrustean methods.

It is as yet hardly possible to form any estimate of the extent to which a species concept, whether purely morphological or more broadly biologically based, is *essentially* inapplicable among the group of flowering plants as a whole, but taking the European flora alone there is already evidence that difficulties of a more or less serious character are to be encountered in some part or other of well over three-quarters of the genera. In many cases trouble arises from aberrations in reproductive method, but even where there is no evidence of anything but a normal breeding system, species circumscription offers difficulties in a disconcertingly high proportion of cases. This situation is to be contrasted with that in mammals and birds, where the level of success is much higher (Mayr, 1942)—although it may be noted that in invertebrate groups the position may be far less satisfactory (Ehrlich, 1961).

To review the many reasons for failure would be beyond the scope of this chapter, but some examples of peculiar significance among flowering plants may be considered.

1. Active evolution, with or without hybridization

A potential source of difficulty in the application of the Linnaean method of which Darwin was very much aware is the group where diversification is actively in progress; here it may be possible to distinguish several modes in the continuum of variation, but no discontinuities permitting the establishment of species boundaries. Frequently the differentiation will be geographically based (compare the polytypic species discussed in the foregoing section), but certainly among plants there is evidence that divergence can occur sympatrically in consequence of very local selection.

It is in practice difficult to distinguish cases of gradual speciation (Valentine, 1949) from those where previously spatially isolated and morphologically divergent populations are becoming re-united through hybridization following resumed breeding contact, especially where ecological barriers have recently been thrown down by man or natural catastrophe. The temperate part of the genus *Salix* is sometimes quoted as an example of a large, polymorphic sexual group in which "speciation" is currently active; this is no doubt true, but there is also every reason to believe that we are witnessing in this genus, as in many others of the north temperate region, the consequences of the wholesale destruction during and after the glacial period of ecological and geographical barriers between incompletely differentiated populations.

In all such cases of incipient divergence or reamalgamation through hybridization, the applicability of a formal taxonomic treatment based upon species diagnosis, whether or not supported by a biological species concept, must necessarily be limited. The alternatives are either to carve up the variation-span arbitrarily around the more conspicuous modes, treating each as a species for nomenclatural purposes, or to cast the species net wide and, if names are required for the diverging populations, to describe them as taxonomic subspecies. Either treatment is incapable of giving anything more than a general guide to the variation pattern.

2. Geographically graded variation

Graded variation has long been familiar to systematists, and Huxley (1938) introduced the term *cline* to describe the situation where grading in one or more characteristics shows geographical regularity. Clinal variation is widespread among flowering plants, and indeed it might be argued that it must be present in some form in all wide-ranging species the distribution ranges of which either span several degrees of latitude or encompass more than one climatic belt. The gradient may affect a number of characters simultaneously, including both vegetative and reproductive features, as in *Alnus glutinosa* in the British Isles (McVean, 1953), or may be observable in only one quantitatively varying characteristic (*Dactylorchis fuchsii*, Heslop-Harrison, 1960a). Clines occur, too, in the proportional representation of variants determined by single genes (*Plantago maritima*, Gregor, 1939). Intersecting clines are also known. Whatever the cause—whether direct phenotypic modification by a graded environmental factor; response to graded selection; or long-range hybridization and introgression—it is obvious that clinal variation cannot be accommodated in a system of discrete categories without distortion of the facts.

3. Reproductive isolation without morphological divergence

Ironically, the biological interpretation of the species category in sexual groups can generate a number of conceptual and practical difficulties by bringing into conflict morphological with cytogenetical criteria. If genetically based reproductive isolation is to be taken as a definitive indication of specific status, as the concept of the biological species would demand, then among flowering plants there would be many examples where morphologically reasonably homogeneous population systems would have to be fragmented because of the existence of intersterile groups within them. Where the subdivisions showed consistent morphological distinctions, then the award of specific rank and a binomial to each would occasion little inconvenience, but where morphological discriminants were not present, or where the variation of the intersterile groups overlapped, the species established would not be determinable infallibly without an experimental test. The occurrence of partial or complete genic or chromosome-structural intersterility between populations of the same accepted taxonomic species has been detected repeatedly, the segments ranging in extent from small local colonies (*Glyceria fluitans*, Borrill, 1958) to whole regional populations (*Datura stramonium*, Blakeslee, Bergner and Avery, 1937). At the chromosome-numerical level, differences which would seem to define sterility barriers are as well documented. The chromosome variation within the common *Cardamine pratensis* is notorious, and morphological study on an heroic scale has as yet failed to find correlated morphological discriminants. Polyploid series offer similar problems. Although the theoretical case for regarding polyploids as specifically distinct because of their putative intersterility is strong (Löve, 1951), correlation between chromosome level and morphology is not invariably discernible, or may be inconsistent in various parts of the range, as in *Valeriana officinalis* (Skalinska, 1951).

Situations are known where sterility barriers have been detected which not only are unmarked by any morphological distinction, but actually intersect with lines of demarcation defined by morphological and ecological properties. Excellent examples have been described in the genus *Clarkia* by Lewis (1953). In the collective species *C. deflexa*, some major morphologically distinguishable groups are interfertile, whilst in the morphologically uniform race *polyantha* populations of different provenance show intersterility due to chromosome structural differences.

The essence of all these situations lies in the fact that in plants, as probably also in many invertebrate groups, reproductive isolation commonly arises without genetic differentiation. What changes is the *genetic*

system, not the *gene complex*, and phenotypic differences are not therefore necessarily to be expected. This kind of situation contrasts strongly with that apparently prevalent among mammals and birds, where the concept of the biological species finds its greatest success. Unless it be simply that lack of knowledge obscures the true condition in these groups (see e.g. Hamerton, 1958), it seems that in them phenotypic differences do generally evolve in step with reproductive barriers. The emphasis which higher animal systematists place upon the *recognition* of members of a species one by another illustrates the point (Mayr, 1955). Ethological barriers depend upon sense stimuli; and what one sentient vertebrate can apprehend, another, in the form of the systematist, can usually do also if he makes enough effort.

So far as practical taxonomy is concerned, it is hardly reasonable to employ the species category to accommodate groups which are not genetically differentiated. Such groups are scarcely even "biological" species, except in the very narrowest sense; they are only potentially so.

If the principle of the species-defining nature of reproductive isolation is pressed, irreconcilable conflict with the demands of Linnaean style classification is inevitable in certain groups. Again the alternative is to accommodate cytogenetical data in special-purpose classifications if they are considered to be of overwhelming importance in any particular context—and if classification is thought necessary.

4. Aberrant breeding systems

The conception of a species as an assemblage of panmictic populations obviously has no meaning when the organisms concerned reproduce asexually or are habitually self-fertilizing. These deviations from the sexual outbreeding condition practically universal in higher animals are widespread among plants, and this is yet one more reason why the biological species concept finds less success in plant than in animal groups.

The kinds of variation pattern to be expected in genera in which apomixis or habitual autogamy have become prevalent are now to be considered as reasonably well understood. The "unit" of variation is a lineage, a clone or homozygous pure line, more or less distinct from others genetically, showing low internal variability and reproducing its like. This unit itself possesses all the properties required of a taxonomic species, and it is entirely logical to argue that each should be treated as such and awarded a binomial. The snag is that where such lineages exist in huge numbers the application of this principle produces a nomenclature so cumbersome as to have little value for any but the extreme specialist. What happens, of course, is that one of the principal functions

of classification—to reduce chaotic data to assimilable order—is defeated. For most botanists the species lists of *Rubus*, or *Hieracium*, or North American *Crataegus*, themselves constitute chaotic data.

The origin of this dilemma lies in the Linnaean conception, enshrined in the *Nomenclatural Code*, that the species shall be the basic unit of classification and the one to be awarded the binomial. For some purposes, including that of providing a working impression of the overall variation, a taxonomic treatment of some apomictic groups based upon species status for each clone simply does not reduce the data enough. The obvious solution is to create subdivisions at a higher level utilizing any discontinuities apparent in the variation range, or, where such discontinuities do not exist, to subdivide by arbitrary boundaries drawn around the main variational foci.

This kind of treatment leads to the conception of the *collective* or *aggregate* species, which itself merits comment. There is a sense in which the taxonomic species in sexual groups and the collective species in agamic complexes are akin: each represents, at one point in time, a collection of individual genotypes considered together because of phenotypic resemblance. In other respects they are evidently quite different kinds of entity. Individuals in the asexual group are linked only by descent; those in the sexual group, also by the capacity for interbreeding. But there is no sacrilege in treating them alike for the purpose of naming, unless the biological species is first deified as the only "true" kind of species. What is important is that the aggregate species should be recognized for what it is, a broad morphological pigeon-hole—neither a biological species comparable with those of a sexual group, nor a single genetical lineage. The addendum *agg.* to the binomial is sufficient to indicate the situation where this is not clearly enough revealed by the context.

It has been remarked above that the agamic lineage possesses all the attributes required in the perfect taxonomic species, and this is true in groups where apomixis is obligate. There are apomictic complexes, however, in which the lineages fail to satisfy the third criterion, that of persistence in time. In these groups variation is gained and lost either by occasional sexual episodes or by internal gene recombination. In some, variable sexual species occur together with both obligate and facultative apomicts. The resulting situations are quite beyond detailed treatment by orthodox taxonomic methods. The variation not only forms a continuum at any one time, but is likely to alter its pattern with time, in the manner which Clausen and associates have so strikingly shown in the genus *Poa* (see discussion by Clausen, 1954). The best that can be done in such groups is to attempt the delimitation of the sexual species and

the more stable apomictic clones for the purpose of naming and classification, and treat the residue as an aggregate.

Much the same considerations arise in relation to autogamy. There are examples of more or less permanent fractionation of inbreeding groups due to the stabilization of a self-pollination mechanism (e.g. in the genus *Epipactis*, Young, 1953), but as Baker (1959) has observed in an important review of reproductive methods as factors in speciation in flowering plants, there is probably no case when a sexual species is *never* outcrossed. Unless the pollen source is from the same colony, each outcrossing is likely to be accompanied by segregation, and the resultant burst of variation may be the starting point for fresh periods of inbreeding and the generation of new more or less homozygous biotypes. The situation is quite similar to that arising with facultative apomicts, and the taxonomic problems are almost identical. Mercifully, however, they are usually on a smaller scale, as exemplified by the Linnaean aggregate *Capsella bursa-pastoris*.

IV. Alternatives to Formal Taxonomy

The principal characteristics of the general-purpose classification in current use are that it is universal in application, hierarchical in structure, and intended to be natural in the sense of being based upon assessments of overall similarity. A classification of this type, whilst undoubtedly the best for the majority of purposes including that of nomenclature, does not necessarily meet every requirement satisfactorily. The usual cause of inadequacy is that, *being* natural, it does not provide the *complete* correlation with certain kinds of data demanded for some purposes. Such purposes need groupings based entirely upon the particular criterion or group of criteria of interest in the special context, i.e. they demand artificial classifications.

All kinds of utilitarian classification of plants are of this nature, and so are all classifications based upon the application of particular biological criteria, like ecological habit, cytological characteristics, and interfertility and intersterility. Three features of artificial classification in application to organisms are of particular importance:

(i) Systems based upon a single criterion, whatever it might be, cannot be expected necessarily to accord with the natural classification of the group under treatment. The correlation may occasionally be found to be quite high, but it is unlikely to be perfect, since it is the nature of natural classification to depend upon correlation patterns amongst numerous criteria. Thus a natural classification gives the class

Angiospermae, in which certainly the majority of plants are green, but an artificial classification of plants based upon the distribution of chlorophyll sorts out such odd bedfellows as *Rafflesia*, *Monotropa* and *Cuscuta* to be classified with the fungi and other heterotrophs.

(ii) The same material may be classifiable in several ways according to the kinds of criteria adopted. Unless a pair of criteria are completely correlated the classifications they produce must necessarily intersect; and for the reason given in (i) they will also intersect with or subdivide the natural groups of orthodox taxonomy.

(iii) Systems based upon special criteria will often be applicable only within a fairly restricted ambit comprising those organisms accessible to test. Special-purpose criteria therefore rarely have pretensions to universality, and this is one reason why they cannot provide the basis for a nomenclature. In general they must be linked at some point or another with the nomenclatural system of orthodox taxonomy, and are usually in fact "hybridized" with the general-purpose taxonomic system, as when a treatise of economic botany lists under latex plants species of Compositae, Euphorbiaceae, Moraceae and Asclepiadaceae.

The use to be made of special criteria at and below the level of taxonomic species has occasioned controversy during the last forty years. The principal issue has been whether or not genetical and cytological data have such transcendental importance that they should be permitted to dominate classification. For example the "experimental concept of the species" formulated by Clausen, Keck and Hiesey (1939) requires the rejection of natural classification at the species level and its replacement by a form of artificial classification based upon experimental tests of interfertility. There have also been proposals that definitions of taxa based upon this kind of criterion should be written into the "International Code of Botanical Nomenclature".

With the development of a fuller understanding of the function of nomenclatural taxonomy as a general-purpose data storage and retrieval system for biology and its utter dependence for this role upon a "natural" classificatory basis, the more extreme viewpoints have lost favour, and it is now generally recognized that even at species level cytogenetical data must be integrated with the others available for the purposes of orthodox taxonomy. The corollary of this is that *should* there be a need for a classification based upon experimental data it must be met by creating a system supplementary to the nomenclatural one.

There have been various proposals for category systems for so-called experimental taxonomy from the time of Turesson's pioneer paper of

1922 in which the well-known *ecotype*—ecospecies—*coenospecies* hierarchy was described, and several reviews and discussions are available (Gregor, 1944; Turrill, 1946; Baker, 1952; Heslop-Harrison, 1960a). The only proposal for a universal category system is that developed by Gilmour and Heslop-Harrison (1954) using the root *-deme* in the matter originally proposed by Gilmour and Gregor (1939). More recent summaries of the system and some of its implications have been given by Gilmour (1960) and Heslop-Harrison (1962), and extended discussion is not merited here. However, the philosophy of the method may be judged from the following passage from the latter paper. "The principal attributes of such a system are largely determined by the nature of artificial as opposed to natural classification. There are as many artificial classifications as there are criteria on which they can be based, so that the category system must make provision for indicating the kind of criterion in use in order that the particular classification can be identified. It follows also that it is imperative that nothing should preclude the inclusion of an individual in more than one category, since the groupings formed will necessarily differ according to the criterion applied. This in turn implies that no nomenclatural system comparable with that of orthodox taxonomy can be devised, but only reference nicknames linked inseparably to the category terms, themselves serving to identify the artificial classification in use. And, finally, only by the overt recognition that 'difference' is always a relative term to be established by context does it seem possible to avoid disputes about category definitions. The validity of an artificial classification is determined solely by its internal consistency, so that the purpose in view can always be allowed to govern the levels of difference to be adopted in establishing the subdivisions."

V. Species Concepts and "Chemical Taxonomy"

The arguments of the last section are obviously relevant to the problem of the use to be made of the data now accruing concerning the distribution of precisely identifiable chemical compounds and families of compounds among flowering plants. The alternatives are to integrate them with other criteria and use them in the same way, or to treat them in some different and special manner. Before a decision can be made, however, it is essential to establish the purpose for which chemical facts are to be used in classification.

If the primary aim is to contribute to the refinement of the general-purpose natural system, the potentialities of chemical data must be assessed against all the other data available. If, on the other hand, it is the *chemical facts themselves* which are to be classified, then chemical

criteria must be adopted to provide the subdivisions of an artificial classification into which the plant populations should be fitted, without necessarily any reference to the groupings of a natural classification. These aims are entirely different, and there is no hope of constructing a chimaerical classification attaining both. In particular, in view of the character of natural classification, it is not admissible to accept the groupings of the natural system when they happen to accord with chemical data, and then demand the re-casting of the system and its nomenclature when one particular set of chemical observations appear to clash with it.

In assessing the relative value of chemical data as criteria for use in general-purpose classification, we need to know whether there is any quality in them which differentiates them as a class. In essence there is not. Biosynthetic pathways leading to particular compounds are expressions of the genome just as are morphological features; indeed so-called morphological features are all in some sense themselves expressions of biosynthetic pathways. It is conceivable, however, that chemical data may form particularly valuable taxonomic criteria because of qualities of consistency and readiness of assessment, and in fact these claims have already been made long ago, even in so lowly a group as the lichens.

Turning to the narrower problem of chemical variation at and around the level of the taxonomic species, it is apparent that here again there are the alternative methods of procedure. The incidence of the compound or compounds under study can be made the basis of a special classification, or the information may be taken as one more kind of evidence for incorporation in the natural system.

At this lowest level there is a difference in what is involved in the construction of special classification. A survey of the distribution of a product which shows a bold pattern of variation among flowering plants will probably reveal subdivisions separating whole orders, families or groups of genera; these taxonomic groups would then become the "units" distributed between the categories of the chemical classification. Where there is variation in the incidence of the compound under study at and below the level of species, it will commonly be populations or population segments which will be defined by its presence or absence. There will then be no pre-existing named taxa to be pigeon-holed. The *chemodemes* would then require informal reference names or code numbers, the whole complex being first linked to the named taxon of nearest rank for the purpose of primary orientation.

The potential contribution of chemical data to orthodox taxonomy at the level of the nomenclatural species and below will depend upon how well they meet the general requirements for taxonomic characters, established now through long years of testing and rejection. *Ease of*

assay is an important criterion, and here we have already reason to believe that the relative unambiguity of modern chemical analytical procedures will be important. *Consistency* is a quality which can only be gauged in relative terms. Bearing in mind the nature of natural grouping which permits exceptions to any generalization, to affirm that a characteristic is consistently present in a taxon implies that it is to be expected in a high proportion of its members—how high being unspecified. To be written into the diagnosis of the taxon, if the salutary advice of Bentham (1874) is to be followed, the characteristic must be such that it is not also consistently present in the taxon of next higher rank, nor so distributed as to be more properly attributed to taxa of next lower rank. Another aspect of consistency concerns the status of a property as an expression of the genotype. A taxonomist can only inspect phenotypes, but he serves Ray's dictum best (p. 22) when he classifies genotypes. For this reason a differential revealed in all environments is likely to have greater classificatory value than one which is conspicuously affected by environmental factors, unless the taxonomist is equipped to detect the property of variability itself and use it taxonomically.

Table I summarizes the questions likely to be asked about chemical properties of plants before their full taxonomic usefulness can be exploited. The subdivision into "observational" and "experimental" categories reflects the fact that what can be determined from individual

TABLE I

Observational	Experimental
Within the individual	
Is the product organ specific?	Does it vary in any systematic way during development?
Between individuals	
Is the variation quantitative (intergrading) or qualitative (discontinuous)?	Is the variation heritable or environmentally determined?
Is the variation correlated with any other characteristics (for example, adaptive features such as habit, indumentum)?	If heritable, is the genetical basis oligogenic or polygenic?
Between populations	
Does the variation between population samples suggest that discontinuities exist, or are the differences only in the average expression in populations?	Do transplant and breeding experiments show the inter-population variability to be genetically based?
Does the variation show (*a*) geographical, (*b*) ecological regularity?	

plants or population samples collected in the wild and brought into the laboratory is limited in its scope. Such material can provide only a basis for inference about such important matters as the genetical status of the observed differences or the extent to which they represent environmentally conditioned fluctuations. It is true, of course, that information such as that solicited in Table I is lacking for many other characteristics which are currently employed as taxonomic criteria, but it has been one of the main contributions of experimental taxonomy to show how significant this information can be for the comprehension of variation at and below the level of the average Linnaean species and how useful it may be in taxonomic practice.

In conclusion, Table II provides a summary of the sources and causes of variation within and between plant populations where a sexual outbreeding habit prevails. This is based upon what is known about morphological and physiological variation in general, but almost all of the situations could now also be illustrated with examples from plant chemistry. What remains is to ensure that in further studies in this field the right kind of information is accumulated to permit a proper interpretation of variation patterns. In this way the ends of taxonomy will be better served, and, where a product of economic significance is concerned, also those of practical exploitation.

TABLE II

Types and sources of variation within and between breeding populations (gamodemes) of outbreeding sexual plants

Within gamodemes	Between gamodemes
Environmentally governed quantitative variation	*Ecologically correlated*
	Environmentally governed quantitative variation.
Polymorphism due to oligogenic systems or the segregation of gene complexes protected from recombination by segmental inversions, etc.	Differences due to selective gene fixation.
	Adaptive differences in the proportional representation of major genes.
Quantitative variation due to segregation in polygenic systems.	Quantitative differences due to selection operating upon polygenic systems.
	Random
	Differences resulting from random gene fluctuation and fixation.
	Quantitative differences due to drift in polygenic systems.

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CHAPTER 3

History of Chemical Taxonomy

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I. Introduction

We are impressed, each time we look into the history of a particular topic, with the difficulty of discovering the real beginnings. Only rarely can we say that "there and then" a named individual "discovered" a particular thing, or initiated a given technique. This difficulty has been well discussed recently by Kuhn (1962) in a paper titled "Historical structure of scientific discovery". Nevertheless we have an urge to give credit to the pioneers of science and to delve back in the literature to

unearth the beginnings of things, and we shall try here to summarize the history of comparative phytochemistry as applied to plant systematics.

II. The Beginnings

Our subject has a respectable antiquity. The early history of botany is largely a history of the uses of plants in medicine, and the root-gatherers and herbalists of the past began, many centuries ago, to group plants having similar "virtues" or medicinal properties.

It was not until near the end of the seventeenth century, however, in the works of Grew, Petiver and Camerarius, that this grouping came to have a modern look.

The first, Nehemiah Grew, who lived from 1641 to 1712, writes in "An Idea of a Phytological History Propounded" (1673), pp. 13–14:

> "From hence likewise the Natures of Vegetables may be conjectured. For in looking upon divers Plants, though of different names and kinds; yet if some affinity may be found betwixt them, then the nature of any one of them being well known, we have thence ground of conjecture as to the nature of all the rest. So that as every Plant may have somewhat of nature individual to it self; so as far as it obtaineth any visible communities with other Plants, so far may it partake of common Nature with those also. Thus the Wild and Garden Cucumers have this difference, that the one purgeth strongly, the other not at all; yet in being Diuretick, they both agree. The Natures of Umbelliferous Plants we know are various; yet 'tis most probable that they all agree in this one, *scil.* in being Carminative. . . . So Tulips, Lillies, Crocuses, Jacynths, and Onions themselves, with many others in their several degrees, are all allied. If therefore Crocuses, Onions, Lillies agree in one or more faculties, then why may not all the rest? as in being anodyne; . . ."

The second, James Petiver, as we may learn from a biographical note in the abridged *Philosophical Transactions of the Royal Society*, was:

> ". . . a zealous cultivator of the science of natural history . . . an eminent London apothecary . . . a Fellow of the Royal Society . . . [who] died at his home in Aldersgate-Street, on the 20th of April 1718."

In the original *Phil. Trans.* we find a paper dated 10 May 1699, "*Some* Attempts *made to prove that* Herbs *of the same* Make *or* Class *for the generallity, have the like Vertue and Tendency to work the same Effects,*" and starting:

> "Having by some *Persons* been asked what Method might be best proposed toward the *discovering* of the *Vertues* of *Plants*, amongst

others I thought this might not prove an altogether unsuccessful conjecture, *Viz.* That *Plants* of the same *Figure* or Likeness, have for the generallity much the same *Vertues* and *Use*: Especially if we consider, that the *Organs* or *Structure* of ye *Plants* of the same *Family* or *Class*, must have much the same *Vessels* and *Ductus's* to consummate that Regular formation, and consequently the *Juices* Circulated and strained thro' them cannot be very *Heterogeneous*; and that as for the most part, the *Scent* and *Tast* have great affinity, so of course their *Vertue* likewise cannot be very *dissonant.*

"1. As for Instance, the *Herbae Umbelliferae.* . . . This *Genus* [family, to us] I generally observe to be endowed with a *Carminative* Tast and Smell, are powerful expellers of Wind, and are therefore good in all flatulent Diseases, and of great use in the *Chollick*, etc. To Instance a few for *Example*, as *Aniss, Caraway, Cummin. Angelica, Smallage, Parsly, Lovage*, etc. . . .

"2. Let us now look into another Class, *Viz.* the *Plantae Galeatae* and *Verticillatae* . . . the *Florae Galeatae* seu *Labiatae.* . . .

"Now whereas the greatest Vertue of the *Umbelliferous Tribe*, were specified to lye in the *Seed* . . . the Sovereign balm of these chiefly consist in their *Leaves* and *Husks*, rather than the *Flowers*; which last, especially all *Authors* has hitherto given the preference to. . . ."

Petiver makes a prediction which sounds very familiar to us:

"I would not be thought to propose this *Hypothesis* for Cheapness sake, for if my assertion holds good, as I doubt not to prove it, I fear they will quickly sell the *Husks* as dear as the *Flowers*, if they find a great vend or a frequent demand for them."

He then says that one can distill more of the active principle from the Husks" (Calyces) than from the flowers, and goes on:

"I look upon the generality of this Tribe, to be a degree Warmer than the last, and their Heat consequently to approach near to the *Aromatae* or Spices, then the *Carminatives*, and the Effects therefore to be more peculiarly appropriated to such Nervous Disease, as are more intense, and the *Umbelliferae* cannot so quickly reach, *Viz. Apolexies, Epilepsies, Palsies*, etc. . . .

"3. We proceed next to those herbs which have a *Tetrapetalose Regular Flower* [the Cruciferae to us]. . . .

"The most Essential Vertue and use of the Herbs of this *Class* I observe are more particularly in the Leaves and Seed, and next them the Roots, and if any parts are slighed [sic. He means slighted?] it's the *Flowers* and *Podds.*

"The Leaves are more particularly used in the *Water* and *Garden Cresses, Sea* and *Garden Scurvy-grass, Hedge-Mustard, Iberis*. . . .

"Others of this *Family* that are more peculiarly eminent for the *Vertue* contained in their Seed, are the common *Mustard* and *Rape*. . . .

". . . I am certain the effects of many of these Herbs . . . are by most, if not all *Physitians*, as well Antient as Mordern, allowed to be extraordinary *Diureticks* and *Anti-scorbuticks*."

The third, Rudolph Jacob Camerarius, is sometimes credited with the authorship of "De Convenientia Plantarum in Fructificatione et Viribus" (1699)—a work which has much the same flavour as that of James Petiver. We have here, however, a problem such as crops up in Linnaeus' "Amoenitates academicae" (1749–90). For the "Convenientia plantarum" is a thesis defended by Georg Friedrich Gmelin with Camerarius presiding. Stearn points out in his introduction to the Ray Society's facsimile edition of Linnaeus' "Species Plantarum" (1957), that theses of this nature were often almost if not wholly the work of the director rather than of the student:

"They were in fact produced under an academic procedure of medieval character accepted in Swedish universities until 1852, and also at one time in north Germany, whereby the student defended in public debate a *thesis for which the professor was primarily or entirely responsible*."

In the early years of the nineteenth century A. P. DeCandolle published his "Essai sur les propriétés médicales des Plantes, comparées avec leurs formes extérieures et leur classification naturelle" (1804). He says that Camerarius (above) was the first to express clearly the connection between forms of plants and their properties and that opinion on the subject was divided until Linnaeus (above):

". . . dans sa dissertation sur les propriétés des plantes, ou il établit que les plantes du meme genre ont la meme propriété, que celles du meme ordre naturel ont des propriétés voisines, et que celles de la meme class ont aussi quelques rapports dans leurs vertus. . . ."

We shall quote but one of DeCandolle's treatments of families:

"53. *Gentianées. Gentianeae* Juss.

"Il est peu de familles ou l'analogie des formes et des propriétés se fasse sentir avec plus de force, que dans celle de gentianées; toutes ces plantes ont une saveur amère, qui réside dans leur herbe et surtout dans leur racine; elles sont conséquemment employées comme toniques, stomachiques et fébrifuges."

This seemed familiar to me and sent me to John Lindley's "The Vegetable Kingdom" (Edn. 3, 1853), where I re-read:

> "The Order [family, we should say] of Gentianworts is not more remarkable for the diversity of its colours than it is for the uniformity of the secretions which its various species exhibit. Bitterness in every part, root, leaves, flowers, fruit, in annuals, perennials, and shrubs, is so much their characteristic that the following account of the purposes to which they are applied is little more than a list of repetitions; with this exception, that they in some cases prove narcotic and emetic."

He had said much the same thing in 1830.

DeCandolle published a second edition of his "Essai" in 1816. Here he has many points of interest to us. On p. 15 he remarks on the relative constancy of composition of plants when grown on different soils:

> "C'est un phénomene continuellement présent a notre examen, que de voir diverse plantes nées dans un sol parfaitement semblable, produire des matieres trés-differentes, tandis que des végétaux analogues, nées dans les sols differens, y forment des produits semblables."

In his first edition he does not separate the *Jasmineae* from the *Oleineae*. In his second edition he does: "82. *Oleinées* (*Oleineae* Hoffm. & Linck. Flore Port.). 83. *Jasminées* (*Jasmineae* Brown. Prod. 520)" and has two significant observations. Firstly, he notes (pp. 22–3) that insects can detect the differences between these groups:

> ". . . les cantharides attaquent d'abord les frênes, puis se jettent sur les lilas et les troènes et jusque sur les oliviers [all members of the *Oleineae*]. . . . Elles n'attaquent au contraire jamais les jasmins, qu'on avais mal-à-propos réunis a la famille des Oleinées, et que forment aujourd'hui une famille particulière [*Jasmineae*]."

We shall return to insects and the comparative chemistry of plants towards the end of this chapter.

Secondly, he states that experiments on grafting also support the split into two families. Lindley (1830) notes this statement and quotes from DeCandolle (but translates):

> "However heterogeneous the Olive tribe may appear as at present limited, it is remarkable that the species will all graft upon each other; a fact which demonstrates the analogy of their juices and their fibres. Thus the Lilac will graft upon the Ash, the Chionanthus and the Fontanesia, and I have even succeeded in making the Persian Lilac live ten years on Phyllirea latifolia. The Olive will take on the Phyllirea,

and even on the Ash: but we cannot graft the Jasmine on any plant of the Olive tribe; a circumstance which confirms the propriety of separating these two tribes."

III. The Modern Pioneers

A pioneer in our field, whose work in its broad generalizations and conclusions is somewhat like that of McNair (see below), was Helen C. de S. Abbott. I have quoted from her papers of 1886 and 1887 in a communication of my own (Gibbs, 1958), but I cannot forbear to quote two brief passages again:

> "The vegetable kingdom does not usually claim our attention for its intellectual attainments, although its members would certainly seem to possess greater chemical skill than a higher race of beings exhibit in laboratories."

and, prophetically:

> "There has been comparatively little study of the chemical principles of plants from a purely botanical view. It promises to become a new field of research."

We may note next the work originating in a great tropical botanical garden—that at Buitenzorg (now Bogor) in Java, which was founded by Reinwardt in 1817. A laboratory for anatomy and physiology was established there in 1884 and one for pharmacology four years later. The early volumes of the garden's "Annales", edited by Melchior Treub until shortly before his death in 1910, contain several papers on plant chemistry.

Thus Eykman reports in 1888 upon his work on alkaloids, and notes their frequency in certain families. He was followed by Greshoff, who (in 1891) summarizes early work in which he is already thinking of the use of comparative chemistry in taxonomy. He found the alkaloid laurotetanine to be a frequent constituent of members of the Lauraceae and then says:

> "Dans les notes jointes à [*Hernandia*, *Illigera*, *Gyrocarpus*, *Cassytha* —in which he also found alkaloids] l'auteur rappelle les opinions divergentes de la place naturelle de ces quatres genres, qu'on a ranges dans les familles très differentes. Peut-être le phytochimiste pourra renseigner le systematicien aussitot que paraîtra l'identité ou l'analogie de structure de ces alkaloïdes . . . avec lauro-tetanine."

A few years later van Romburgh published quite extensive work on the occurrence in plants of acetone, methyl salicylate, and HCN. Treub

himself was interested in the role of HCN and we find four papers of his here on this subject (1904, 1907a and b, 1910). A paper by de Jong on HCN in *Pangium* and *Phaseolus* appeared in 1908, while Gorter (1910) investigated chlorogenic acid and its distribution in nature.

After Treub's death few papers of interest to us came from the Buitenzorg garden. Those mentioned above are a valuable contribution to comparative phytochemistry, particularly as the plants studied—those of the wet tropics—are not available to workers in other regions. They contribute, too, in a large way to our knowledge of distribution of certain substances—a very necessary part of our field.

Greshoff, whom we mentioned above, subsequently worked at Kew for some time and published (1909) a summary of his work there. He looked for tannins, alkaloids, cyanogenetic compounds, and saponins in a wide variety of plants. He has a striking way of emphasizing the high proportion of HCN in *Platanus*:

> "Indeed, in the ordinary plane-tree of the London streets (*P. acerifolia*), there is so much hydrocyanic acid present that the amount from every London plane-leaf would be enough to kill a London sparrow."

He defines "Comparative phytochemistry" as "the knowledge of the connection between the natural relationships of plants and their chemical composition" and says:

> "Strictly speaking one might demand that every accurate description of a genus or of a new species should be accompanied by [I should say 'should *include*'] a short 'chemical description' of the plant."

In a long series of papers published between 1917 and 1945 McNair attempted to apply comparative chemistry generally to taxonomy. We must examine his work in some detail because it carries a warning that should be kept in mind by all workers in our field.

In his first paper McNair notes that the fruit-coat fats of two species of *Rhus* native to North America are similar to the so-called "Japan wax" (actually a fat) obtained from the fruits of Asiatic species of the genus. Fats and oils and their taxonomic significance were to prove of continuing interest to him and in a paper appearing in 1929 he considers more than 300 oils, fats, and waxes occurring in eighty-three families, in relation to climate and taxonomy. He divides the oils into drying, semi-drying, and non-drying, and notes other characteristics such as iodine numbers, saponification values, and specific gravities. He concludes that in general plants of tropical habitats tend to store fats or non-drying oils of higher melting-points than the plants of temperate regions. He says that the fats and oils of closely-related plants are closely similar.

In 1935 he turned his attention to alkaloids. He remarks on the fact that each species of a genus such as *Aconitum* may have a different member of a group of closely-related alkaloids: that any one alkaloid rarely occurs in more than one family but may occur in many members of one family (e.g. protopine in the Papaveraceae).

This general occurrence of protopine in the *Papaveraceae* (s.l.) is of great interest. Hutchinson (1959) argues for the separation of the Fumarioideae from the Papaveraceae (s.l.) as a family Fumariaceae, and says that:

> "In his [own] opinion it is quite distinctly separated as a group from Papaveraceae proper, and nearly as closely allied to certain genera of Berberidaceae, such as *Epimedium*, *Aceranthus* and *Bongardia*. That there is close affinity with certain Papaveraceae is quite evident, especially with *Chelidonium* and allied genera. But it is probable that this alliance is more apparent than real, and that the Fumariaceae have not arisen directly from the ancestors of the present Papaveraceae [s.s.]."

Manske (1944, cf. 1954) says:

> ". . . the alliance is more real than apparent, and it is his [Manske's] opinion that the nature of the contained alkaloids can already decide the issue. No plant in the entire Papaveraceae family [i.e. including *Fumarioideae*] has yet been found to be devoid of alkaloids and at least one alkaloid, namely protopine, is present in every plant. What is equally significant is that protopine has never been found in any plants of other families."

Since Manske wrote the above new facts have come to light, including the isolation by Ohta (1949) of protopine from the seeds of *Nandina domestica* (Berberidaceae), and the report in a brief paper by Majumder, Sarkar, and Dutta (1956) of protopine in *Zizyphus jujuba* (Rhamnaceae). Manske (in a personal communication dated 30 October 1962) says that he is quite convinced by Ohta's work; which, we may add, would be in line with a relationship between the Papaveraceae and Berberidaceae. He is still a little sceptical about *Zizyphus*.

A second paper by McNair in 1935 deals with "Angiosperm phylogeny on a chemical basis". Here he tries to use alkaloids, glycerides (fats, oils, etc.), and volatile oils as aids to taxonomy:

> "Plants can be classified chemically in accordance with the substances made by them. Such a chemical classification may be compared with or used as a supplement to morphological classification and may be of some importance in the development of the true natural system of angiosperm phylogeny."

He claims that the chemical products of more highly evolved plants have larger molecules, and that the iodine number of glycerides (a measure of unsaturation) is higher in the more highly-evolved groups. These "facts" are used to support the argument that trees are more primitive than herbaceous plants—that Magnoliaceae and Berberidaceae are more primitive than Ranunculaceae Table I).

TABLE I

"Are the Magnoliaceae pre-ranunculacean?" (McNair's title)

Family	Dominant form	Alkaloids (Mol. wt.)	Glycerides (Iodine number)
Magnoliaceae	Shrub-tree	—	95·5
Lardizabalaceae	Shrub	—	78·4
Berberidaceae	Herb-shrub	330	139·1
Ranunculaceae	Herb	543	145·0

He concludes that:

"In the taxonomic revision of various plant groups, the serum diagnostic method of Mez [1926] and the electrophoretic method of Moyer may give correct taxonomic sequences, but the use [of molecular weights] of alkaloids, [of iodine numbers] of glycerides and [of specific gravities or refractive indices] of volatile oils gives not only these sequences but also (because they deal in numerical values) gives an idea as to the relative degree in evolution of various groups, e.g. the palms versus the iris, or the palms versus the Rubiales."

In the paper discussed above McNair contrasts publications by Standley (1931) and Rusby (1931–2). Rusby's paper is a scathing criticism (by an old man—he published on *Cinchona* in 1887!) of Standley's work. He points out that:

"It is doubtful if any other genus of equal size has received such thorough study, as to gross and microscopical structure, chemistry, reproduction, embryology, horticulture, ecology and geography, as has *Cinchona* . . . [yet] In the most recent publication on the Bolivian cinchonas, Standley's *The Rubiaceae of Bolivia*, all this information is ignored, with the result of so many errors that I can regard the publication only as a misfortune to *Cinchona* literature."

This may be an unfair statement but it *does* indicate the recognition

by Rusby, at least, of the need of taxonomists to use all criteria, including chemical ones, in their work.

In 1945 McNair has a paper on chemical ontogeny and chemical phylogeny. In the seed chemical ontogeny is often carbohydrate → saturated oil → unsaturated oil. This may represent recapitulation (see also p. 74) and we may argue that carbohydrate storage is primitive, oil storage more advanced. Accordingly, McNair has a table which is supposed to indicate support for the view that monocotyledons are more primitive than dicotyledons and that the Sympetalae are the most advanced of the dicotyledons.

TABLE II
(from McNair, 1945)

Group	Embryo		Albumen		General contents
	% Starchy	% Oily	% Starchy	% Oily	% Oily
Monocotyledons	12	45	57	27	15
Dicotyledones					
Archichlamydeae	15	45	16	30	50
Sympetalae	9	42	2	40	54

He discusses cyanogenetic glycosides, too, from this point of view (see Gibbs, 1958, and below).

A second paper in 1945 reviews work on plant fats in relation to environment and evolution. Much of what he says has been dealt with above. He considers it to be a "fact" that "The more highly evolved the plants the larger are the iodine values of their seed fats provided the plants grow in the same climate . . ." and he makes sweeping generalizations as to what is primitive and what advanced in the flowering plant.

We can only conclude that while McNair was correct in stressing the importance of comparative chemistry for taxonomy, he was unfortunate in the applications which he made of it.

It is not profitable here, any more than in traditional history, to follow a simple chronological sequence. We must, therefore, take up a few of the topics which have received most attention from phytochemists and use these to illustrate the history of our subject. I propose to include some reference to visible chemicals, to glycosides as represented by those yielding HCN, to serology and plant systematics, to some studies at the genus level, and to chromatography as a powerful tool, and then to conclude with some general remarks.

IV. The Use of Certain Chemical Criteria in Plant Taxonomy

The systematic botanist is used to dealing with the visible, morphological characters of plants. He uses visible chemical characters when he notes flower-colour, presence or absence of green pigments, and the types of crystals to be seen in sections of plant material. Some of these last are constantly to be found in certain families, or are just as constantly absent (Metcalfe and Chalk, 1950, pp. 1343–6).

A. Raphides

Crystals of calcium oxalate are perhaps the commonest of plant inclusions and prominent among them are *raphides*. These, in the sense used here (and some writers are less exact) are *slender, needle-shaped crystals arranged parallel to each other in tight bundles and occurring in special raphide-sacs.*

Robert Brown (1773–1858) was an early user of presence or absence of raphides as a diagnostic character. Thus we find him stating, in a paper read in 1831 but published in 1833:

> "My concluding observation on Orchideae relates to the very general existence and great abundance, in this family, of Raphides or acicular crystals in almost every part of the cellular tissue."

And again, in a paper read in 1834, but appearing in a volume dated 1845 (!) he notes that the reticulated covering through which the flower of the parasitic *Rafflesia* bursts might be derived from the host plant or might be part of the parasite. He then uses presence or absence of raphides to decide the point:

> "That the whole of this covering belongs to the stock, is proved by its containing those raphides or acicular crystals which are so abundant in the root of the *Vitis* or *Cissus*, and which are altogether wanting in the parasite."

We may observe, in parenthesis, that we looked for raphides in many genera of the Orchidaceae and have numerous records from the work of others. Raphides are, indeed, widespread in the family, as Brown remarks, and they may be universal. We have not found them, however, in *Arpophyllum*, but have not studied the genus at all fully. They do occur in two species of *Meiracyllium*, which Dressler (1960) believes to be closely related to *Arpophyllum*, and in every other genus that we have examined.

Gulliver, a British anatomist and microscopist who lived from 1804 to 1882, made a careful study of raphides, defining them as I have done,

and published a long series of papers from 1861–80. He gave much attention to the possibility that raphides might vary in occurrence with differing conditions and concluded that they are thoroughly reliable:

> "In short, I know of no means by which a raphidean plant can be grown in health, if at all, so as to extinguish this character, nor by which a plant regularly devoid of raphides can be made to produce them."

He found that:

> "Only 3 orders [we should say families] of British Dicotyledons can as yet be characterized as raphis-bearers, and these are—Balsaminaceae, Onagraceae, and Rubiaceae."

We should agree with him today. Not all Rubiaceous plants have raphides, as he realized, but those of Britain do. *Trapa*, which occurs just south of Britain, and which has been included in the Onagraceae (Oenotheraceae) lacks raphides. This is one argument for making it the type of a separate family, Trapaceae or Hydrocaryaceae. Eames (1953) says that comparative anatomy is another argument for this separation. *Montinia*, too, a South African endemic, has been included in the Onagraceae. Gulliver notes that it lacks raphides "and perhaps does not really belong to the order Onagraceae". We should agree and place it elsewhere. But where? Nakai (1943) would have a separate family *Montiniaceae*; Phillips (1951) has it in the Saxifragaceae; Hutchinson (1959) would say Escalloniaceae. Perhaps a further study of its chemistry and that of *Grevea* which Milne-Redhead has placed in Montiniaceae with it, would help us to decide!

Gulliver credits Lindley with using raphides as a character as early as 1839. I have not seen this work but Lindley was apparently a close second to Robert Brown. In 1880, in what seems to be his last botanical paper, Gulliver says that he has never seen raphides in the many members of the Saxifragaceae which he has examined but that they are in *Hydrangea*:

> "Here then is a natural and sharp diagnostic between the Saxifrages and Hydrangeas."

This, he says, would support Lindley's recognition of a family Hydrangeaceae (1853). Actually Dumortier seems first to have "made" a family of this name in 1829, including *Hydrangea* and *Deutzia*, Lindley included *Hydrangea*, *Cardiandra*, *Platycrater*, *Schizophragma*, *Jamesia*, *Broussaisia*, *Dichroa* and *Bauera*.

Other taxonomists have recognized a family Hydrangeaceae and we may note: Martius (1835), who names only *Deutzia* and *Hydrangea*;

Bessey (1915), who names only *Hydrangea* and *Philadelphus*; van Tieghem and Constantin (1918), who are the only ones, I think, to use raphides at all as a diagnostic character—they include fourteen genera but name only six:

> "D'après la présence ou l'absence de raphides, la conformation de l'inflorescence et la déhiscence du fruit, les genres se groupent en deux tribus:
>
> 1. *Philadelphées*—Pas de raphides. Fleurs toutes fertiles. Capsule septicide: Deutzie [*Deutzia*], Seringat [*Philadelphus*], Carpenterie [*Carpenteria*], etc.
> 2. *Hydrangées*—Des raphides. Fleurs de la périphérie stériles. Capsule loculicide ou baie: Hydrangée [*Hydrangea*], Broussaisie [*Broussaisia*], Décumarie [*Decumaria*], etc."

Nakai (1943); Moldenke (1944); Gundersen (1950), who says he would include forty genera but names only a few; Metcalfe and Chalk (1950), who name only the genera they are describing; Skottsberg (1940); Boivin (1956); Cronquist (1957); and Hutchinson (1959). Between them they name twenty-two genera, but I have no information about two of these (*Fendlerella*—mentioned only in a personal communication by Cronquist, and *Pterostemon*—included only by Gundersen?). That leaves twenty genera, most of which I have examined myself for raphides, a few of which have been examined by others. It seems that raphides are present in seven of these genera and absent or probably absent from thirteen. They occur in *Hydrangea* itself and if we assume occurrence of raphides to be a family character then the seven raphide-bearing genera (*Broussaisia*?, *Decumaria*, *Deinanthe*, *Dichroa*, *Hydrangea*, *Pileostegia* and *Schizophragma*) might be considered to constitute the family. It is amusing to score taxonomists on their success in including raphide-bearing genera (A) in and excluding raphideless ones (B) from the Hydrangeaceae, giving the results as a fraction A/B. The "ideal" would be $A(7)/B(0) = \infty$. Nakai has a score of 3·5 (that is he includes all seven of the raphidean genera and only two of the non-raphidean ones and thus gets $7/2 = 3{\cdot}5$). In Table III we give the full list. It will be seen that several taxonomists include all seven of the raphidean genera but most of them are "guilty" of including non-raphidean ones as well. It would be of the greatest interest, of course, to include other chemical characters, too, and we hope to do that at a later date. It may be mentioned in passing that the presence or absence of cyanogenetic glycosides in these genera (they occur in *Hydrangea* itself, for example) does *not* seem to be closely correlated with presence or absence of raphides.

TABLE III

Raphides and the Hydrangeaceae

	A Genera with raphides (7)	*B* Genera without raphides (13)	Score *A*/*B*
"Ideal"	7	0	∞
Nakai (1943)	7	2	3·50
Hutchinson (1959)	5	2	2·50
Skottsberg (1953)	4	3	1·33
Van Tieghem and Constantin (1918)	(3)	(3)	(1·00)
Bessey (1915)	(1)	(1)	(1·00)
Martius (1835)	(1)	(1)	(1·00)
Dumortier (1829)	(1)	(1)	(1·00)
Moldenke (1944)*	7	9	0·78
Boivin (1956)*	7	9	0·78
Metcalfe and Chalk (1950)	6	8	0·75
Lindley (1853)	3	4	0·75
Cronquist (1957)*	7	10	0·70
Gundersen (1950)	(1)	(6)	(0·17)

Bracketed figures indicate that only partial lists are available.

* Genera included confirmed by personal communication, 1962.

As we were completing this section a most interesting paper by Tomlinson appeared (1962). He is using morphological and anatomical evidence as aids in working out the taxonomy an dphylogeny of the Scitamineae. In the eleventh edition (1936 by Diels) of "Engler's Syllabus der Pflanzenfamilien" the Scitamineae is considered to be an order of four families—Musaceae, Zingiberaceae, Cannaceae and Marantaceae. There is at endency in more recent work, to subdivide some of these and so to recognize more families. Thus Hutchinson (1959), who calls the order Zingiberales, has six and Nakai (1941), who uses the name Anomales, is said by Tomlinson (1962) to have eight (I have not seen Nakai's paper).

After discussing some characters which seem rather haphazard in their distribution Tomlinson then says (italics are mine):

> "In contrast to the randomness just discussed, three features suggest that the eight families fall into four natural groups. These are the structure of the guard cells, *the presence or absence of raphide sacs*, and the structure of the root stele. These three features seem to be quite unrelated, and each may thus be considered an independent indicator of taxonomic affinity. The first of the four groups includes *Heliconiaceae*, *Musaceae*, and *Strelitziaceae, which have raphide sacs*, symmetrical

guard cells . . . and anomalous root structure at least in the last two families. The second includes Costaceae, Marantaceae, and Zingiberaceae, which have asymmetrical guard cells . . . *and lack raphide sacs* and anomalous root structure. . . . The fourth group consists of Lowiaceae *with its raphide sacs*, asymmetrical guard cells, and normal root structure."

Tomlinson decides, in discussing the various secretory elements, that raphide sacs are a primitive feature, absent from the more specialized families. It is of interest to note that there is some evidence that raphides are primitive in the dicotyledons, too (see Gibbs, 1954). It seems that Nakai's conclusions are supported by Tomlinson's work.

B. Crystals of Calcium Oxalate other than Raphides

I have restricted my own studies and records essentially to raphides, but the other forms of crystals (mostly of calcium oxalate) occurring in plants have sometimes been used as taxonomic characters, and we may note one or two examples. Thus Jaccard and Frey (1928) and Kharchenko (1928), whose papers I have not seen in the original, found the different shapes of crystals in *Allium* to be useful taxonomically. Very recently Dormer (1961) has studied the "crystals" occurring in the ovary-walls of the *Cynareae* (Compositae). Of some, at least, of them he says:

"These bodies are not simple crystals [of calcium oxalate], but organized inclusions of the cell."

In a later paper (1962) he found that in *Centaurea* the two main shapes ("prismatic" and "curvilinear") of the crystals are very closely correlated with the pollen-types, both not agreeing at all well with the present views on taxonomic relationships within the genus.

C. Lapachol

More than a century ago a yellowish powder in "Taigu" wood from Paraguay was investigated by Arnaudon (1858) who named the substance "acide taigutique". Its structure was worked out much later by Hooker (1892, 1896) and others. It is 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphtho-quinone and is now known as "lapachol". Masses of it may be seen in cells of the woods of a number of bignoniaceous plants (*Tecoma*, *Paratecoma*, *Tabebuia*). It occurs, too, in the mangrove *Avicennia* of the Verbenaceae (or, if one recognizes a separate family, Avicenniaceae). Apart from these its occurrence is uncertain. There are reports of it in *Bassia* (*Illipe*?) of the Sapotaceae, and in several genera (*Amyris*, *Tonka*

and *Adenanthera*) of the Leguminosae, but I have seen no confirmation of these reports. Metcalfe and Chalk (*loc. cit.*) say that except for *Avicennia* lapachol is not known to occur outside the Bignoniaceae; while Thomson, in his book on the naturally-occurring quinones (1957), seems to accept the record of *Bassia*, but does not list the leguminous genera. Lapachol would seem to be characteristic of the Bignoniaceae, at least.

There are many naphthaquinones in plants but they are not visible solids though they may colour tissues. Lapachol, then, is quite unusual in this respect.

D. Silica

Small silica-bodies occur in many plants, and these, like raphides, may be of considerable value to the taxonomist. Metcalfe and Chalk (*loc. cit.*) describe them from a large number of families of the dicotyledons, but only in a few do they seem to be universal or highly-characteristic in shape and distribution. In the monocotyledons, on the other hand, they are important in two of the great families which have recently been monographed from the anatomical point of view.

Metcalfe (1960) has discussed the Gramineae. In this family silica is very prominent in the epidermis of the leaf, where "silica-cells" containing "silica-bodies" have long been known. He distinguishes twenty types of silica-bodies and says:

> "The silica-bodies in the silica-cells assume very characteristic forms when the grass leaf is mature and are of considerable value for diagnostic and taxonomic purposes. . . ."

Tomlinson (1961) deals with the anatomy of the Palmae. Here, too, silica-cells (stegmata) occur, each with a silica-body. Hat-shaped or conical bodies are characteristic of the Bactroid, Caryotoid, Chamaedoroid, Iriartoid, and Nypoid palms, he says, while spherical, rather irregular, and somewhat ellipsoidal types are found in the Arecoid, Borassoid, Cocoid, Lepidocaryoid, Phoenicoid, Phytelephantoid and Sabaloid groups, and in those palms of uncertain position. *Latania* has both types! Tomlinson notes, too, that raphides occur in most, if not in all, palms.

E. Gypsum

Crystals of gypsum ($CaSO_4 . 2H_2O$) occur in some plants, in spite of a statement to the contrary by Poli (1882), and they have been found to be of some use taxonomically. Thus Brunswick (1920) found such crystals in every member of the Tamaricaceae available, but did not

find them in the nearly-related (?) Frankeniaceae and Fouquieriaceae which did, however, have crystals of calcium oxalate.

Gypsum is not confined to the Tamaricaceae. It has been recorded in several members of the Capparidaceae, in the *Compositae*, in the Polygonaceae (*Eriogonum*), and in the Loasaceae (*Mentzelia*).

F. Other Crystals

Solereder (1908) says that all members of the little family Salvadoraceae contain crystals which appear to consist of "an organic salt of lime". I know of no later work on these but it would be interesting to learn of what they consist and if similar crystals ever occur in related families such as Aquifoliaceae and Oleaceae.

G. Starch

That a visible storage-material such as starch, occurring in a vast array of plants, should have attracted attention over a long period, should come as no surprise.

We are told by Reichert (1913) that:

> ". . . our knowledge of this substance had its origin practically in the microscopical examination by Leeuwenhoek in 1716 . . . [but that] . . . Fritzsche . . . seems to have been the first to study the form and structure, and the mechanism of formation of the starch grain."

Fritzsche published a paper "Uber das Amylum" in 1834. In it he describes starch grains from several different plants. Schleiden in his "Principles of Scientific Botany" (translated from the German by E. Lankester, 1849) says that the only previous work deserving of notice is that of Fritzsche. Schleiden lists the forms of starch with which he was acquainted as: "Amorphous" (seeds of *Cardamomum minus*, bark of Jamaica sarsaparilla—but there perhaps due to the drying involved); "Simple Granules" (the majority of plants. He lists many varieties of simple grains); and "Compound Granules" (with several forms).

The works of Nägeli (1858) and of Meyer (1895) have not been available to me, but their classifications of starch grains are listed by Reichert (*loc. cit.*). Muter (*ca.* 1900), whose work, too, has not been seen by the writer, classified starch grains; Reichert saying that he listed them as of potato type, legume type, wheat type, and so on.

Reichert's imposing work of 1913 has an equally imposing title: "The differentiation and specificity of starches in relation to genera, species, etc. Stereochemistry applied to protoplasmic processes and products,

and as a strictly scientific basis for the classification of plants and animals." In the preface to this monograph he says (the italics are his):

> ". . . one may lay down the dictum *that each and every form of protoplasm existent in any organism is stereochemically peculiarly modified in specific relationship to that organism, and that, as a corollary, the products of synthesis will be modified in conformity with the molecular peculiarities of the protoplasm giving rise to them.* It follows, therefore, that if the plastids of any given plant be of different stereochemical structure from those of others, *the starch produced will show corresponding variations, and hence be absolutely diagnostic in relation to the plant.* Abundant evidence will be found in the pages which follow in justification of this statement. Moreover, if such differences are diagnostic, it is evident that they constitute *a strictly scientific basis for the classification of plants.*"

In this, and in another large volume published in 1919, Reichert gives the results of his detailed study of starches. He not only shows that nearly-related plants often have starch-grains which are very much alike in appearance, but that when starches are tested by a variety of means a sort of spectrum of properties may be drawn up for each starch. The spectra when compared may then indicate nearness or remoteness of relationship, the spectra from starches of nearly-related plants being very similar.

There is no doubt that Reichert's conclusions are in part, at least, correct, and that the types of starch-grains present may sometimes yield useful taxonomic information.

H. Cyanogenetic and other Glycosides

Plants manufacture a remarkable variety of glycosides. We may omit di-, tri-, and oligo-saccharides from the present discussion, though they are, of course, of very great importance in the plant.

Of the types that are readily hydrolysable into one or more sugar units and an aglycone—there are many groups. My own list, and it does not pretend to be complete, includes mustard oil, cardiac, saponin, cyanogenetic, coumarin, phenol, flavone, isoflavone, flavanone, naphthaquinone, anthraquinone, alkaloidal, chalcone, dihydrochalcone, catechin, anthocyanidin, xanthone, stilbene, terpene, and furan glycosides. Whole books have been written about them (Armstrong and Armstrong, 1931; McIlroy, 1951, for example). We shall deal here with but one group, those yielding HCN upon hydrolysis.

It was recognized long ago that some plants (a minority) yield under

some conditions hydrocyanic acid, and that this character is of use in taxonomy, while it may also make plants toxic to man and stock. Such plants may be termed "cyanogenetic" and we know today that the hydrocyanic acid comes almost always from "cyanogenetic glycosides".

The earliest reference that I have been able to find to taxonomic use of this character is in Lindley (1830). Here he says that *Amygdaleae* [Amygdalaceae] are:

> "Distinguished from Rosaceae and Pomaceae by their fruit being a drupe, their bark yielding gum, and by the presence of hydrocyanic acid; from Leguminosae by the latter character, and . . . from Chrysobalaneae [Chrysobalanaceae] by their hydrocyanic acid. . . ."

He notes also that cyanogenetic plants may be toxic:

> ". . . as, for example, the Cerasus capricida [*Prunus undulata*?] which kills the goats of Nipal; and the C. virginiana [*P. serotina*?], which is known in North America to be dangerous."

Endlicher, in his great work of 1836–40, also uses presence or absence of HCN as a character. Thus we find:

> "Ordo [family] 273. *Amygdaleae*. Ordo fructus indole, acidi hydrocyanici in foliis praesentia, et ejusdem in pericarpiis nonnullarum copia ab affinibus facillime distinctus, quibusdam notis Terebinthaceas Anacardieas revocat."
>
> "Ordo [fam.] 274. *Chrysobalaneae*. C. in regionibus tropicis totius orbis, imprimis Americae indigenae, ab Amygdaleis, quibus proxime affines, calyce saepissime basi ob adnatum ovarii stipitem inaeqali, staminibus alterius floris lateris imperfectioribus, ovulis et seminibus erectis, nec non acidi hydrocyanici defectu facillime distinguunter."

During the next century a large number of papers appeared recording plants that yield HCN. We may note Jorissen (1884); Lehmann (1885, I have not seen this); Jorissen and Hairs (1891); Lutz (1897); Jouck (1902), who lists more than a hundred cyanogenetic plants; Treub (*loc. cit.*); Guignard (1905, 1906); Henry (1906), who with Dunstan (see below) isolated some of the cyanogenetic glycosides; Greshoff (1906, 1907); Guérin (1907, 1929); Petrie (1913–20), Smith and White (1918), Finnemore and Cox (1929, 1930), and Finnemore and Cooper (1936, 1938), who examined many Australian plants; Mirande (1913); Moran, Briese, and Couch (1940); Plouvier (1948); Gibbs (1945, 1954, 1961); Honeyman (1956); Gardner and Bennetts (1956); and Hegnauer (1958, 1959a, b, 1961a).

Few of these have used cyanogenesis as a taxonomic character, but it is obviously a good character and will become more useful when we know

TABLE IV

Cyanogenetic glycosides

Dates	Glycosides	Constitution or hydrolysis products	Plants	References
1830	Amygdalin	D(−)-Mandelonitrile-β-gentiobioside	*Prunus amygdalus Stokes*	Robiquet and Boutron-Charlard (1830)
1830	Linamarin (*b*) (Manihotoxin (*a*); Phaseolunatin (*c*))	Acetone-cyanohydrin-β-glucoside	(*a*) *Manihot utilissima* Pohl (*b*) *Linum usitatissimum* L. (*c*) *Phaseolus lunatus* L.	Ricord-Madianna (1830) via Karrer (1958) Jorissen and Hairs (1887, 1891) Dunstan and Henry (1904)
1839	Prulaurasin (*b*) (Amorphous amygdalin (*a*))	DL-Mandelonitrile-D-glucoside	(*a*) *Prunus laurocerasus* L.; (*b*) *P. padus* L.	Winckler (1839) via Karrer (1958); Simon (1839); Hérissey (1905). Secondary (?) (Plouvier, 1936, 1937)
1884	Lucumin	Arabinose + HCN + benzaldehyde (?)	*Lucuma mammosa* Gaertn.	Jorissen (1884) (?); Bachstez *et al.* (1948)
1900 (1901)	Lotusin	Lotoflavin + maltose-cyanohydrin (?)	*Lotus arabicus* L.	Dunstan and Henry (1900) (1901)
1902	Dhurrin (*a*) (Phyllanthin (?) (*b*))	β-Glycoside of *p*-hydroxymandelonitrile	(*a*) *Sorghum vulgare* Pers. (*b*) *Phyllanthus gasstroemi* Muell. Arg.	Dunstan and Henry (1902) Finnemore, Reichard and Large (1937)
1903	Corynocarpin		*Corynocarpus laevigata* Forst.	Easterfield and Aston (1903) via Greshoff (1906)

TABLE IV—*continued*

Dates	Glycosides	Constitution or hydrolysis products	Plants	References
1904	Gynocardin	Glucose + a diketone ($C_6H_8O_4$) + HCN (?)	*Gynocardia odorata* R.Br.	Power and Gornall (1904)
1905	Sambunigrin	L(+)-Mandelonitrile-D-glucoside	*Sambucus nigra* L.	Bourquelot and Danjou (1905)
1906	Vicianin	Mandelonitrile-vicianoside	*Vicia angustifolia* Roth	Bertrand (1906)
1907	Prunasin	D(−)-Mandelonitrile-D-glucoside	*Prunus padus* L.	Hérissey (1907)
1935	Acacipetalin	Isobutyric acid + glucose	*Acacia stolonifera* Burch	Rimington (1935)
1936	Zierin	*m*-Hydroxymandelonitrile-β-glucoside	*Zieria laevigata* Sm.	Finnemore and Cooper (1936)
1937	Acalyphin	(?)	*Acalypha indica* L.	Rimington and Roets (1937)
1937	*p*-Hydroxymandelonitrile-glucoside	*p*-Hydroxybenzaldehyde + glucose + HCN	*Goodia lotifolia* Salisb.	Finnemore and Large (1937)
1938	Lotaustralin	Glucose + methyl ethyl ketone + HCN	*Lotus australis* Andr. v. *pubescens*	Finnemore and Cooper (with Stanley) (1938)

TABLE V

Pseudo-cyanogenetic glycosides, etc.

Dates	Substances	Constitution or hydrolysis products	Plants	References
1873	Karakin	Glucose + 3 hiptagenic acid	*Corynocarpus laevigata* Forst.	Skey (1873)
1921	Hiptagin	Perhaps = Karakin	*Hiptage madablota* Gaertn.	Ritsema quoted in Gorter (1921)
1921	Hiptagenic acid	β-Nitropropionic acid	*Indigofera endecaphylla* Jacq.	Gorter (1921), Carter and McChesney (1949), Morris, Pagán and Warmke (1954)
1941	Macrozamin	Primeverose and hydroxyazoxymethane	*Macrozamia spiralis* Miq.	Cooper (1941)
1955	Cyasin	β-Glucosyloxyazoxymethane	*Cycas revoluta* Thunb.	Nishida, Kobayashi and Nagahama (1955)
1959	Neocyasin A	3-*O*-β-D-glucopyranosylcycasin	*Cycas revoluta* Thunb.	Nishida *et al.* (1959)
1959	Neocyasin B	β-Gentiobiosyloxyazoxymethane	*Cycas revoluta* Thunb.	Nagahama, Numata and Nishida (1959)
1960	Neocyasin C	(?)	*Cycas revoluta* Thunb.	Nishida (1960)

what particular cyanogenetic substances occur in the plants we are investigating. In most cases we know only that a plant is cyanogenetic, not what the cyanogenetic substance is. More than a dozen "cyanogenetic glycosides" have now been isolated; it is known that benzyl cyanide occasionally occurs in plants; and that a number of what Lythgoe and Riggs (1949) have called "pseudo-cyanogenetic glycosides" are also to be found. We have listed these, more or less in historical sequence from amygdalin (1830) to neocyasin C (1960) in Tables IV and V. Some of them are known from but one or a few plants; others may be of more general occurrence. Dillemann (1958) says that amygdalin is confined to the seeds of the Rosaceae—and we have noted above that Lindley and Endlicher both would exclude the family Chrysobalanaceae from the Rosaceae, in part because it lacks HCN.

My own records (which include the reports of others) list production of HCN from members of more than eighty families of angiosperms. It is given also by a few gymnosperms (*Taxus*, *Metasequoia*), by several ferns (*Asplenium*, *Lindsaya*, *Schizaea*, etc.), by some fungi (Singer, 1949), and by a myriapode (*Fontaria*) which is said to give off HCN and benzoic aldehyde when excited (Jorissen, 1884)! Is this last made possible by cyanogenetic plants upon which the animal may feed?

There are beautiful examples of chemical relationships here. Every member of the Passifloraceae and of the closely-related Turneraceae of which there are records, or of which I have tested fresh material (*Modecca*, 2 spp.; *Ophiocaulon gummifer* Harv.; *Passiflora*, 38 spp.; *Tacsonia*, 3 spp.; and *Tetrapathaea tetrandra* Cheeseman, of the *P.*: *Erblichia* (*Piriqueta*) *odorata* Seem and *E. Standleyi* Steyermark; *Piriqueta*, 3 spp.; *Turnera*, 6 spp., of the *T.*) yields HCN. On the other hand no member of the Cucurbitaceae or of the Violaceae, so far as I know, has ever been found to be cyanogenetic. There are taxonomists who would group these families with the Passifloraceae and Turneraceae; others who see no such relationship. The latter would seem to find support here.

Almost a hundred years ago Skey (1873) isolated from the nut of *Corynocarpus laevigata* Forst. ("Karaka") a bitter, toxic substance which he named "Karakin". This was shown much later to be a glycoside yielding on hydrolysis glucose and hiptagenic acid (Carrie, 1934; Carter, 1943). Meanwhile another (or the same?) glycoside was isolated by Ritsema and Gorter (1921) from *Hiptage madablota* Gaertn. of the Malpighiaceae, and named "hiptagin". The hiptagenic acid from this was shown by Carter and McChesney (1949) to be β-nitropropionic acid. It is interesting that this last substance seems to occur free in *Indigofera endecaphylla* Jacq. and to be its toxic component (Morris, Pagán and Warmke, 1954). It is produced, also, by *Aspergillus flavus*.

In 1941 Cooper reported the isolation from the seeds of a cycad—*Macrozamia spiralis* Miq.—of a toxic glycoside which she named "macrozamin". This could be hydrolysed by dilute alkali; but not by dilute acids, by preparations from almonds, or by yeast. Acidification after hydrolysis with alkali resulted in the liberation of HCN. The structure of this interesting substance was elucidated by Lythgoe and Riggs (1949) and Langley, Lythgoe and Riggs (1951). It may be general in the cycads for it has been reported in *Bowenia* (probably in both species), in two species of *Cycas*, in *Encephalartos*, and in several species of *Macrozamia* (Riggs, 1954). I do not know that it has been found in the American genera (*Zamia*, *Microcycas*, *Dioon*, *Ceratozamia*) but it is likely to be in these, too.

In the last few years a group of related substances have been found in *Cycas revoluta* Thunb. by Nishida and his co-workers. These are "cycasin" (1955), "neocycasin B" (1959), "neocycasin C" (1960). They seem all to be azoxy glycosides. We may well expect that such an isolated group as the cycads will prove to have some peculiar chemical characters, and if this group of "pseudo-cyanogenetic glycosides" is indeed confined to the cycads, it will provide one example of such chemical characters to set the Cycadales off from other plants.

V. The Use of Certain Techniques in Plant Taxonomy

A. Serology

It seems probable that each kind of living organism has its own set of proteins; that the proteins of nearly-related species are nearly alike; that those of more distantly-related ones are unlike. It has been estimated that variations in structure even in a single protein might be sufficient to provide such differences. Thus Reichert and Brown (1909) say:

> "The possibilities of an inconceivable number of constitutional differences in any given protein are instanced in the fact that the serum albumin molecule may, as has been estimated [by Mieschor, Reichert says elsewhere] have as many as 1000 million stereoisomers."

If, then, a method could be devised to detect such differences in the proteins of plants, it would seem to hold promise of "measuring" relationships. Such a method, it has been claimed, has indeed been devised in "serology" in all its forms. This subject was well-reviewed some time ago by Chester who has a bibliography (in 1937!) of 392 papers. His review has been of great help in preparing the brief account which follows.

Towards the end of the last century it was noticed that extracts of some

plants but not of others, can haemolyse the washed red corpuscles of blood. When an animal is immunized with an active plant extract it may produce anti-bodies capable of inhibiting normal haemolysis. Thus Ehrlich (1891) was able to immunize animals to ricin and abrin. In other cases acquired agglutinins have been demonstrated.

Perhaps the best-known reaction is that known as the "precipitin reaction". There has been much discussion as to the antigens responsible for the production in the animal of precipitins. Most often proteins seem to be responsible, but other substances have been given the credit in some cases, and Chester (*loc. cit.*) says "lipids and carbohydrates also have an important part to play in serological reactions". If one is to make use of precipitin and other reactions in taxonomic studies it is essential that one should work with the substances that are specific to the taxon under investigation. Storage proteins and lipids, for example, may be almost, if not quite identical in relatively distantly-related organisms, and in some cases where immunological differences have been found with these they have been shown to be due to contaminating body proteins.

Kowarski (1901) was among the first to use the precipitin reaction with plant proteins. He injected an "albumose" extract from wheat-meal into rabbits and says:

> "Das Blutserum nun zeigte auf Zusatz der oben beschriebenen Albumosenlösung eine ziemlich starke Trübung, welche beim Stehen oder Centrifugiren einen weisslichen kleinflockigen Niederschlag bildete."

The classic work of Nuttall at about the same time on the animals related to man (1902) brought widespread attention to serological methods. Much of this early work was reviewed by Janchen (1912), but I have not been able to see his papers.

Investigation of plant systematics by serology began on a large scale with the experiments of the so-called "Königsberg school" of Mez and many others. Gohlke (1913), whose work I have not seen, was one of the first of this school. Papers followed in rapid succession, dealing with large groups of plants, and the results were summarized in the "Königsberg Stammbaum" (see Mez and Ziegenspeck, 1926, and Gortner, 1938, pp. 542–5). This "tree", which purports to show the more-or-less exact relationships within the plant kingdom, naturally aroused great interest, and it was criticized, sometimes bitterly, by many people.

In the first place a group of workers in Berlin claimed to "refute" the tree. Notable among the members of this "Berlin School" were Gilg and Schürhoff (1927), but many others were involved (see Table I, pp. 170–1, in Chester, 1937). It is impossible to anyone but a specialist to follow the

controversy between the two schools, and this controversy delayed the acceptance of serology as a taxonomic weapon; for a second group of critics, the pure systematists, were influenced by it, as Chester points out:

> "The attack on the Königsberg work by the Berlin investigators also has had its effect on the acceptance of the Königsberg Stammbaum by systematists, the latter being unversed in serological techniques and often accepting as just criticism the not wholly unprejudiced and sometimes intemperate contradictions of the Berlin School. A consequence of the unfortunate controversy between the two schools has been to impair the repute of the sero-systematic method, although the impartial and soundly scientific attitude of such recent workers as Boom in Holland, Moritz in Kiel, and Krohn in Finland have fortunately had a stabilizing effect on the disturbed state of sero-systematics in Europe."

The work of Krohn referred to above, appeared in 1935 and deals with the "Sympetalae" of the Königsberg Stammbaum. Krohn was careful to follow exactly the methods of the Königsbergers and concludes by saying that his results support the classification in the Königsberg work, in opposition to the publications of the Berlin school.

The third group of critics were such as those mentioned in the last few lines above—men unconnected with the two schools, who made honest efforts to understand the points at issue and to judge between the contestants by studying the results of careful and critical experimentation.

Chester, whose own early work (1930–3) was in this general field, marvels at the spectacle:

> "It is indeed remarkable that, while thirty investigators at Königsberg all found the serological methods suitable for the study of plant relationships, a score of workers at Berlin have nearly all come to opposite conclusions, stated in the following terms of Gilg and Schürhoff [the translation from their German is ours]; '. . . serodiagnosis is entirely useless for botanical research on relationships. We are of the opinion that this single reflection suffices to show that one cannot attach far-reaching deductions to the results of unspecific reactions'."

He believes that much of the trouble was due to differences in techniques—the Berlin group, in their anxiety to interfere as little as possible with the original plant proteins, not eliminating many of the interfering substances. On the whole he strongly favours the Königsberg school and says:

> "In no case is it to be concluded that the Berlin work constitutes a decisive refutation of that of the Königsberg school."

There seems to have been a revival of interest in recent years in plant serology. We have time only for a few brief references—and our selection is not critical.

Hyun (1949), whose work is only available to me through his summary in *Biological Abstracts*, studied extracts from germinating seeds of fifteen species of *Quercus* and found general agreement between serological results and traditional taxonomy. He includes some work on the related genus *Castanea*.

An active group has been studying serology at Rutgers during the past few years, and some of their energy has been devoted to the higher plants. Boyden, in 1954, introducing three of these papers—on Magnoliaceae, Cucurbitaceae and Solanaceae respectively—says:

> "Since the earlier extensive work in plant serology of Mez and associates and the contemporary studies of the Berlin workers . . . there seem to have been published no significant studies by serological means of the higher plants."

This is in line with our own observations.

The first of the three papers introduced by Boyden is that of Johnson (1954) on the Magnoliaceae. He studied *Magnolia*, *Michelia*, *Talauma*, *Liriodendron* and *Illicium* and concluded that the first three are serologically fairly close to one another, that *Liriodendron* is serologically distinct from these, and that *Illicium* might well be placed elsewhere. This is in line with Dandy's (1927) treatment of the family. He would make *Liriodendron* the sole member of a tribe Liriodendreae, and would exclude *Illicium*.

The second paper, by Baum (1954), dealing with the Cucurbitaceae, calls for no further mention here. The third of this series, by Hammond (1955b), is concerned with the Solanaceae, and reports work originally done some eight years earlier. It suggests that further work is desirable from all viewpoints, on relationships within the family.

Hammond has another paper in this field, on the Ranunculaceae (1955a). In this he uses serological, morphological, and cytological evidence to decide upon degrees of relationship between genera. He concludes that much work, with more genera and many more species, needs to be done—a conclusion that we must draw all too often in our work in comparative phytochemistry!

Moritz and his co-workers have made a whole series of contributions to our subject from about 1928 on. In 1956 he and Rohn studied members of the Rhoeadales (*Chelidonium*, *Papaver*, *Glaucium* and *Sanguinaria* of the Papaveraceae; *Capparis* and *Cleome* of the Capparidaceae; *Sinapis* and *Sisymbrium* of the Cruciferae; *Reseda* of the Resedaceae; and *Moringa*

of the Moringaceae) and concluded that these families form a natural group. This is of interest in the light of Hutchinson's (1959) treatment of the same families, the non-serological but plant biochemical paper by Hegnauer (1961b) on the *Rhoeadales*, and our own long-standing concern with the order (unpublished). Yet another paper, this time by Moritz and Frohne, appeared in 1958.

Gell, Hawkes and Wright have used immunological techniques in their studies of *Solanum* (1960). They were concerned with fifteen spp. from Mexico, and twenty-two from South America and they say that their methods:

> ". . . depend on the fact that different antigen-antibody systems in a mixture form separate bands of precipitate when allowed to diffuse toward one another in a thin sheet of agar gel. Thus, a characteristic 'spectrum' of precipitin lines or bands is formed for every species or group of species, and further, one species may be directly compared with another by observing how many of these lines join up when protein extracts of each are placed side by side and allowed to diffuse towards a common antiserum."

They conclude that:

> "The results show in their main outline a remarkably close agreement with those obtained from the classical taxonomic methods, and with the general conclusions arrived at from cytological and genetical studies."

A paper on advances in the serology of fungi by Seeliger appeared in 1960. He says that when he collected the papers in this field in 1958 there were already about five hundred, and that some fifty have appeared in the next two years! He uses the black yeasts and some other yeasts to illustrate the applications of serology to the systematics of the fungi.

To bring us right up to date we may report further papers from the Rutgers group whose earlier work was referred to above. Fairbrothers and Johnson (1961) used the precipitin reaction as an indicator of relationships of some grasses. Finally, an abstract of a paper presented at the "International Conference on Taxonomic Biochemistry, Physiology, and Serology" held 4–6 September 1962, has come to hand and may be noted. This is also by Fairbrothers and Johnson and deals with some members of the Cornaceae and Nyssaceae. The full paper is to appear later. It is not out of place, perhaps, to note that our own work (unpublished) upon the comparative chemistry of the Cornaceae suggests that it is a very mixed group that is by no means fully understood. It requires study from all viewpoints.

B. Chromatography

Progress in any field is made possible by the tools available to the workers in that field, and progress in comparative phytochemistry has been vastly speeded up as chromatographic methods have been developed and applied.

The varied techniques of chromatography are so much used today that we are apt to take them for granted. We are apt, too, to regard them as of very recent origin. Actually if we include the use by Pliny (lived A.D. 23–79) of papyrus impregnated with an extract of gall-nuts (tannins) for the detection of ferrous sulphate, they date back some nineteen hundred years!

The real beginnings are, perhaps, a century ago in the work of Schönbein (1861) and those who followed him, such as Goppelsroeder (1901), on "capillary analysis". But even this did not lead directly to the popular techniques of today. We have to await for Day and Tswett and for the taking-up of their work by still other investigators before chromatography grows up. Day, in a paper on the origin of Pennsylvania petroleum dated 1897, noted the change in colour of the oil when filtered through finely divided clay. In a later paper (1903) he used this method of "fractional diffusion" and at his suggestion Gilpin with several others pursued the matter further (1908, 1910, 1913).

Independently of Day work in substantially this field was being pursued by Tswett. His earliest paper (1903) was in Russian. Later (1906a and b) he published two papers in German on the separation of plant pigments, and his work (we translate) has a very modern sound:

> "There is a certain adsorption-series by which substances can be arranged. On this law rests the following important application. If one filters a petrol-ether solution of chlorophyll through an adsorption column (I used chiefly calcium carbonate, densely packed in narrow glass tubes) the colouring matter is separated into zones from top to bottom, according to the adsorption series. . . . This separation becomes practically complete, if after the passage of the coloured solution into the adsorption column, one then uses a stream of the pure solvent. . . . Such a preparation I call a chromatogram and the corresponding method a chromatographic method."

Even after this there was no rush to use chromatography. Block, Durrum and Zweig, in the second edition of their manual of chromatography (1958), say that in their opinion, "the great popularity of the present-day chromatography is due . . . to A. J. P. Martin of Cambridge

and London and his co-workers, R. Consden, A. H. Gordon, and R. L. M. Synge." Let us note briefly the contributions of these men.

In 1941 Martin and Synge published two papers on the separation of amino-acids by liquid–liquid counter-current techniques. Three years later Consden, Gordon, and Martin described a paper chromatography method, using water in cellulose (filter-paper) as the stationary phase. They found phenol, collidine and n-butanol : benzyl alcohol mixture (1 : 1) to be useful as mobile phases. They also introduced two-dimensional chromatography. To detect the amino acids they employed ninhydrin.

After this came the flood! It is no exaggeration to say that chromatography in all its forms has provided a tool which for usefulness may be likened to the microscope. In the use of this tool plant biochemists and plant physiologists have played big parts: and this seems only natural since much of the earliest work was done on plant materials.

Recent examples of chromatographic work are mentioned elsewhere in this paper.

VI. Some Phytochemical Studies at the Genus Level

It would be nice to include in this historical survey a systematic account of the application of comparative phytochemistry to taxonomic groups of different levels—classes, orders, families, genera, species. We have mentioned the work of Moritz and Rohn on the Rhoeadales (p. 67), and our own preliminary survey of the Tubiflorae (Gibbs, 1961) is a second example of application at the order level. The serological papers by Johnson (1954), Baum (1954), and Hammond (1955a, b) mentioned above (p. 67) are examples at the family level, while the discussion of protopine (p. 48) involves the possible splitting of a family.

We shall deal here with *Eucalyptus* and *Pinus* to illustrate attempted applications of phytochemistry on a large scale to the taxonomic problems of large and difficult genera.

A. Eucalyptus (L'Heritier, 1788)

The trees known as *Aromadendron* (*Aromadendrum*) (1840)—names having obvious reference to a chemical character, the aromaticity of the leaves and twigs—are now included in *Eucalyptus*. The genus is practically confined to Australia, only a handful of its hundreds of species occurring outside the continent. In Australia it grows almost everywhere —from the Karri forests of the south-west, to the hardy species of Tasmania and the Snowy Mountains of south-east Australia, and the Ghost Gums of central Australia and to the north.

The earliest known chemical work on *Eucalyptus* has been called to my attention by an interesting and informative article—"Chemistry of the Australian Bush" by Read (1944). This is in the Appendix to White's (1790) "Journal of a Voyage to New South Wales, etc.", where we find under the name *Eucalyptus piperita*:

> "The name of Peppermint Tree has been given to this plant by Mr. WHITE on account of the very great resemblance between the essential oil drawn from its leaves and that obtained from the Peppermint (*Mentha piperita*) which grows in England. This oil was found by Mr. WHITE to be much more efficacious in removing all cholicky complaints than that of the English Peppermint, which he attributes to its being less pungent and more aromatic. A quart of this oil has been sent by him to Mr. *Wilson*."

Thus one of the first specific names in the genus is indicative of a chemical character, and a cursory glance through my list of species (not at all complete) reveals other similar names—*astringens, camphora, citriodora, dextropinea, laevopinea, melliodora, resinifera.*

The Australians early realized that *Eucalpytus* is one of their great assets. Much has been done to unravel the taxonomy of the genus, but it is very imperfectly understood even today. We can refer here only to a selection of the chemical work which has contributed so largely to our present knowledge.

Maiden was one of the pioneers in the study of plant products in Australia and we find him writing of "tan-substances" in 1888, including *Eucalyptus* and the related *Angophora* (see below) among the genera investigated. Many Eucalyptus yield kinos and the nature of these may aid in classification, as Maiden, Smith and the two together point out in a series of papers (1889, 1892, 1895, 1897). Such kinos are still being studied (Hillis, 1951, 1952).

From about 1897 we find Baker and Smith investigating the essential oils of *Eucalyptus* and its relatives. They soon realized that there is a close connection between the chemistry of the oils and the taxonomy of the plants yielding them. In 1901, they remark that the venation of the leaves of the Eucalypts known as "Blood woods" is characteristic also of species of *Angophora* and say:

> "The chemical evidence shows that the connection with the Angophoras is directly associated with those Eucalypts that have this particular venation in their leaves.
>
> "This venation . . . appears to be indicative of a predominance of pinene in the oil . . . while phellandrene is quite absent, and in the oil

that we distilled from the leaves of *Angophora lanceolata*, pinene was also found. . . ."

In 1902 Baker and Smith published their book, "A research on the Eucalypts, especially in regard to their essential oils". This dealt with well over 100 species. A second edition followed in 1920, summarizing work on 176 species! I have discussed this work briefly in a previous paper (Gibbs, 1958).

Baker and Smith concluded that *Eucalyptus* arose in the north from *Angophora* and spread southward; that primitive species had (and have) feather-veined leaves and much pinene; that more advanced ones have intermediate venation and essential oils with pinene and cineole; and that the most recent species have butterfly-wing venation and oils which contain phellandrene, piperitone, and/or geranyl acetate.

Baker and Smith considered the chemistry of any one species to be comparatively stable and frequently it is, but later work has shown that there is sometimes great variation. This is brought out in some of the papers of Penfold and Morrison. In 1928–30, for example, they discuss the chemical varieties of *Eucalyptus dives* Schauer, recognizing a Type. and vars. A, B and C:

"It was, therefore, apparent that several varieties or forms of *Eucalyptus dives* existed, distinguishable only by chemical means. We are perfectly satisfied that morphologically the trees are all undoubtedly *E. dives*, which opinion is founded not only on our own field observations, but upon careful examination made by experienced botanists."

We have summarized their findings in Table VI.

TABLE VI

Composition (as percentages) of the essential oils of chemical varieties of *Eucalyptus dives* Schau. (Penfold and Morrison, 1928–30)

	Piperitone	Phellandrene	Piperitol	Cineole
Type	40–50	40	—	—
Var. A	5–15	60–80	Present	—
Var. B	10–20	Present	—	25–50
Var. C	< 5	Little or none	—	45–75

Penfold and Morrison (1928) used differences in the essential oils to

separate *E. micrantha* from *E. haemastoma* and (in 1934) recognized a piperitone-rich variety of *E. micrantha*.

McNair, whose work we have considered in some detail elsewhere in this paper (p. 47), get sin on the Eucalypt story, too, with a publication in 1942. It is largely a discussion of the work of Baker and Smith referred to above, but has a note on the idea that ontogeny recapitulates phylogeny:

> "The *Eucalypti* furnish an excellent chemical counterpart to the morphological theory of Haeckel that ontogeny recapitulates phylogeny. . . . Oil from the younger seedlings contains more d-pinene and less cineole (that is more hydrocarbon and less oxygenated products) than does that from the saplings two or three years old, and the maximum cineole content is reached in the oil collected from older trees."

We shall meet this idea that saturated organic compounds are more "primitive" than unsaturated ones again below (p. 74).

Eucalyptus is economically important not only for its essential oils but also for its wood, and much work has been done upon the structure and chemistry of the latter. We may mention here the papers by Cohen (1935) on the identification of coloured woods of the genus by chemical tests, and by Hillis (1956) on leucoanthocyanins as possible precursors of the extractives of the wood. Among recent work is that of Hathway (1962) who used chromatography to study the occurrence of *cis*- and *trans*-resveratrol and the corresponding glycosides in the heartwoods of Eucalypts of the sub-section Longiores. They were found to be present in many species but to be absent from *Eucalyptus guilfoylei* of the sub-series Neocorymbosae and from species placed in the sub-series Ochrophloiae (both of the series Corymbosae), and from the series Transversae. Hathway says that *E. guilfoylei* might be transferred to the Transversae and that the Ochrophloiae might be removed from the Corymbosae. These changes, he feels, would be in agreement with the views of Carr and Carr (1959).

The Eucalypts have been dealt with in detail by Penfold and Willis in their recent monograph (1961). It is disappointing to find that they make but little use of comparative chemistry in the taxonomic treatment—prompted, no doubt, by the observations of Penfold and Morrison (above) that in some species there is very considerable variation in chemistry. They use, almost without change, the system based primarily upon stamen types, of Blakely (1955).

It is to be hoped that some synthesis of knowledge from the various viewpoints may lead ultimately to a better understanding of this remarkable and difficult genus.

B. Pinus (Tourn.) L.

Eucalyptus, which we have discussed above, is an angiospermous genus native to the southern hemisphere. *Pinus*, a genus of about 100 spp., is gymnospermous and confined to the northern hemisphere. Confined is, perhaps, not the right word, for it ranges from well within the Arctic circle (*Pinus sylvestris* in northern Sweden) to the Philippine Islands (*P. insularis*) and Mexico (several species). It probably does not occur in Borneo despite early reports to the contrary, and may not cross the equator, though Mirov (1961) includes a station for *P. merkusii* just south of the equator in Sumatra. Classification within the genus is not easy, and many pines have been variously treated—considered to be identical, or treated as varieties, or as distinct species.

They are economically important for their resinous exudates—the basis of the "naval stores" industry—and for their woods, and it is natural that their chemistry has been much investigated. The most extensive work has been upon their volatile "gum turpentines", and upon heartwood constituents. We may note, too, the less extensive work on alkaloids, on leucoanthocyanins, and on cyclitols.

The systematic examination of the turpentines has been largely the task of Mirov and his associates. We shall not deal here with the pre-Mirov work. Mirov's first paper in this field (with Foote) appeared in 1933, and considered the turpentine of *P. monticola*. In 1938 he discusses the phylogenetic relationships of *P. jeffreyi* and *P. ponderosa*. Here we find reference to the possible value of degree of unsaturation as a phylogenetic guide. Mirov (1938) quotes Simonsen and Rau (1922) as suggesting that saturated constituents are evolutionarily older than unsaturated ones. The iodine value is a measure of unsaturation of fats and oils, and Mirov quotes figures obtained by Ivanov, Adams and Holmes, and himself for several gymnosperms, in support of Simonsen and Rau's theory. He then gives iodine values for oil samples from the seeds of *P. jeffreyi* (av. 134) and *P. ponderosa* (av. 151) in line with the view that the former is "older" than the latter.

In 1942 Mirov has a paper on simple biochemical tests for differentiation of species of pine. Other papers followed in 1946 (two) and 1948. The last is a review in which Mirov discusses our knowledge of pine turpentines at that date. He refers to the earlier work of Dupont, Simonsen, Schorger and others relating turpentine chemistry to taxonomy. Several interesting examples of pine chemistry are discussed, such as the biochemical differences between *P. jeffreyi* and *P. ponderosa* which are morphologically so alike; the fact that *P. monticola* has n-undecane in its turpentine while the very similar *P. strobus* has none; that *P. pithyusa*

and *P. halepensis* are considered by Shaw (1914) to be identical though turpentine of the former has 70% L-α-pinene and 24% Δ^3-carene and that of the latter 95% D-α-pinene. Mirov concludes that in 1948:

> "It would be a futile task even to attempt to devise a biochemical classification of pines that would replace the existing botanical classification, but at the same time the biochemical characters of a pine may well be used to establish or clarify relationships that are not discernible by morphological characters alone."

Further papers followed in 1949 and 1953 (on taxonomy and chemistry of the white pines), in 1954, 1955 (at least two), 1956 (one on lodge-pole × jackpine hybrids, and several others with Iloff,) 1958, 1959 (with Stanley), and in 1961. This last is a bringing together of Mirov's work on *Pinus* and is a very interesting little book of some 158 pages. It deals with the gum turpentines of no fewer than ninety-two species and two varieties! Few genera of comparable size can have been so thoroughly investigated.

Mirov (1961) has followed Shaw's (1914) treatment of the genus in general but finds it advisable in the light of his knowledge of turpentine chemistry to make a number of taxonomic changes. We quote:

> "Essentially, the species are arranged according to the system used in Shaw's monograph of 1914 . . . which contained 66 species. Since that time, Shaw's treatment of the genus *Pinus* has somewhat changed. Several new pines have been described . . . and some 20 pines have been elevated from varietal to species rank.
>
> "In the light of recent investigations [by Wu, 1947] I prefer, instead of the highly heterogeneous *Pinus chinensis* . . . to use the name *P. tabulaeformis*.
>
> "I considered *Pinus jeffreyi* and *P. oaxacana* as valid species and moved them from the group Australes to the group Macrocarpae. Then I moved the whole group Macrocarpae from the end of Shaw's classification closer to the head of the subgenus Diploxylon, so that instead of Shaw's classification: Leiophyllae, Longifoliae, Pineae, Lariciones, Australes, Insignes, and Macrocarpae, I arranged the Diploxylon pines in the following order: Longifoliae, Leiophyllae, Pineae, Macrocarpae, Lariciones, Australes, and Insignes.
>
> "Such a re-arrangement of the pines is not as drastic as it may appear. As a whole Shaw's system has been retained, but the re-arrangement places all aliphatic hydrocarbon pines together, and closer to the monospecific group Pineae. . . . Shaw's arrangement of the subgenus Haploxylon . . . has not been changed."

The heartwood constituents of woody plants are of great complexity—and of great taxonomic interest. Much work has been done on those of the conifers in general and we may note here that done on *Pinus* in particular. It has been summarized by Erdtman in a chapter "Organic Chemistry and Conifer Taxonomy" in "Perspectives in Organic Chemistry" (edited by A. Todd, Interscience, New York, 1956). I had hoped, when arranging a symposium for the 1959 Botanical Congress held at Montreal, to get Mirov and Erdtman together, but that did not prove possible. As far as I know there has been no attempt as yet to synthesize their work and to relate it, with other chemical investigations, to the taxonomy of *Pinus*.

It is comforting to read how cautious Erdtman is in applying chemical data to taxonomy:

> "Finally, chemical arguments should be taken only as contributions to a taxonomic discussion which are valuable because they represent a completely different approach. Nothing can discredit the chemical approach to taxonomy more than the uncritical over-estimation of the chemical method."

He found constant chemical differences between the Haploxylon and Diploxylon pines and says:

> "The observed differences between Haploxylon and Diploxylon are of such a nature that the Haploxylon pines have an oxidation–reduction system at their disposal which has disappeared or is defective in the case of the Diploxylon pines. Since 'loss' mutations are more common than progressive mutations it is probable that Haploxylon is more primitive than Diploxylon."

The Gymnosperms are not rich in alkaloids but they are said to occur in *Keteleeria*, *Picea*, *Taxus*, *Cephalotaxus*, *Podocarpus*, *Ephedra* and *Gnetum* (Willaman and Schubert, 1961). Only in a few cases are the actual alkaloids known. *Pinus* is said to have pinidine and (+)-α-pipecoline, both of which are members of the "pyridine group". Tallent, Stromberg and Horning (1955) obtained positive tests for alkaloids (using Mayer solution and silicotungstic acid) in extracts of fresh leaves and twigs from four species of *Pinus* (both reagents); from a further five spp. (silicotungstic acid only); while another eighteen spp. were negative to both reagents. Pinidine was found in three species, and Tallent and his coworkers say:

> "It is a striking fact that the three species found to contain appreciable amounts of pinidine (*P. sabiniana*, *jeffreyi*, and *torreyana*) are

also unusual in that they do not contain bicyclic terpenes; both α- and β-pinenes, as well as Δ^3-carene, are absent from the turpentine fraction of these pines."

Kariyone and his co-workers have also published on the occurrence of alkaloids in conifers (1956). They tested eighty-five species and say that 11 had alkaloids—including four species of *Pinus* in which alkaloids had not been reported before. Hsu (1958) has also tested conifers for alkaloids, and reports that they may be present in yet one more species (*P. massoniana*). Obviously much remains to be done in this field.

Recent work on the leuco-anthocyanins of *Pinus* has appeared in a paper by Krugman (1959). This is rather ambiguously titled "The leuco-anthocyanin distribution in the genus *Pinus*", which led me to hope that it might help in taxonomy within the genus. Actually Krugman investigated needles, bark, and stem-wood of some thirty-seven species; roots of seven of these; and pollen of twelve. He found leuco-anthocyanins yielding cyanidin and delphinidin in the needles and bark of all species; while the roots and stem wood contained leuco-anthocyanins giving cyanidin only. He found no leuco-anthocyanins at all in the pollen. While this does not help us in intra-generic taxonomy, it does suggest that possession of leuco-anthocyanins yielding cyanidin and delphinidin is a generic character of *Pinus*.

Plouvier must be one of the most active workers in the field of comparative phytochemistry, producing since 1936 (at least) a stream of papers which describe the detection, isolation, and identification of many plant constituents. What is more Plouvier usually follows up his initial work by studying the distributions of the substances isolated and using the facts so assembled as taxonomic weapons. Our field would be the richer if it had more phytochemists like Plouvier.

Several of his papers deal with cyclitols such as those of the Sapindaceae (1948, 1949b); pinitol of the Leguminosae (1949a, 1950a and b, 1955c) and of the conifers (1952, 1953, 1957, 1958b); liriodendritol of the Magnoliaceae (1955a); bornesitol and ononitol (1955b, 1958a); sequoyitol, dambonitol, viburnitol and quebrachitol (1960); and, most recently, leucanthemitol (1962).

We shall concern ourselves here only with pinitol and sequoyitol. Plouvier has found pinitol in the following conifers: Cephalotaxaceae—*Cephalotaxus* (2 spp.); Cupressaceae—*Chamaecyparis* (3), *Cupressus* (2), *Libocedrus* (1), *Thuya* (2) and *Thujopsis* (1); Pinaceae—*Abies* (10), *Cedrus* (3), *Larix* (2), *Picea* (15), *Pinus* (26), *Pseudolarix* (1), *Pseudotsuga* (1) and *Tsuga* (4); Taxodiaceae—*Cryptomeria* (1), *Cunninghamia* (1) and *Sequoia* (2). Thus it is very common, if not universal, in the Pinaceae,

including *Pinus*, and may be general also in Cephalotaxaceae, Cupressaceae and Taxodiaceae. It appears to be absent from the Araucariaceae Podocarpaceae and Taxaceae.

Plouvier says it occurs in at least one cycad (*Cycas revoluta*) and in *Ginkgo biloba*. It is not restricted to the Gymnospermae, being found in many members of the Leguminosae, in the *Cistaceae*, and perhaps elsewhere.

Sequoyitol (or sequoitol, both spellings appear) was looked for by Plouvier more particularly in those genera having little or no pinitol. He found it in all families of gymnosperms except Ephedraceae, and (he says) it is *only* in gymnosperms. He found it in old male cones of the Austrian pine. It had been found by others in the heartwood of *Pinus lambertiana* and in pollen of *P. montana*.

VII. The Present

In the above pages we have tried first of all to give some idea of the early history of our subject—the past. Then, to illustrate a few of the major fields and to give recognition to the workers in those fields, we have added notes on visible chemicals used as taxonomic characters, on substances yielding HCN, on two important techniques—serology and chromatography, and, finally, two examples of studies at the genus level.

It is becoming increasingly clear that the higher plants, at least, have, chemically speaking, a rather uniform basic pattern. We feel strongly that they must be monophyletic. Within this basic uniformity, however, we may recognize relatively minor differences that are detectable by serology, for example.

It seems not unlikely that the body lipids in higher plants are relatively uniform, too. Storage fats and oils, on the other hand, seem to vary widely and we might well have based a large section of this paper upon the known occurrence and distribution of the fatty acids of the fats in the seeds of plants and the application of this knowledge to taxonomy. Earlier work in this field was laborious in the extreme, and some of the earlier analyses are suspect. Today, modern chromatographic methods make the investigation of seed-fats much easier and more accurate, and new fatty acids are being found quite frequently. It is invidious to pick out for mention the work of one person rather than of another, but that of Hopkins and Mary Chisholm comes to mind (1959, 1960, 1962).

Certain plants are expert at producing fatty acids with acetylenic linkages and these acids are taxonomically extremely interesting. We note without further comment a long series of papers by Sörensen and co-workers from 1941 on dealing with acetylenic compounds in

the Compositae (summarized in part by Sörensen, 1953); a review on the occurrence of acetylenic compounds in nature by Wailes in 1956; and the papers by Hatt and his colleagues on the acetylenic acids from fats of the Olacaceae and Santalaceae (1954, 1956, 1959, 1960).

Alkaloids, like fats, are of great importance taxonomically, and so, too, are saponins. There is a medical interest in these and surveys of large groups for sources of them are not uncommon. We cannot deal with them here.

Glycosides have been referred to above (p. 58) and we have considered one group of glycosides—those that are cyanogenetic—in some detail. Many other groups have received attention in recent years, but most of these, again, must be ignored in this paper.

The phenolic substances that occur in plants are legion and some of them are of restricted distribution. Many of them occur as glycosides. They are easily investigated by chromatographic methods and consequently are receiving much attention. This is evidenced by the holding of a Plant Phenolics Group Symposium in 1960, the proceedings of which appeared under the editorship of Ollis (1961). The most interesting paper in this to the writer is that by Dreiding on the betacyanins.

For many years it has been known that the red pigments of beetroot (*Beta*) and of some related plants contain nitrogen and so are not normal anthocyanins. They have been called "nitrogenous anthocyanins", and recent work confirms that they do contain nitrogen. They are now called betacyanins. Their restriction to a group of families included in, or near to, the Centrospermae is a striking example of chemical relationship which I have discussed in an earlier paper (Gibbs, 1945), and which has been the concern, too, of Reznik (1955). It is remarkable that betacyanins and anthocyanins seem never to occur in the same families. Dreiding lists the known occurrence of the betacyanins as follows:

Chenopodiaceae—*Beta*, *Chenopodium* (4 spp.), *Atriplex* (3), *Corispermum*, *Kochia*, *Suaeda*.

Amaranthaceae—*Amaranthus* (5), *Celosia* (4 or 5), *Alternanthera* (6), *Mogiphanes*, *Aerva*, *Iresine* (2), *Gomphrena*.

Nyctaginaceae—*Oxybaphus*, *Bougainvillea* (2), *Mirabilis* (2), *Boerhaavia* (2), *Cryptocarpus*.

Phytolaccaceae—*Phytolacca* (2), *Rivina* (2), *Trichostigma*.

Aizoaceae—*Sesuvium*, *Tetragonia*, *Mesembryanthemum*, *Conophytum* (17), *Lampranthus* (2), *Pleiospilos* (2), *Fenestraria*, *Lithops*, *Gibbaeum* (2), *Trichodiadema* (2), *Malephora*, *Dorotheanthus*.

Portulacaceae—*Portulaca* (2 or 3), *Calandrinia*, *Anacampseros*.

Basellaceae—*Basella* (2).

Cactaceae—*Pereskia*, *Mammillaria* (7), *Neoporteria*, *Melocactus*, *Aylostera*, *Hariota*, *Rebutia* (4), *Parodia* (3), *Lobivia* (2), *Cleistocactus*, *Notocactus* (2), *Gymnocalicium* (3), *Ariocarpus*, *Chamaecereus*, *Cereus* (3), *Selinocereus*, *Hylocereus*, *Opuntia* (2 or 3), *Zygocactus*, *Thelocactus*, *Monvillea*, *Nopalxochia*.

The family Caryophyllaceae is conspicuous by its absence from this imposing list. It is noteworthy that raphides, which have been seen in at least four of the above families—Nyctaginaceae, Phytolaccaceae, Aizoaceae, Cactaceae—have not been found in the Caryophyllaceae.

Among the more recent papers on phenolics and their use in taxonomy are those of Swain and Bate-Smith (1956), Bate-Smith and Metcalfe (1957), Bate-Smith (1958, 1961, 1962), and Ibrahim, Towers and Gibbs (1962).

Many of the substances—such as the alkaloids, the cyanogenetic glycosides, the phenolics, and so on, that seem to be so useful as taxonomic guides—have no obvious usefulness to the plant. We are apt to think of them as "secondary substances" which arise, as it were, by accident rather than by design. When we know more of them we may think differently. A paper that is pertinent to this paragraph is that of Fraenkel (1959) which has as its title, "The raison d'être of secondary plant substances". He points out that insects are mono-, oligo-, or polyphagous but that they "never feed on all plants", and he goes on to say of the secondary substances:

> "Their role in the metabolism of plants has never been satisfactorily explained, but in view of their sporadic occurrence [we might differ here!] and of the differences in their chemical constitution, it is almost inconceivable that they play a function in the basic metabolism of plants. . . . It is suggested that the food specificity of insects is based solely on the presence or absence of these odd compounds in plants, which serve as repellants to insects (and other animals) in general and as attractants to those few which feed on each plant species. . . . Most, if not all, secondary plant substances possess characteristic odors or tastes and thus may elicit sensory reactions to the food."

Fraenkel then gives examples of plants drawn from several families, including the Cruciferae and the Umbelliferae (so we are back to the groups recognized by Grew, Petiver and Camerarius!); of the significant secondary substances that they contain; and of experimental work upon their susceptibility or resistance to different groups of feeders.

I have mentioned above the meeting of the Plant Phenolics Group.

It is symptomatic of the tendency today to hold small symposia for groups of specialists in our field. Other examples are the Symposium on Phytochemistry held at Kuala Lumpur (*Proceedings*—Anon., 1957); the Symposium on Biochemistry and Taxonomy held by the Linnean Society of London in July, 1957 (*Proc. Linn. Soc.* **169**, 198–239, 1958); the International Conference on Taxonomic Biochemistry, Physiology and Serology held in September this year in Kansas; and the meeting of which this volume constitutes the Proceedings. There will be others!

Included in the Kuala Lumpur symposium are reports of phytochemical surveys of Australia (Hatt), Hong Kong (Arthur), India (Chatterjee, Chopra and Handa), Indonesia (Bisset), Japan (Kariyone), Malaya (Douglas), New Zealand (Briggs), Philippines (Santos) and Viet-Nam (Chom). These, in part at least, are brief summaries of more extensive work. Thus for Australia we have the survey by Webb (Pts. I and II, 1949 and 1952) and by Simes, Tracey, Webb and Dunstan (Pt. III, 1959); for Papua–New Guinea by Webb (1955); and for New Zealand by Cain, Scannell and Cambie (Pt. I, 1961). A survey for alkaloids in plants of Hawaii, by Swanholm, St. John and Scheuer, appeared in 1959 and 1960.

VIII. The Future

The historian looks back, he considers the present time, and (being human) he likes to wonder about the future. If he is wise he refrains from writing about the future, but he can often be reasonably certain of some features of it.

In our own case we may be sure: that the pace will accelerate; that more and more plants will be investigated as travel becomes quicker and easier; that more and more chemicals will be discovered as techniques for recognition, isolation, and characterization improve; that automation will be necessary to process the vast bulk of information resulting from all this activity.

Will it be a better world for the chemo-taxonomist?

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CHAPTER 4

Some Aspects of Chemotaxonomy

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I. Introduction

During the first International Symposium on the Chemistry of Natural Products, Lord Todd (1961), in his Presidential Address, made some remarks that at first may have disturbed some of his audience. With regard to the structural elucidation of natural products he said: "I doubt myself whether it will nowadays lead to any major advances in science however convenient it may remain from the standpoint of producing exercises for Ph.D. students." It is possible that Lord Todd will not prove to be a true prophet, but, of course, his intention was not to discourage anybody from dealing with such problems in the future.

The elucidation of the structure and configuration of natural compounds will always remain a matter of great importance, but it is true that the main interest in natural product research is now gradually changing from problems of a purely chemical character to those of a biochemical and biological one; this is what Lord Todd wanted to emphasize. It is interesting to compare monographs on plant colouring matters, terpenes or alkaloids, produced fifteen to twenty years ago

with their recent equivalents. In spite of the many variations of the basic structures that have been discovered, the descriptive parts of the modern publications, useful as they are, appear somewhat monotonous.

Very often recent work has simply extended the number of variations of a well-established basic structure. For example, the flavones have been shown to contain from nought to eight hydroxyl or alkoxyl groups attached to the basic 2-phenylchromone nucleus; about a hundred aglycones and almost as many glycosides have been isolated and, probably, there are hundreds yet to be found. The chemical interest in the flavone field has already culminated and the major importance of these compounds now lies in elucidating their mode of biosynthesis, relations to other natural products, distribution in the vegetable kingdom, biological function, and physiological properties. The days have gone when the reputation of a chemist was proportional to the number of structural problems he had solved, just as that of a Bornean head-hunter used to depend upon the number of his trophies.

The elucidation of the structure of a natural product should no longer then be regarded as an end in itself, but as a contribution to the clarification of general biological problems. To state this, does not imply a discrimination against organic chemistry, nor does it denigrate the value of the work done by earlier generations of chemists.

The problem of biosynthesis has always attracted the interest of organic chemists, and early fruits of work in this field were the biogenetic generalizations intimately connected with the names Winterstein, Robinson and Ruzicka. Their ideas, which embrace a certain amount of taxonomic experience, appeared when a number of natural products had been structurally elucidated and the need for unifying principles other than those of a purely systematic, chemical nature became apparent.

Real advances in the field of biosynthesis could only be made after the introduction of the tracer techniques and it is only fair to give credit to the biochemists who, often in collaboration with geneticists, made the fundamental discoveries. Even tracer methods are not free from ambiguities and some organic chemists have certainly shown a tendency to overlook that the successful incorporation of a presumed precursor *per se* only shows that it can act as such and not necessarily that it constitutes a true biosynthetic intermediate.

II. General Principles of Chemotaxonomy

Very early in the development of natural products chemistry it occurred to many botanists and chemists that it should be possible to characterize and classify plants on the basis of their chemical consti-

tuents. It is not surprising that this idea came at an early stage, since many of the first students of natural products were pharmaceutical chemists having considerable biological, and particularly botanical, training. Interest in the relationship between plant constituents and plant classification long remained insignificant, mainly because the number of natural products of known structure was small and the knowledge of their distribution was very scanty. Moreover, botany and chemistry became more and more separated due to increasing specialization of their respective devotees. With our growing knowledge of the structure of natural products and their occurrence in plants the potentialities of "chemotaxonomy" is now becoming increasingly obvious.

The early classifications of plants were artificial and designed to serve practical purposes. After Darwin, "natural systems" founded on real relationships were proposed but even today one is still unable to construct a true "phylogenetic" system. The main reason for this is that the vast majority of extinct species are unknown. The natural systems are essentially based on comparative studies of the genetically controlled, morphological and anatomical (i.e. supermolecular) characteristics of plants. Some of these characteristics are of a very general nature and serve for the separation of large systematic categories such as divisions, classes and orders. Others are less general but suitable for the delimitation of smaller groups of plants; families, genera, subgenera, etc. The classification of plants thus rests upon very thorough considerations of many very different, biological characteristics.

Evolution depends upon a combination of internal and external factors such as mutation, recombination of genic differences and selection. During evolution it may happen that unrelated groups of plants give rise to morphologically similar ones. This is called "convergence" or "parallel development". Conversely, related plants may give rise to very dissimilar descendants ("divergence"). Such phenomena can cause considerable taxonomic difficulties.

Chemical contributions to the classification of plants are based on their chemical constituents, that is, on their "molecular characteristics". These characteristics are genetically controlled, and have the advantage over morphological ones that they can be very exactly described in terms of definite structural and configurational chemical formulae. The elucidation of the structures and configurations of naturally occurring organic compounds paves the way to an understanding of their biosynthesis which is a matter of fundamental systematic importance. The method of "chemical taxonomy" is thus simple in principle, and consists of the investigation of the distribution of chemical compounds, or groups of biosynthetically related compounds, in series of related, or supposedly

related, plants. It is possible that in future the enzymes will be found to be more important for the chemical classification of plants than the low molecular-weight "secondary" products. This implies that some information may ultimately be gained from the investigation of the nucleic acids, but for the time being we shall have to rely on the more "trivial" plant constituents.

The greatest virtue of the chemical method is that it is entirely independent of the classical biological methods. It will therefore be possible for organic chemists not only to assist the botanists but also to check their conclusions and to point out problems which may not occur to them.

An inherent limitation of the chemical method, however, lies in the fact that as a rule, only recent plants can be examined. Moreover, the isolation and structural elucidation of plant constituents is often very difficult and time consuming. Up to the present time the structures of only a few thousand natural products have been established and these obviously represent only a very small group as compared to those that remain to be discovered. Still worse, we know far too little about the distribution of these compounds in Nature.

However, rapid progress in these fields can be expected in the immediate future owing to the powerful analytical methods now available. The isolation of new compounds in a pure form and the routine examination of plant materials is greatly facilitated by the chromatographic, electrophoretic and counter current distribution techniques, and the availability of highly efficient columns for the fractional distillation of mixtures of volatile compounds. Structural work has been simplified by the discovery of new degradative and synthetic methods based on highly specific chemical reactions, deepened insight into the mechanism of chemical reactions, and the introduction of new and powerful physical techniques. For example the combination of gas-liquid chromatography and mass spectroscopy is likely to cause a revolution in several fields of natural product chemistry. We may also expect that many of the most difficult problems will be solved by the X-ray crystallographers.

Many substances such as proteinogenic amino acids, some fatty acids and sugars occur in almost all plants and are therefore of little or no taxonomic interest (Erdtman 1952, 1956, 1959). Enzymatic conditions for their production must have been developed at a very early stage. These compounds are probably as old as life itself. Compounds found in only a single species are also taxonomically useless if not biosynthetically related to plant constituents of intermediate distribution. It is among the latter substances that we may expect to find compounds of the highest taxonomic value.

There are relationships between the "biosynthetic complexity" of a

substance and its taxonomic significance. Many chemical compounds, even those of quite complex nature, may be formed by relatively simple biosynthetic processes. Such substances are, of course, less interesting from a taxonomic point of view than related compounds which have undergone re-arrangements or other secondary changes. Compare, for example, normal fatty acids and their acetylenic analogues; cinnamic acids and lignans; flavones such as quercetin and the highly reduced flavones of *Primula* and *Dionysea* species (both Primulaceae); flavones and the re-arranged isoflavones; isoflavones and rotenoids; isoquinoline alkaloids and bisisoquinoline alkaloids; diterpenes of the normal, regular labdane or pimarane structures and the rearranged or otherwise modified diterpenes of the abietane, totarane and podocarpane type.

Different plants sometimes contain substances which, although belonging to different classes of chemical compounds, appear to be biosynthetically analogous. Such plants probably contain similar enzyme systems, and the compounds which they produce may therefore indicate that a relationship exists between the relevant plants.

Optical antipodes of a compound, or structurally related compounds belonging to antipodal series, have frequently been found in unrelated plants, e.g. (+)- and (−)-borneol, and (+)- and (−)-camphor, sinomenine and the morphine alkaloids, but they have sometimes also been isolated from closely related species. (+)-Spartein, (−)-spartein and (±)-spartein have been isolated from *Cytisus* and *Lupinus*; oenanthotoxin from *Oenanthe* and cicutoxin from *Cicuta*. (+)-Pinene occurs in some pines and (−)-pinene in others, some pines even contain mixtures of both antipodes; (+)- and (−)-δ-cadinol have also been found in different pine species. Some *Podocarpus* species produce (+)-kaurene and others (−)-kaurene and it is interesting to note that some conifers, e.g. *Sciadopitys*, are able to produce both phyllocladene and (−)-kaurene which belong to antipodal series. It would seem very improbable from a biological point of view that closely related species contain very different enzyme systems and the fact that antipodes are sometimes produced by such plants might reflect a spatial flexibility of an enzyme, in principle similar to that of, for example, tri-*o*-thymotide.

Relatively small changes caused by mutations can give rise to large differences in the production of secondary plant products due, for example, to the blocking of some synthetic routes which may thereby become "dormant" for long periods of time. If such changes interfere with the early stages of a biosynthetic route, plants may arise having a very abnormal chemistry. By analogy with similar biological phenomena they could be regarded as examples of "chemical divergence". Such chemically abnormal plants cause great chemotaxonomic difficulties.

Identical compounds are often found in quite unrelated plants and this has frequently puzzled chemists interested in the use of chemical characteristics in plant classification. This phenomenon is not as serious as it may seem. It is easy to conceive that during evolution, conditions for the production of some compounds or groups of biosynthetically related substances have been developed separately in many plants. However, it is highly improbable that totally unrelated plants would be in the possession of the enzymatic prerequisites for synthesizing several chemically unrelated compounds of intermediate distribution.

Chemotaxonomic studies should therefore include the investigation of the patterns of compounds occurring in plants and preferentially in all the various individual parts of plants such as the bark, wood, leaves, roots, cuticles and seeds. The chemical constituents generally vary considerably from one organ to another. Such integrated investigations are necessary in order to obtain really convincing evidence for the relationship or non-relationship of plants. It is always dangerous to draw taxonomic conclusions from the occurrence or non-occurrence of a single compound in a single part of a plant. Although compounds of considerable taxonomic value may be found in any part of a plant, it is reasonable to assume that the most important ones occur in phylogenetically old, conservative, little specialized organs.

Some complications can arise due to the fact that plant organs are not homogeneous and if, for example, a resin acid is not found in the wood of a species where one might expect it to occur, this may be due to the lack of resin ducts in that particular species. It is also advisable to make sure that the organs compared are biologically homologous. For practical reasons chemists often extract whole plants. This reduces the general value of the studies and it is highly desirable that such work should be complemented by an investigation of the localization of the substances isolated.

All living organisms are subject to variation, and different individuals of the same species sometimes differ considerably. Certain compounds may be missing in some of them or occur in such small amounts that they escape observation. This can be due to the effect of soil conditions, or seasonal or climatic factors, and one should therefore always examine several individual plants of the same species, if possible grown under different conditions. Dead tissues such as the heartwood of trees usually show a more constant chemical composition than living organs, since they are much less subject to the influence of environmental factors.

In this connection an interesting observation of changed metabolism due to an infection should be mentioned. Hasegawa and Shirato (1959) have found that the wood of a *Prunus* species which had been attacked

by a fungus (*Coriolus* (= *Polyporus*) *versicolor*) contained considerably fewer flavonoids than the sound wood but, instead, a large amount of a lignan, isoolivil, a compound that normally occurs neither in the wood nor in the fungus.

At the present stage chemists should probably limit themselves to investigating as carefully as possible the various patterns of compounds present in different organs of series of botanically related, or supposedly related, plants. They will often find compounds which are frequently or constantly present in a whole genus or even bridge the gap from one genus to another. Individual compounds or groups of substances may be missing in some species but other constituents may provide the link. In this way a chemical plant classification might ultimately be accomplished. Chemists may also be able to assist the botanists in solving some of their problems such as those due to convergence or divergence. Naturally, it is very improbable that, for example, a morphological convergence would be accompanied by "chemical convergence". The chemical examination of such critical groups of plants should clearly be of great interest.

As mentioned earlier botanists are able on morphological grounds to differentiate, more or less successfully, between large taxonomic categories such as divisions, classes and orders. This is at present generally beyond the capacity of the chemists. Biologists can also discern lines of progression within systematic groups. No chemical analogies are known with certainty, although it has sometimes been argued that there is some relationship between the "complexity" of the chemical constituents and the "lower" or "higher" status of the relevant plants.

Since chemotaxonomy rests on the occurrence of specific substances in plants it may be of interest to discuss briefly a problem which may at first appear strange to chemists and that is how to define a substance in chemotaxonomic contexts.

To a chemist, benzoic acid is C_6H_5COOH regardless of whether it has been isolated from a natural source or obtained by a Grignard reaction, by hydrolysis of benzonitrile or by the oxidation of toluene. However, in chemotaxonomy it is the biosynthesis of benzoic acid which is a matter of very great concern. Benzoic acid formed either from shikimic acid, or by degradation of a larger molecule, or by a cyclization reaction is the result of quite different biochemical processes, and benzoic acid molecules arising from such different biosynthetic pathways must evidently be considered to constitute different objects from a chemotaxonomic point of view. An extreme illustration is benzoic acid from gum benzoin and from hippuric acid isolated from urine.

Lysine is formed in some micro-organisms from α,α'-diaminopimelic acid but in others from α-aminoadipic acid. These lysines are thus

biologically different compounds. We still know very little about the synthesis of lysine in higher plants, but it is at least conceivable that lysine (and many other compounds) is also formed in plants in different ways. If this is the case then chemically identical alkaloids arising from "ontogenetically" different lysines are biologically different. At present we do not know whether this possible complication is anything but a nightmare for it would seem very unlikely that the biosynthesis of a compound in related plants follows very different pathways. However, these reflections may at least serve to emphasize again the fundamental importance of biosynthesis in chemotaxonomy.

III. Applications of Chemotaxonomy

A. Separation of Higher Systematic Categories

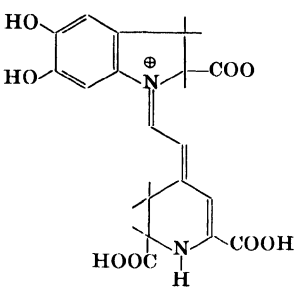
Although there are great differences between the chemistry of bacteria, fungi, and algae there is no possibility of characterizing these categories by chemical tests. It is very unfortunate that the various divisions of the algae have been so little investigated. They constitute an interesting series of plants of which most have remained aquatic, and this is presumably one of the main reasons why they differ considerably from those plants which have been adapted to a terrestrial life.

Bacteria, fungi and algae are all able to produce compounds according to the acetate and shikimic acid pathways of biosynthesis but apparently they seldom combine them; flavonoids, for example, have never been found in any of these organisms. The alleged occurrence of rutin in *Chlamydomonas* has recently been withdrawn (Kuhn and Low, 1960).

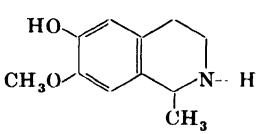
Flavonoids are, however, found in the Bryophyta. Quite recently Bendz and Mårtensson (1961) and Bendz *et al.* (1962) reported the isolation of crystalline anthocyanins from a red *Bryum* species which on hydrolysis gave luteolinidin. It is still uncertain whether the mosses contain substances which could be regarded as lignins, but from *Polytrichum commune* Holmberg (1958) has obtained a product which appears to be of a lignin type. On oxidation with nitrobenzene and alkali it gave large amounts of *p*-hydroxybenzaldehyde, vanillin and syringaldehyde. No lignins have been found in plants lower than mosses. Lignins and flavonoids are common, however, in almost all plant groups higher than the bryophytes. Although ill-defined chemically, the lignins appear to have some very general taxonomic interest. Lignins from gymnosperms, mono- and dicotyledons usually exhibit characteristic chemical differences (cf. Brauns and Brauns, 1960).

There are at least one or two angiosperm orders that seem to be characterized by specific compounds of very general occurrence. Apart

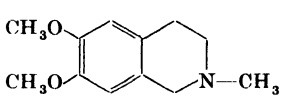
from the Caryophyllineae, all the Centrospermae contain betacyanins (Dreiding, 1961), highly coloured substances which were long supposed to be related to the anthocyanins. The Caryophyllineae contain anthocyanins and it is possible that they should be separated from the order Centrospermae. From a systematic point of view the betacyanins, "nitrogenous anthocyanins", have been investigated by the Robinsons and others, but it is only recently that some insight into their structure has been achieved, thanks to the painstaking work of O. Th. Schmidt and A. Dreiding. The best known of these pigments is betanin, from red beet (Chenopodiaceae) and Dreiding has proposed the unique structure (I), or a closely related alternative, for its aglycone, betanidin (Whyler and Dreiding, 1962; Mabry *et al.*, 1962).



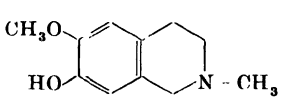
(I)
Betanidin



(II)
Salsoline



(III)
Carnegine



(IV)
Corypalline

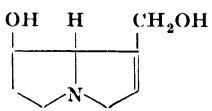
Betacyanins, even betanin itself, also occur in the order Opuntiales (Cactaceae) and this is important since it has long been assumed by botanists that the orders Centrospermae and Opuntiales are phylogenetically related.

There are also other chemical connections between those two orders of which the occurrence of the isoquinoline alkaloids salsoline (II) in a *Salsola* species (Chenopodiaceae) and carnegine (III) in a Cactaceae could be mentioned. Corypalline (IV) from a *Corydalis* species (Papaveraceae) has a similar structure but this, of course, does not invalidate the argument.

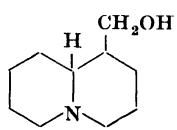
B. Similar Enzyme Systems in Related Plants Producing Analogous Compounds

The alkaloids of the pyrrolizidine ("senecio") type and the analogous alkaloids of quinolizidine ("lupin") type are interesting since they have been found in some botanically related genera.

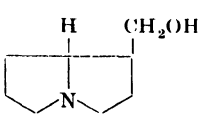
"Senecio alkaloids" occur in *Crotalaria* (Leguminosae, Papilionatae). "Lupin alkaloids" have been isolated from some of the Papilionatae, e.g. *Lupinus* and *Cytisus* both belonging to the group Genisteae which also includes *Crotalaria*. The presence of these alkaloids in the group of related genera Genisteae is interesting. The necine portion of monocrotaline from *C. spectabilis*, retronecin (V), is analogous to lupinine (VI) of some *Lupinus* species. The "pyrrolizidine alkaloids" can be derived from ornithine, and the quinolizidine alkaloids from lysine, and apparently in the Genisteae there are similar enzyme systems some adapted to ornithine and some to lysine. *Cytisus laburnum* can use both of these amino acids and contains the pyrrolizidine derivative laburnine (VII) as well as the quinolizidine derivative cytisine (VIII).



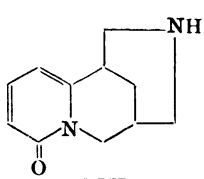
(V)
Retronecine



(VI)
Lupinine



(VII)
Laburnine



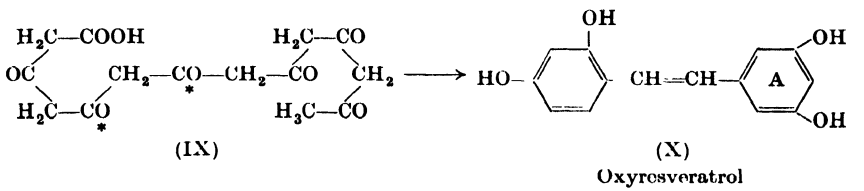
(VIII)
Cytisine

The furo- and pyranoquinolines of Rutaceae and the Amaryllidaceae alkaloids provide further examples (see Chapters 14 and 15).

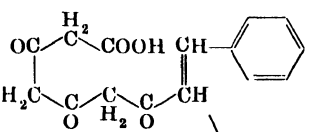
C. Different Pathways to Similar Products

The natural stilbenes constitute a small but intriguing group and their structures and distribution in plants are given in Table I. Two biosynthetic routes have been discussed for these compounds. Robinson (1955) has suggested that they are built up from acetic acid units and this is a

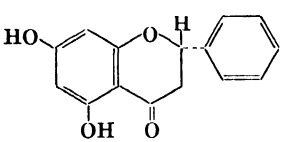
particularly attractive hypothesis in the case of two stilbenes, oxyresveratrol (X) and its geranyl derivative chlorophorin (Table I), found in Moraceae. According to this hypothesis oxyresveratrol (X) would be formed as follows.



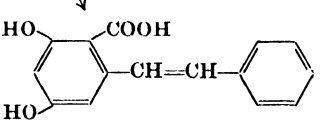
(IX) (X) Oxyresveratrol



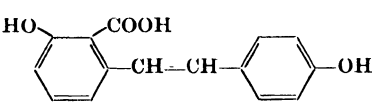
(XI)



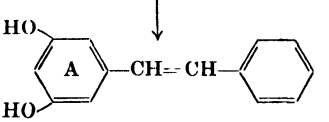
(XIV) Pinocembrin



(XII)



(XV) Hydrangeic acid



(XIII) Pinosylvin

The cyclization of the hypothetic intermediate (IX) is followed by reduction of the two carbonyl groups marked * leaving, after aromatization, the remaining hydroxyl groups correctly oriented. Pinosylvin (XIII) could be formed in a similar manner, but in this case it is necessary to assume that two more carbonyl groups have been reduced yielding an unsubstituted B-ring.

TABLE I

Distribution of Natural Stilbenes

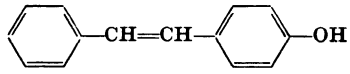
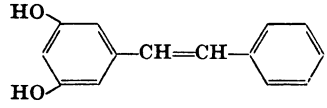
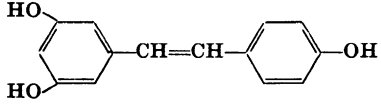
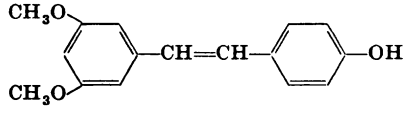
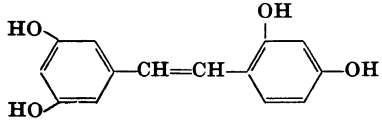
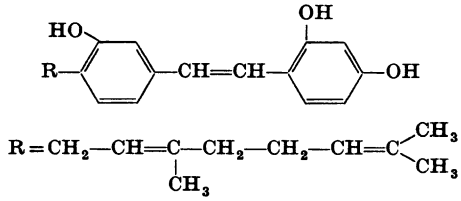
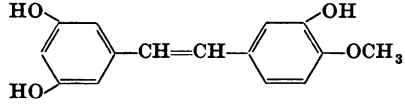
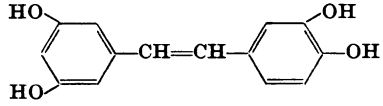
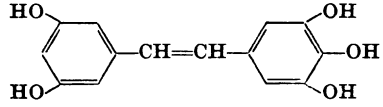
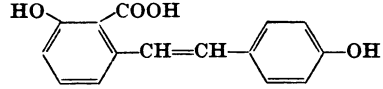
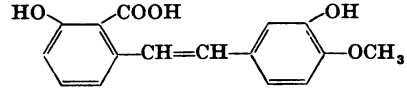
Name	Structure	Order	Family	Genus	Species, etc.
4-Hydroxystilbene (also its methyl ether)		Pinales	Pinaceae	*Pinus*	In one out of about 100 species; probably in other species also. Heartwood
Pinosylvin (also its mono- and dimethyl ethers)		Pinales	Pinaceae	*Pinus*	In about 50 species; probably all. Heartwood
Resveratrol		Pinales	Pinaceae	*Picea*	In 3 out of about 40 species. Needles
		Liliflorae	Liliaceae	*Veratrum*	In one out of about 50 species. Root
		Myrtiflorae	Myrtaceae	*Eucalyptus*	In many species. Heartwood
Pterostilbene		Rosales	Leguminosae (Papilionatae)	*Pterocarpus*	In 5 out of about 20 species. Heartwood
Oxyresveratrol		Liliflorae	Liliaceae	*Veratrum*	In one out of about 50 species. Root
		Urticales	Moraceae	*Morus*	In 2 out of 12 species. Heartwood
				Toxylon (*Maclura*)	In one species. Heartwood
				Artocarpus	In one out of about 60 species. Heartwood

TABLE I—*continued*

Name	Structure	Order	Family	Genus	Species, etc.
Chlorophorin		Urticales	Moraceae	*Chlorophora*	In one out of 35 species. Heartwood
Rhapontigenin		Polygonales	Polygonaceae	*Rheum*	In 3 or more out of 20 species. Root
				Polygonum	In one out of about 300 species. Rhizome
		Myrtiflorae	Myrtaceae	*Eucalyptus*	Leaves
3,5,3′,4′-Tetrahydroxystilbene		Rosales	Leguminosae	*Cytisus* (*Laburnum*)	In one out of 3 species. Heartwood
			(Papilionatae)	*Vouacapoua* (*Andira*)	In 2 out of about 30 species. Heartwood
3,5,3′,4′,5′-Pentahydroxystilbene		Rosales	Leguminosae (Papilionatae)	*Vouacapoua* (*Andira*)	In one species. Heartwood
Hydrangeic acid		Rosales	Saxifragaceae	*Hydrangea*	In one out of about 80 species. Leaves
Phyllodulcic acid		Rosales	Saxifragaceae	*Hydrangea*	In one species. Leave-

Birch, on the other hand, has suggested that the stilbenes result from a combination of the acetic acid and shikimic acid pathways (Birch and Donovan, 1953; Birch, 1957). He assumes that, for example, in the biosynthesis of pinosylvin (XIII) one molecule of cinnamic acid is condensed with three molecules of acetic acid to give the intermediate (XI) which is cyclized to pinosylvin carboxylic acid (XII) and finally decarboxylated. This elegant hypothesis receives considerable support from the fact that two stilbene carboxylic acids (hydrangeic acid (XV) and phyllodulcic acid, Table 1), either as such or in the form of the corresponding isocoumarin derivatives, have been found in *Hydrangea* species. With these compounds in mind we searched for stilbene carboxylic acids in many pine heartwoods but without success.

Birch's hypothesis has the further merit that it explains the co-occurrence of pinosylvin (and its ethers), pinocembrin (XIV) and other flavonoids in the heartwood of all pines, and from some of which cinnamic acid has been isolated. This hypothesis has recently received experimental support from Billek and Kindl (1961, 1962) as well as Ibrahim and Towers (1960, 1962), who studied the biosynthesis of pinosylvin and hydrangeic acid using radioactively labelled acetate and glucose. Acetate was incorporated into the A-ring (XIII) and glucose took part in the formation of the B-ring.

By substituting hydroxylated cinnamic acids for cinnamic acid, Birch's hypothesis also serves to explain the biosynthesis of those stilbenes which are hydroxylated in the B-ring. It is possible that even oxyresveratrol is formed in a similar manner since the flavonol morin, which has meta oriented (2′,4′) hydroxyl groups in ring B, has been isolated from *Morus* and *Artocarpus* species. If this is true then the hydroxyl group in position 2′ should be a secondary introduction analogous to that assumed to occur during the biosynthesis of, for example, umbelliferone. It is interesting that resveratrol and oxyresveratrol have been found to occur together in *Veratrum*. From no *Veratrum* species, however, has any compound analogous to morin yet been isolated, and a study of the biosynthesis of resveratrol and oxyresveratrol using tracer methods would be of great interest.

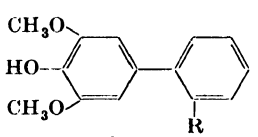
The biosynthesis of 4-hydroxystilbene is unclear. It is probable, that it follows a route similar to that of pinosylvin since the compound occurs in pine heartwood, but then one has to postulate, for example, both hydroxylation and reduction of a common intermediate. As a matter of fact it is at present impossible to differentiate between the A- and B-rings in this stilbene.

The geranyl group in chlorophorin is probably introduced at an early pre-aromatic stage. The anionoid reactivity of pinosylvin seems to be

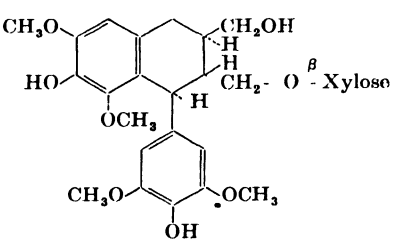
highest in the 2-position as shown by a detailed study of the stepwise bromination of pinosylvin dimethyl ether (Erdtman and Leopold, 1948).

It is seen from the Table I that except for *Pinus* and *Eucalyptus* only a few species of the stilbene-producing genera have been investigated. This is unfortunate, and a careful investigation of *Morus*, *Artocarpus* and related genera of the Moraceae as well as several genera of Leguminosae, particularly the Papilionatae, would certainly be rewarding. Such investigations should, of course, include compounds which on biogenetic grounds are likely to be related to the stilbenes. It is interesting to note that a stilbene derivative has been isolated from laburnum heartwood (Erdtman, unpublished), and the flavanone pinocembrin (XIV) from the related genus *Sarothamnus* (Matas, 1960). It is possible that both genera contain similar enzyme systems, but that in the former the routes to flavones are blocked, and in the latter those leading to stilbenes.

From the heartwood of *Sorbus aucuparia* (mountain ash, Rosaceae, Pomoideae) we have recently isolated some compounds which may be mentioned in connection with the stilbenes (Erdtman *et al.*, 1961). These are the biphenyl derivatives aucuparin and methoxyaucuparin (XVI).



(XVI)
Aucuparin (R = H)
Methoxyaucuparin (R = OCH_3)



(XVII)
Lyonia-xyloside

The sapwood contained a completely different compound (Arya *et al.*, 1962) which turned out to be lignan xyloside (XVII) previously found in some unrelated plants *Lyonia* (Ericaceae) and *Alnus* (Betulaceae). *Sorbus intermedia* was next investigated, and the *Lyonia*-xyloside was isolated but neither of the aucuparins. This is probably due to the fact that no specimens of this large tree contained true heartwood. Dr. Rudloff has

kindly investigated several Canadian *Sorbus* species (*S. scopulina*, *S. americana*, *S. decora*) and they were all found to contain the aucuparins in the heartwood and *Lyonia*-xyloside in the sapwood and, of course, we now wish to investigate the whole northern, circumpolar genus *Sorbus*. In *S. intermedia* the lack of aucuparins is compensated by the presence of the *Lyonia*-xyloside. It would have been impossible to allocate this species to the genus *Sorbus* without reference to the botanical classification. It is possible, however, that this could have been achieved had we included in our investigation the examination of other constituents such as those present in the bark of the *Sorbus* species. It is interesting to note that the aucuparins and the *Lyonia*-xyloside are all pyrogallol derivatives.

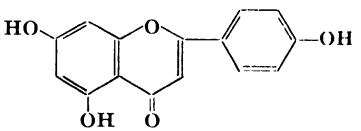
D. Structural Complexity and Restricted Occurrence

Many examples could be mentioned from several groups of compounds including the alkaloids which demonstrate relationships between the complexity of a compound and its systematic value. The biflavonyls provide good illustration.

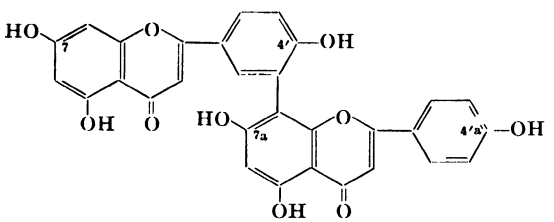
Apigenin (XVIII) and its methyl ethers, genkwanin (5,4′-dihydroxy-7-methoxyflavone), acacetin (5,7-dihydroxy-4′-methoxyflavone) and 5-hydroxy-7,4′-dimethoxyflavone, as well as many glycosides of the three former compounds, are widely distributed in the vegetable kingdom. Compounds containing two apigenin molecules connected by a carbon–carbon, or by an ether linkage are the so-called biflavonyls. The first member of this group, ginkgetin, was discovered some thirty years ago by Furukawa in the leaves of the maiden hair tree *Ginkgo biloba*, and several other biflavonyls were isolated later by Japanese chemists especially Kariyone and his collaborators. The structures of these compounds have been investigated and finally elucidated by Baker, Nakazawa, Kariyone and their collaborators (cf. Baker and Ollis, 1961). The two fundamental structures of the biflavonyls are (XIX) and (XX) representing respectively the recently discovered amentoflavone (Hsü, 1959) and hinokiflavone which was first isolated by Kariyone and Sawada. Several methyl ethers of amentoflavone are known. Sotetsuflavone is a monomethyl ether (OCH_3 at 7a), ginkgetin and isoginkgetin are dimethyl ethers (OCH_3 at 7,4′ or 4′,4′a), and sciadopitysin and kayaflavone are trimethyl ethers (OCH_3 at 7,4′,4′a or 4′,7a,4′a). No methyl ethers derived from hinokiflavone have yet been found.

The biflavonyls have mainly been found in leaves of Gymnosperms. Hsü (1959) has found apigenin together with amento- and sotetsuflavone in *Selaginella tamariscina*; *Cycas* species are reported to contain sotetsuflavone; *Ginkgo* contains both ginkgetin and isoginkgetin. Among

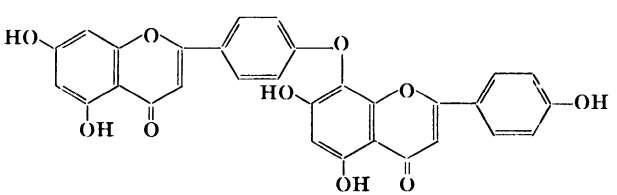
the genera of Taxaceae some *Taxus*, *Torreya* and *Amentotaxus* species have been investigated, and apigenin and sciadopitysin found in *Taxus*, kayaflavone in *Torreya* and amentoflavone in *Amentotaxus*. Two *Cephalotaxus* species have also been studied; one contained sciadopitysin and an apigenin glycoside, and the other kayaflavone.



(XVIII)
Apigenin



(XIX)
Amentoflavone



(XX)
Hinokiflavone

Of the true conifers, a few genera of the families Araucariaceae and Podocarpaceae have been examined. From one out of four *Araucaria* species sciadopitysin has been isolated; three contained apigenin but no biflavonyls. Several *Podocarpus* species studied contained kayaflavone. Biflavonyls are common in the order Cupressales, both in the heterogeneous family Taxodiaceae and in the Cupressaceae. In these families hinokiflavone is a very common biflavonyl and has been found in all genera except *Cryptomeria* (Taxodiaceae) which contains sciadopitysin, kayaflavone and sotetsuflavone. In some other genera of Taxodiaceae (*Taxodium*, *Metasequoia*, *Sequoia* and *Glyptostrobus*) only hinokiflavone has been observed; in others there are mixtures of biflavonyls of amento-

and hinokiflavone type. The same applies to those genera of the family Cupressaceae which have been investigated.

Surprisingly enough, in no genera of the Pinaceae have any biflavonyls been detected, although in some cases (*Abies*, *Picea* and, especially, *Pinus*) many species were investigated. It is possible that the inability to synthesize biflavonyls is characteristic of the order Pinales.

Many conifer genera remain to be screened for the occurrence of biflavonyls but it is clear that, especially in connection with other constituents, these compounds are very important for the chemical classification of the gymnosperms and further advances in this field are awaited with great interest.

The reported isolation of hinokiflavone from an angiosperm, *Casuarina stricta*, was unexpected. It has sometimes been assumed that there are relationships between the gymnosperms and the casuarinas, but there are no valid botanical reasons for this view as the similarities are only superficial. However, a careful investigation of a large number of *Casuarina* species is now highly desirable. It would not be surprising if, in the future, biflavonyls were found in several groups of angiosperms.

The biflavonyls are presumably formed by oxidative coupling of two apigenin molecules, and it is therefore interesting that apigenin has been found in leaves containing biflavonyls. Flavones other than apigenin, for example quercetin, have been found in many plants, even in the leaves of some conifers but no trace of the corresponding biflavonyls has yet been found.

IV. Integrated Investigations of Groups of Plants

A. Introduction

Few systematic investigations have been carried out with the intention of characterizing large groups of plants such as orders or families by means of their patterns of chemical constituents in different organs and it is easy to understand why, since most of the plant groups are discouragingly large.

However, some such information can be gained by a compilation of references in the literature, for example, about the distribution of acetylenic compounds and sesquiterpenes in Compositae, flavonoids and alkaloids in Leguminosae, unusual fatty acids, acetylenic compounds, and coumarins in Umbelliferae, etc.

B. Coniferae

We have concentrated our work on the conifers (cf. Erdtman, 1952, 1955, 1956, 1959), a class of gymnosperms which includes only about

600 species. They are often considered as being merely an order. The conifers have the advantage over the angiosperms that they constitute an isolated, old and conservative group of plants dating back some two hundred million years. The history of the conifers is relatively well known and they have been much studied by botanists who, however, have not reached unanimity on some points of classification. There are some large genera suitable for comparative chemical investigations, and several small ones of unclear classification, and the chemical examination of the latter could at least give results of interest to the botanists.

Another advantage was that a great deal of chemical work had already been carried out on this group of economically important plants.

1. Natural order Pinales

The conifers have been divided into several orders such as Araucariales, Podocarpales, Pinales and Cupressales. Pinales has only one family, Pinaceae, divided into several genera, e.g. *Abies*, *Cedrus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga*.

Our work started with an investigation of the phenolic constituents of the heartwood of a large number of pine species (over 50% of the recent species). A series of new or known compounds was isolated including stilbenes, flavanones and flavones. The results of this work, which was largely carried out by Dr. Lindstedt (Lindstedt and Misiorny, 1951), are recorded in Table II.

What we learned from this investigation is briefly the following. Pines can be characterized by their specific pattern of heartwood phenolics. The two subgenera Diploxylon and Haploxylon could easily be recognized. Smaller groups, however, could not be discerned except Strobi and Gerardianae (Haploxylon).

The Diploxylon pines contained only stilbene derivatives (the pinosylvins) and flavanones [e.g. pinocembrin (XIV) = dihydrochrysin and pinobanksin (XXI) = 3-hydroxypinocembrin]. The Haploxylon pines contained the same substances as well as dihydropinosylvins and flavones such as chrysin. This indicates that in Haploxylon there are hydrogen-transferring enzyme systems operating at some stage of the biosynthesis of the pinosylvins and flavonoids, which are absent or blocked in Diploxylon. The Haploxylon pines have more powerful methylating systems than Diploxylon (pinostrobin is 7-methylated pinocembrin and tectochrysin 7-methylated chrysin). Some groups of Haploxylon (Strobi and Gerardianae) even contained carbon-methylated flavonoids (strobobanksin, stropinin and crystostrobin). It is interesting to speculate what would have happened had we only investigated *Pinus silvestris* and *P. peuce*. We might have missed pinocembrin in the former species and

TABLE II

Heartwood constituents of pines

(Classification according to Shaw)

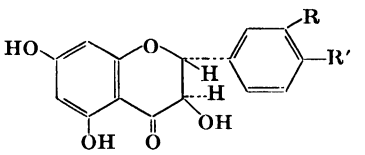
Section (1)	Subsection (2)	*Pinus* species (3)	Pinosylvin = P (4)	P-monomethyl ether = PSM (5)	Dihydro-P (6)	Dihydro-PSM (7)	Pinocembrin (8)	Chrysin (9)	Pinostrobin (10)	Tectochrysin (11)	Pinobanksin (12)	Strobobanksin (13)	Strobopinin (14)	Cryptostrobin (15)
		HAPLOXYLON												
Cembra	Cembrae	1 *koraiensis* S. and Z.	+	+	+	+	+	+	+	+	+	–	–	–
		2 *cembra* L.	+	×	+	+	×	×	+	×	?	–	–	–
		3 *albicaulis* Eng.	+	×	+	×	+	+	+	+	+	–	–	–
	Flexiles	4 *flexilis* Jam.	?	+	+	+	+	+	+	+	+	–	–	–
	Strobi	5 *ayacahuite* Ehr.	+	+	+	+	?	+	?	+	+	–	–	–
		6 *lambertiana* Dougl.	–	–	–	–	+	?	×	×	+	×	×	?
		7 *parviflora* S. and Z.	+	+	+	+	+	+	–	–	+	–	+	+
		8 *formosana* Hay	+	+	?	+	+	+	+	–	+	+	–	–
		9 *peuce* Gris.	–	–	–	–	+	+	+	+	?	–	+	+
		10 *griffithii* M'Cl.	+	×	–	+	×	×	+	×	×	–	–	–
		11 *monticola* Dougl.	+	×	–	+	+	×	+	×	?	–	×	+
		12 *strobus* L.	+	×	–	+	+	×	×	×	×	×	×	×
Paracembra	Cembroides	13 *cembroides* Z.	–	+	–	+	+	+	+	?	?	–	–	–
	Gerardianae	14 *bungeana* Z.	+	+	–	–	+	–	+	–	+	+	+	?
		15 *gerardiana* Wall.	+	+	–	–	+	–	+	–	+	+	–	–
	Balfourianae	16 *balfouriana* A. Murr	+	+	+	+	+	+	+	+	+	–	–	–
		17 *aristata* Eng.	?	×	–	+	×	×	+	×	+	–	–	–

TABLE II—*continued*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
		DIPLOXYLON												
Parapinaster	Leiophyllae	1 *leiophylla* S. and Ch.	+	+	−	−	+	−	−	−	+	−	−	−
		2 *lumholtzii* R. and F.	+	+	?	+	+	−	−	−	+	−	−	−
	Longifoliae	3 *canariensis* Sm.	+	+	−	−	+	−	-	−	+	−	−	-
		4 *longifolia* Rox	+	−	−	−	+	-	−	−	+	−	−	−
	Pinaea	5 *pinea* L.	+	×	−	−	×	−	−	−	+	−	−	−
Pinaster	Lariciones	6 *resinosa* Ait.	+	×	−	−	?	−	−	−	?	−	−	−
		7 *massoniana* Lamb	+	+	−	−	+	−	−	−	+	−	−	−
		8 *densiflora* S. and Z.	+	+	−	−	+	−	−	−	+	−	−	−
		9 *silvestris* L.	×	×	−	−	+	−	−	−	+	−	−	−
		10 *mugo* Turr.	×	×	−	−	×	−	−	−	?	−	−	−
		11 *nigra* var. *poiretiana* Sch.	+	×	−	−	+	−	−	−	−	−	−	−
		12 *nigra* var. *austr.* (H). Bad.	+	+	−	−	+	−	−	−	−	−	−	−
	Australes	13 *montezumae* Lamb	+	+	−	−	+	−	−	−	+	−	−	−
		14 *ponderosa* Dougl.	×	×	−	−	×	−	−	−	×	−	−	−
		15 *jeffreyi* Balf.	+	×	−	−	×	−	-	−	+	−	−	−
		16 *occidentalis* Sw.	+	+	−	−	+	−	−	−	+	−	−	−
		17 *palustris* Mill.	?	+	−	−	+	−	−	−	+	−	−	−
		18 *caribaea* Mor.	−	+	−	−	+	−	--	−	+	−	−	−
		19 *taeda* L.	+	×	−	−	×	−	−	−	×	−	−	−
		20 *glabra* Walt.	+	+	−	−	+	−	−	−	+	−	−	−
		21 *echinata* Mill.	+	+	−	−	+	−	−	−	+	−	−	-
Pinaster	Insignes	22 *halepensis* Mill.	×	×	−	−	+	−	−	−	+	−	−	−
		23 *pinaster* Ait.	+	+	−	−	×	−	−	−	×	−	−	−
		24 *virginiana* Mill.	?	×	−	−	×	−	−	−	×	−	−	−
		25 *clausa* Vas.	+	+	−	−	×	−	(+)	−	×	−	−	−
		26 *rigida* Mill.	+	+	−	−	+	−	−	−	+	−	−	−
		27 *pungens* Lamb.	+	+	−	−	+	−	−	−	+	−	−	−
		28 *banksiana* Lamb.	×	×	−	−	×	−	−	−	×	−	−	−
		29 *contorta* var. *latif.* Eng.	×	×	−	−	×	−	−	−	×	−	−	−
		30 *muricata* D. D.	+	+	−	−	+	−	−	−	+	−	−	−
		31 *attenuata* Lemm.	?	+	−	−	+	−	−	−	+	−	−	−
		32 *radiata* D.D.	−	×	−	−	×	−	−	−	×	−	−	−
		33 *radiata* var. *insignis* Dougl.	+	+	−	−	+	−	−	−	+	−	−	−
	Macrocarpae	34 *sabiniana* Dougl.	+	+	−	−	+	−	−	−	+	−	−	−
		35 *coulteri* D. D.	+	+	−	−	+	−	−	−	+	−	−	−

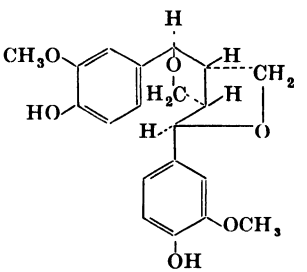
(+ and −: presence or absence of compound as demonstrated by paper chromatography; ×: compound isolated.)

the pinosylvins in the latter, and thereby concluded that heartwoods are no good for the chemical characterization of pines.



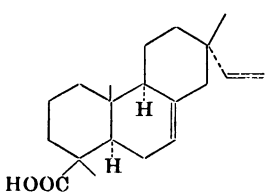
(XXI)

Pinobanksin (R = R′ = H)
Aromadendrin (R = H; R′ = OH)
Taxifolin (R = R′ = OH)



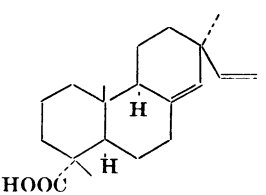
(XXII)

Pinoresinol



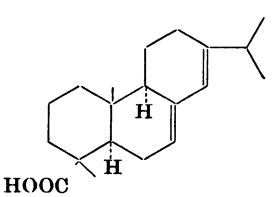
(XXIII)

Isopimaric acid



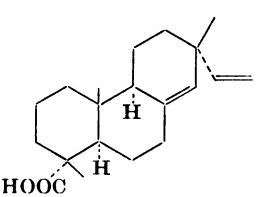
(XXIV)

Pimaric acid



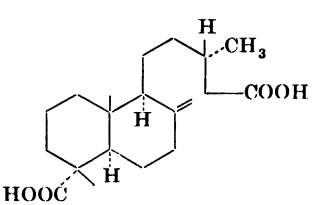
(XXV)

Abietic acid



(XXVI)

Sandaracopimaric acid

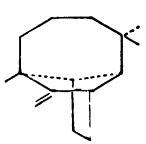


(XXVII)

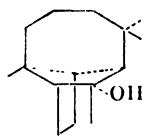
Pinifolic acid

Other pine substances of interest are the lignan pinoresinol (XXII), which is also found in *Picea*, and resin acids such as those of pimarane (XXIII, XXIV, XXVI) and abietane (XXV) type. These acids have also been found in *Abies*, *Larix*, *Picea* and *Pseudotsuga* (Daniels and Erdtman,

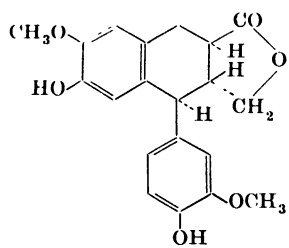
unpublished). (The acids of pimarane type have been found in other orders of the conifers but those of abietane type only in Pinaceae.) All the diterpene acids hitherto found in Pinales have the same configuration, the carboxyl group at C_4 being equatorial. Recently we have isolated the hydrocarbon corresponding to pimaric acid, pimaradiene, from the oleoresin of the wood of *P. silvestris* (Erdtman and Westfelt, unpublished). From the leaves of *P. silvestris* a new bicyclic resin acid, pinifolic acid



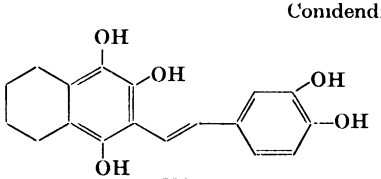
(XXVIII)
Longifolene



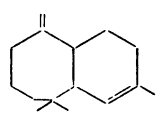
(XXIX)
Juniperol
(Longiborneol)



(XXX)
Conidendrin



(XXXI)
Piceatannol



(XXXII)
α-Himachalene

(XXVII) (Enzell and Theander, 1962) was isolated. It, too, has a normal "Pinales configuration". Most of the mono-and sesquiterpenes found in the oleoresin of *Pinus* are spread over the whole class of Coniferae. No sesquiterpenes such as those of eudesmane or guaiane type, which are believed to be formed from *trans*-farnesol, have been found in Pinales, only sesquiterpenes of "*cis*-farnesyl type". It is possible that in this order longifolene (XXVIII) and the related juniperol (XXIX) (Akiyoshi *et al.*, 1960) are restricted to *Pinus* but this is by no means certain. The same applies to thunbergene (cembrene), a novel macrocyclic diterpene (Dauben *et al.*, 1962; Kobayashi and Akiyoshi, 1962). Several overlappings from genus to genus of heartwood and bark constituents are known

in Pinales. The lignan conidendrin (XXX) is common in the wood of *Picea* and *Tsuga* and has been found in one *Larix* (Nair and von Rudloff, 1960) and possibly one *Abies* species. Several other lignans have been found in wood and bark of the Pinales genera. All of them contain guaiacyl groups. Flavanones such as pinobanksin and the related aromadendrin and taxifolin provide bridges between most of the genera. It is possible that some novel tetraline compounds from spruce bark, e.g. piceatannol (XXXI) (Endres and Leppmeier, 1961; Grassmann and Endres, 1959), are restricted to *Picea* and the himachalenes, sesquiterpenes of a novel type (XXXII), to *Cedrus* (Bredenberg and Erdtman, 1961; Joseph and Dev, 1961).

Much work remains to be done before we can get a clear picture of the chemistry of the various species and their organs. We should therefore at present refrain from speculating about the more or less close relationships between the various genera of Pinales. However, it already appears justifiable to speak in a broad way about a characteristic "Pinales chemistry" and this supports the opinion of the botanists that Pinales constitutes a group of conifers of common origin.

2. Natural order Cupressales

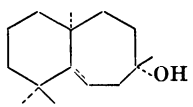
It is interesting to see whether the same can be said about the order Cupressales. This order is generally divided into two families, Taxodiaceae and Cupressaceae. The Taxodiaceae embrace a series of only distantly related, essentially northern hemispheric conifers. They are subdivided into several small or even monotypic genera, e.g. *Athrotaxis*, *Cryptomeria*, *Taiwania*, *Cunninghamia*, *Sciadopitys* and *Sequoia*. They are the last remnants of very old groups of conifers. Due to the paucity of species they are not very suitable for comparative chemical studies.

Cunninghamia and *Sciadopitys* are known to contain cedrol which is a typical "Cupressales compound". *Cryptomeria* is interesting because it produces several compounds occurring in Cupressales as well as in Pinales, Araucariales and Podocarpales, e.g. eudesmane, pimarane and phyllocladane (Kondo *et al.*, 1960) derivatives.

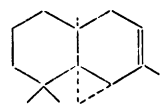
Athrotaxis is a small southern genus (three species from Tasmania) and *Taiwania* is northern (one species from Formosa and China) and they are considered to be botanically related. The wood of *Taiwania* contains a cadinol and hinokiol (Lo, private communication) and we (Erdtman and Vorbrüggen, 1960) have also found cadinols and hinokiol in the *Athrotaxis* species. This does not mean very much but at least it is in agreement with the views of the botanists.

During our studies of the family Cupressaceae we have isolated many compounds, mainly of terpene nature. Some of these were new and we

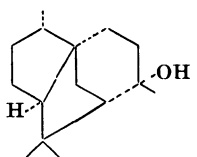
have elucidated the structures of several of them. Other compounds were already well known. Examples of new substances or compounds of incompletely known structure or configuration, or which had previously been assigned erroneous structures are: tropolones of thujaplicin-"C_{10}-type" (Erdtman and Gripenberg, 1948a, b), tropolones of noot-



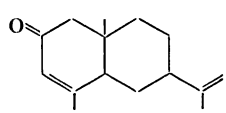
(XXXIII)
Widdrol



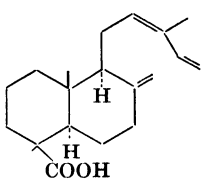
(XXXIV)
Thujopsene



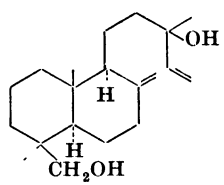
(XXXV)
Cedrolic acid



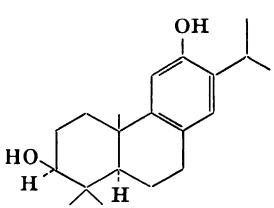
(XXXVI)
Nootkatone



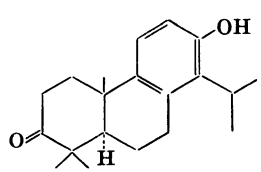
(XXXVII)
Communic acid



(XXXVIII)
Torulosol



(XXXIX)
Hinokiol



(XL)
Totarolone

katin-"C_{15}-type" (Duff *et al.*, 1954), thujic acid (Erdtman and Gripenberg, 1949; Gripenberg, 1949), chamic and chaminic acids (Carlsson *et al.*, 1952; Erdtman *et al.*, 1956), cuparene and cuparenic acid (Enzell and Erdtman, 1958), widdrol (XXXIII) (Erdtman and Thomas, 1958; Enzell, 1961a, 1962), thujopsene (XXXIV) and hinokiic acid (Erdtman and Thomas, 1958; Erdtman and Norin, 1960; Norin, 1961),

TABLE III

Some heartwood constituents of the subfamily Cupressoideae

Species	Carvacrol	Hydrothymoquinone derivatives	C_{10}-Tropolones	C_{15}-Tropolones	1-Rhodinic acid	Dehydrogeranic acid	Myrtenic acid	Chamic, chaninic acid	Thujic acid	Shonanic acid	Cadinanes	Thujopsanes	Widdranes	Cuparanes	Cedranes	Eudesmanes	Occidanes	Guaianes	Manoöl	Ferruginol	Hinokiol	Totarol
CUPRESSEAE																						
Chamaecyparis																						
lawsoniana			+?	−							+											
thyoides			+?	−								+	+	+	+							
obtusa			−								+?	+									+	
pisifera			−								+											
formosensis			?				+				+					+						
taiwanensis			+		+						+	+?			+							
nootkatensis	+		+	+				+								+						
Cupressus																						
bakeri	+		+	+																		
macnabiana	+		+	+																		
macrocarpa	+		+	+																		
arizonica	+		+	+								+	+	+	+							
sempervirens	+		+	+															+			
torulosa	+		+	+								+		+					+	+	+	
JUNIPEREAE																						
Juniperus																						
communis	+		+	+							+	+	+	+	+					+		
cedrus	+	+	+	+							+	+	+	+	+							

TABLE III—*continued*

Species	Carvacrol	Hydrothymoquinone derivatives	C_{10}-Tropolones	C_{15}-Tropolones	1-Rhodinic acid	Dehydrogeranic acid	Myrtenic acid	Chamic, chaminic acid	Thujic acid	Shonanic acid	Cadinanes	Thujopsanes	Widdranes	Cuparanes	Cedranes	Eudesmanes	Oppositanes	Guaianes	Manoöl	Ferruginol	Hinokiol	Totarol
JUNIPEREAE—*cont.*																						
Juniperus—cont.																						
foetidissima											+		+		+							
virginiana			—	—								+	+	+	+							
californica			+	+								+	+	+	+							
utahensis	+		+	+								+	+	+	+							
thurifera	+		+	+								+	+	+	+							
phoenicia			+	+								+	+	+	+							
procera	+			+										+	+							
chinensis	+	+	+	+								+	+	+	+							
THUJOPSIDEAE																						
Thuja																						
plicata			+						+													
occidentalis			+						+							+	+					
standishii			+																			
Thujopsis	+		+		+							+	+	+	+							
Platycladus (*Biota*)			+						+			+	+	+	+							
Calocedrus decurrens	+	+	+																			
Calocedrus formosana			+					+	+	+												
Fokienia hodginsii																+						

TABLE IV

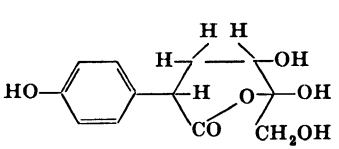
Some Heartwood Constituents of the Subfamily Calllitroideae

Species	Carvacrol	Hydrothymoquinone derivatives	C_{10}-Tropolones	C_{15}-Tropolones	1-Rhodinic acid	Dehydrogeranic acid	Myrtenic acid	Chamic, chaminic acid	Thujic acid	Shonanic acid	Cadinanes	Thujopsanes	Widdranes	Cuparanes	Cedranes	Eudesmanes	Occidanes	Guaianes	Manoöl	Ferruginol	Hinokiol	Totarol
Callitroideae																						
Callitris																						
calcarata					+													+				
glauca					+	+												+				
intratropica					+													+				
morrisonii																		+				
preissii																		+				
propinqua																		+				
rhoei																+						
rhomboidalis																		+				
verrucosa																		+				
macleyana					+													+				
sulcata																+		+				
Neocallitropsis						+					+					+		+				
Widdringtonia																						
cupressoides																+						
dracomontana												+	+	+	+	+						
juniperoides												+	+	+	+							
schwarzii												+	+	+	+							
whytei												+	+	+	+							
Tetraclinis	+	+	+												+						+	+
Pilgerodendron											+											
Austrocedrus	+		+															•				
Papuacedrus			+																			

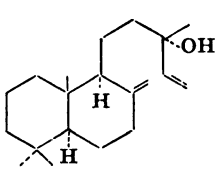
cedrolic acid (XXXV) (Runeberg, 1961a), nootkatone (XXXVI) (Erdtman and Hirose, 1962), occidentalol (XLIV) (von Rudloff and Erdtman, 1962), communic acid (XXXVII) (Arya *et al.*, 1961a, b), torulosol (XXXVIII) (Barreto and Enzell, 1961; Enzell, 1961b), hinokiol (XXXIX) (Chow and Erdtman, 1960a, 1962b; Chow, 1960, 1962), totarol and totarolone (XL) (Chow and Erdtman, 1960b, 1962c).

The family Cupressaceae has been divided in different ways. It has two subfamilies, Cupressoideae and Callitroideae. Cupressoideae is essentially northern and is divided into three tribes, Cupresseae, Junipereae and Thujopsideae. Callitroideae is essentially southern, an exception being *Tetraclinis* (North Africa). Some of our results on the heartwood constituents of Cupressaceae and those of several others are summarized in the Tables III and IV. In order to make these Tables more easy to follow only skeletons of the sesquiterpenes are mentioned, not the individual compounds. It should also be noted that in many cases the investigations are very incomplete having been carried out before modern analytical methods had been introduced and that many species have still to be investigated.

Of the tribe Cupresseae, the genus *Chamaecyparis* still appears to be heterogeneous from a chemical point of view. Most sesquiterpenes are of the "*cis*-farnesyl type", e.g. cadinane, thujopsane, widdrane, cuparane, and cedrane derivatives but there are also some of "*trans*-farnesyl type", e.g. eudesmane derivatives. The most aberrant species is certainly *Ch. nootkatensis*, which contains C_{15}-tropolones and some monoterpene acids of unusual type, e.g. chamic acid. Botanically it also differs in several respects from the other *Chamaecyparis* species and long ago Ørsted considered it to constitute a genus of its own, which he called *Callitropsis*. The heartwood of *Ch. pisifera* contains a phenolic substance, sawaranin (XLI), the structure of which has recently been elucidated by Imamura (1962), and seems to indicate that sawaranin has been formed from a biphenyl derivative by cleavage of one of the rings.



(XLI)
Sawaranin



(XLII)
Manoöl

The genus *Cupressus* seems to differ considerably from the "normal" *Chamaecyparis* species. The C_{15}-tropolone nootkatin was present in all

species investigated. Surprisingly the Eurasian species were found to contain the diterpene alcohol manoöl (Enzell and Erdtman, 1957), earlier found in *Dacrydium* (Podocarpales); it has not been found in any of the American species investigated. *Cupressus torulosa* also contained the hydroxymanoöl torulosol (XXXVIII) (and the corresponding aldehyde) in which the CH_2OH group is axially oriented as is the COOH group at C_4 in agathene dicarboxylic acid (XLIII) and podocarpic acid.

Of the tribe Juniperae several species of *Juniperus* have recently been investigated by Runeberg (1961b) and so far they appear to be more similar to *Cupressus* than to *Chamaecyparis*. This has recently caused us (Arya and Erdtman, unpublished) to investigate some juniper barks and the results of this very preliminary study, which also includes one *Cupressus* species, are given in Table V. It was interesting to find that the bark of *C. arizonica* like that of the *Juniperus* species contained communic acid and the closely related agathene dicarboxylic acid (XLIII). Both have axial carboxyl groups at C_4.

TABLE V

Bark constituents of *Juniperus* and *Cupressus* species

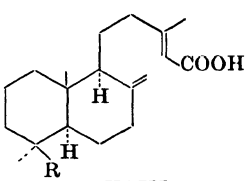
Genus	Longifolene	Juniperol	Hinokiic acid	Communic acid	Agathenedicarboxylic acid	Ferruginol	Sugiol	Totarol
J. communis	+	+		+				+
J. foetidissima				+	+			
J. procera				+				
J. californica			+	+				
J. utahensis				+	+			
J. mexicana				+	+		+	
C. arizonica				+	+	+	+	

The Thujopsideae appear to be a very heterogeneous group of which *Thujopsis* and *Platycladus* have several constituents in common. It is very unfortunate that most of the genera of this tribe contain only a few species or are monotypic. This weakens the taxonomic value of at least some of their chemical constituents. The chemical investigation of such

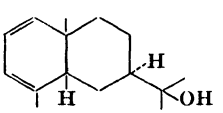
genera, however, is often justified since they also give the botanists similar troubles.

Passing over to the Callitroideae it is seen that *Callitris* and *Neocallitropsis* (in the chemical literature often "*Callitropsis*") have guaiol and eudesmol in common. Guaiol is a sesquiterpene which has not been found in any other conifers. The two genera are botanically related. The heartwood of *Neocallitropsis* may also contains cadinols (von Rudloff, 1962). The investigation of the South African *Widdringtonia* species was important in order to find out whether they exhibit similarities to *Callitris* or to the Northern Cupressoideae. The latter was definitely the case although the *Widdringtonia* species differed in not containing tropolones. The North African *Tetraclinis* was once lumped together with the Australian *Callitris*; chemically they are totally different (Chow and Erdtman, 1962a). *Tetraclinis* resembles the northern Cupressaceae. The resin from this species contains sandaracopimaric acid (XXVI) which possesses an equatorial carboxyl group at C_4.

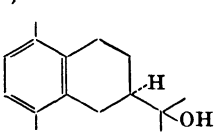
The genus *Libocedrus* has been subjected to repeated revisions by the botanists. It used to include the genera *Calocedrus* (e.g. "*Libocedrus decurrens*", the incense cedar), *Pilgerodendron*, *Austrocedrus* and *Papuacedrus*, etc. What is known about the chemistry of these very small, new genera supports the view that the old genus *Libocedrus* (*sensu lato*) was heterogeneous.



(XLIII)
Agathene dicarboxylic acid (R = COOH)
Agatholic acid (R = CH_2OH)



(XLIV)
Occidentalol



(XLV)
Occidol

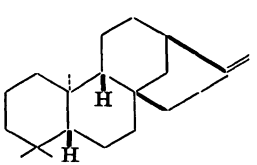
The phenolic constituents of the Cupressaceae have been less investigated than the terpenes. Flavonoids such as taxifolin have been found in the wood of some genera. Lignans have been isolated, particularly from the leaves. No lignan of the "guaiacyl type" has been described, only those containing methylenedioxy groups (sesamin, hinokinin, savinin).

The needles of *Chamaecyparis*, *Austrocedrus*, *Calocedrus* and several *Juniperus* species were found by Hartwell to contain the tumour damaging lignans, podophyllotoxin and deoxypodophyllotoxin (Fitzgerald *et al.*, 1957).

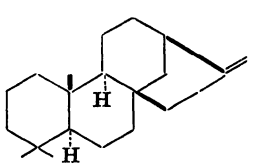
It is easy to see how very different the orders Pinales and Cupressales are from a chemical point of view. They have several constituents in common but there are also certain compounds of specific "Cupressales type" such as the tropolones and several sesquiterpenes. Some of these and other compounds are fairly widely distributed in the order, others are restricted to a few genera. There are also some substances such as occidentalol (XLIV) (von Rudloff and Erdtman, 1962) and occidol (XLV) (Hirose and Nakatsuka, 1959) both of modified eudesmane type, which have been found in a single species only. It remains to be seen whether they also occur in other species of the genus *Thuja*.

3. Natural orders Araucariales and Podocarpales

The orders Araucariales and Podocarpales have been insufficiently studied. The former contains the genera *Araucaria* and *Agathis*. The discovery of the "Pinales lignan", pinoresinol, in the South American *Araucaria angustifolia* was unexpected. The leaves of *A. excelsa* contain phyllocladene (XLVI) (also found in the leaves of *Cupressus macrocarpa*). The wood of *A. bidwillii* contains agathene dicarboxylic acid. The kauri resin of *Agathis australis* also contains this acid as well as the similar but less highly oxidized agatholic acid (Enzell, 1961b). The leaves contain (−)-kaurene (XLVII), a compound of the phyllocladane type but having *the opposite configuration* at C_5, C_9 and C_{10}.



(XLVI)
Phyllocladene



(XLVII)
(—)-Kaurene

The order Podocarpales embraces seven genera among which *Dacrydium*, *Phyllocladus* and *Podocarpus* may be mentioned. The "leaves" of *Phyllocladus* species contain phyllocladene. Due to their geographical distribution and still uncertain classification *Dacrydium* and *Podocarpus* are of particular interest. *Podocarpus* is the largest conifer genus and like *Dacrydium* it is heterogeneous. The wood of only a few *Podocarpus* species has been investigated. Several structurally modified diterpenes such as podocarpic acid (*axial* COOH at C_4), ferruginol, sugiol and totarol

(even 16-oxidized totarols—with *axial* CH_2OH, CHO and COOH at C_4) have been isolated from *Podocarpus* species belonging to the sections *Eupodocarpus*, *Stachycarpus* and *Dacrycarpus* (Taylor, 1961; Briggs *et al.*, 1959; Cambie and Mander, 1962). These, or similar compounds, also occur in Cupressales; isopimaric acid (from *P. ferrugineus*) is the only "normal" diterpene acid encountered (it is also present in Pinales and Cupressales). The New Zealand *P. spicatus* (matai) is completely aberrent. The wood contains a series of non-terpenic substances such as aromadendrin, taxifolin and the isoflavones, genistein and podospicatin, as well as lignans, e.g. matairesinol and conidendrin. The leaves of *P. ferrugineus* contain phyllocladene and (+)-kaurene but those of *P. macrophylla* contain (−)-kaurene.

Surprises are also to be found in the *Dacrydium* species. The wood from both *D. bidwillii* and *D. biforme* contains manoöl and that from the latter also contains isopimaric acid (leaves; phyllocladene). The wood of *D. colensoi* contains manoyl oxide and the strange ketomanoyl oxide (with a carbonyl group at C_2); the leaves phyllocladene, longifolene and juniperol, but the wood of *D. cupressinum* juniperol, ferruginol, sugiol, totarol, and podocarpic acid. The presence of these compounds suggests that *D. cupressinum* should be placed together with *Podocarpus*. The Tasmanian *D. franklinii* differs from these New Zealand species just as distinctly as does *Podocarpus spicatus* from the other podocarps, as it is apparently lacking in higher terpenic compounds. The wood of *Dacrydium franklinii* contains eugenol methyl ether and other constituents presently being investigated include eugenol, elemicin and several other phenolic compounds (Erdtman and McLean, unpublished).

As can be seen our knowledge of the chemistry of the conifers is still very incomplete and unexpected discoveries will certainly be made in the future.

V. Conclusions

Investigations in the conifer field have convinced us, as we expected, that there is no easy chemical route to plant classification. Chemical taxonomy does not differ fundamentally from classical taxonomy, it simply utilizes entirely different characteristics. There is no doubt, however, that chemical investigations can be used, and in future will be used, to an increasing extent in our attempts to shed light on the phylogenetic problems which belong to the most difficult yet intriguing ones that science has faced.

The biologists have a great handicap, and at the present stage chemical investigations should therefore be carried out with the biological

classification of plants as a background. Considering the large number of known plants—the angiosperms alone number some 250,000 species divided into almost 300 families—the task ahead of the chemists is enormous. It will therefore be important for them to concentrate upon suitable groups of plants. These may be found more or less by chance, or may become obvious by a perusal of compilatory works, such as for example that of Karrer (1958), and many valuable suggestions can be furnished by the botanists.

Universities have now been founded, and chemical studies commenced, in many hitherto technically undeveloped countries. Although their chemists will certainly not start work with the very best equipment they should be able to make valuable contributions by means of simple techniques, such as paper chromatography, and chemotaxonomy may serve as a breeding ground for the development of chemistry in those parts of the world.

The time has come when every student of natural products should have a handbook on plant taxonomy on his desk (e.g. Willis, 1960). He will certainly find it stimulating at least if taken in small doses and it will help him to see relationships between the genera. It is to be recommended that chemists adopt the habit of adding, to the name of the plant, the name of the family to which it belongs.

One of the great difficulties in all chemotaxonomic work is the procuring of plant material. Many genera are cosmopolitan and it is, of course, of special interest to investigate the species of such genera in order to find out the extent to which they differ from each other. Although many herbs can be raised from seeds obtainable from botanical gardens all over the world, unfortunately one cannot always rely upon the identity of such seeds. All plant material, therefore, must necessarily be checked by botanical experts. Commercial products such as essential oils are often convenient starting materials useful for the isolation of sufficient quantities of certain substances for structural investigations. However, essential oils only contain volatile components and they are not infrequently adulterated. It is therefore necessary to complete the investigation by an examination of products isolated from the plant in question, and whenever possible freshly prepared extracts of plants should be used.

We may end with a note on nomenclature. For various reasons botanists sometimes have to change the scientific names of plants. In order to avoid confusion chemists should, therefore, mention not only the name of the plant, but also the name of the author responsible for it. *Callitropsis araucarioides* Compton is the same plant as *Neocallitropsis araucarioides* (Compton) Florin. Both names define the plant but the latter, new name is preferred. Unfortunately chemists are frequently very careless in their

spelling of Latin names and their unfortunate "misprints" are often, naïvely, cited by later generations of chemists leading to much confusion. It has even happened that chemists have mistaken the botanical author name for that of an author of a chemical paper (e.g. Ansell, 1961). "L. Endl." for example is not a chemist; L. stands for Linnaeus and Endl. for Endlicher, two prominent botanists.

Compilations of natural products are generally arranged according to their chemical classification and plants from which the substances have been isolated are often barely mentioned. In the main, little attention is paid to the organs in which they have been found. "Occurring in *Pinus silvestris*" or "in the essential oil of *Thuja occidentalis*" does not tell the reader very much. Chemotaxonomic studies would be greatly facilitated if compilations of natural products were produced based on a botanical classification as is the case in some classical works such as Wehmer's "Die Pflanzenstoffe" (1929, 1931) and Kariyone's recent "Annual Index of the Reports on Plant Chemistry" (1960).

This paper was read at the Second Symposium on Natural Products held in Prague, 1962. It is also to appear in *Pure and Applied Chemistry*.

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CHAPTER 5

Usefulness of Chemistry in Plant Taxonomy as Illustrated by the Flavonoid Constituents

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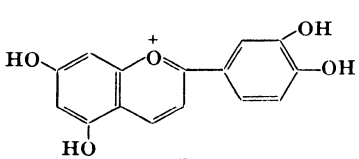
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I. Introduction

The flavonoid compounds are now taken to include not only those substances having the true flavonoid structure, but also such closely related classes of compounds as the chalcones, isoflavones, and aurones, the stilbenes, and the cinnamic acids and coumarins which are demonstrably associated with the true flavonoids in being formed in plants by a single synthetic pathway (Fig. 1).

Flavonoid compounds are rarely found in any but vascular plants; in fact the first authentic report of their occurrence in a non-vascular plant is extremely recent, that of an anthocyanidin lutolinidin (I), in a moss



(I)
Luteolinidin

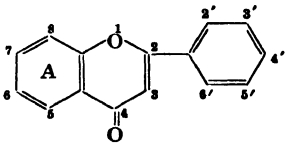
(Bendz, Martensson and Terenius, 1962). It is notable that this is also the first recorded occurrence of this particular anthocyanidin in nature. Ferulic and *p*-coumaric acids (Table I) have also recently been reported

to be present in mosses (Ibrahim, Towers and Gibbs, 1962), but the author has been unable to confirm this. Leucoanthocyanins are certainly completely absent from mosses (Cambie, Cain and La Roche, 1961), whereas these and other flavonoid compounds are overwhelmingly present in the vascular plants, occurring equally in the ferns, the gymnosperms, the monocotyledons and the dicotyledons.

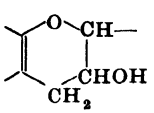
The association of flavonoid compounds with vascularity in plants is, of course, not fortuitous; the linkage through lignification is inescapable. Lignins may, in fact, be regarded as flavonoids in the wider sense, although their chemistry is as complicated as that of synthetic resins and more difficult to infer, because the *actual* building-stones are not yet known. It is now generally agreed that these precursors are phenylpropane units in some shape or form (coniferyl alcohol is strongly supported by many chemists) (Freudenberg, 1955), so that of the regular flavonoid constituents (Table I) the cinnamic acids are the closest in structure to them.

II. Importance of Flavonoid Compounds

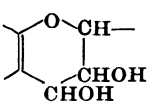
Consideration of the possible function of plant constituents is of the utmost importance in discussing their bearing on plant relationships, and therefore on classification. Constituents which are actively concerned in essential metabolic processes may be present in larger or smaller amounts (or even totally absent) depending on the momentary balance of those processes of metabolism in which they are involved. Constituents such as citric, malic and tartaric acids, certain sugars, and particular nitrogenous bases do occur in exceptional concentrations in many plants in quite unrelated genera for reasons which cannot easily be ascertained. The exceptional usefulness of the flavonoid constituents as taxonomic guides however is due to the fact that they are not actively concerned in cellular metabolic processes. If, subsequent to their formation, they are involved in some normal physiological processes, these must proceed at a relatively low rate, so that any particular flavonoid constituent can be relied on to be present in more or less constant amounts, in the same tissues of the same species so long as the plants are grown under normal physiologically healthy conditions. The only conspicuous exception to this rule is that the balance between mono- and dihydroxy substituted constituents, such as *p*-coumaric and caffeic acids, or kaempferol and quercetin (Table I) can vary rather widely, without significance from the taxonomic point of view. This is not true, however, of the balance between di- and trihydroxy constituents: the biosynthetic pathways must be quite different for these two types of end product.



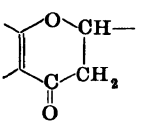
Flavone



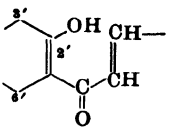
Catechins
[Flavan-3-ols]



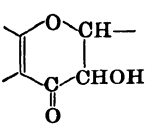
Leucoantho-
cyanins
[Flavan-3,4-diols]



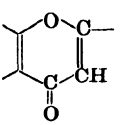
Flavanones



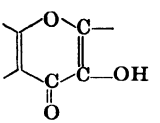
Chalkones
[note numbering]



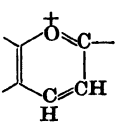
Flavanonols



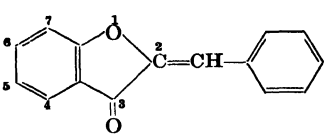
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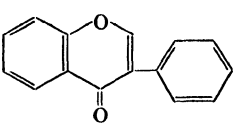
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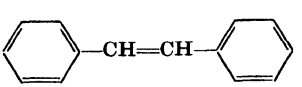
Anthocyanidins



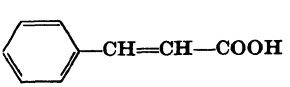
Aurones



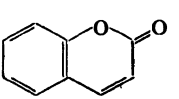
Isoflavones



Stilbenes



Cinnamic acids

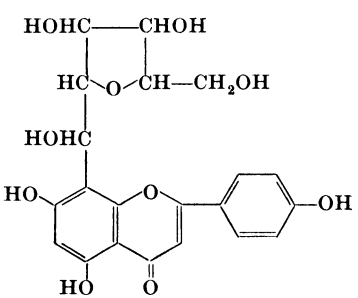


Coumarins

FIG. 1. Types of flavonoid and related compounds.

This raises another important question of taxonomic interpretation. The loss of a single step in a biosynthetic sequence might occur without any significant morphological consequences, so that plants which are judged to be closely related on these grounds might differ widely in their chemical constitution. On the other hand, the loss of a step in biosynthesis might have so profound an effect on the physiological development of a plant, that marked morphological differences would ensue, and the taxonomic consequences would then be considerable. The more we can learn, therefore, about the physiological function and relationship of the different constituents, the better shall we be able to evaluate the importance of the differences we observe in the chemical constitution of species, genera, families, and other classes of plants. While chemical characters can already, demonstrably, be valuable aids to the taxonomist, their value will be enormously increased when we can link differences in chemistry with the morphological differences on which the whole of present-day taxonomy is based.

Several instances can be given by way of illustration of the great diversity of flavonoid compounds. Fuller details of these compounds and their systematic distribution in the dicotyledons have been given elsewhere (Bate-Smith, 1962). The first instance is that of the occurrence of the glycoflavone vitexin (II) in *Crataegus* species. The general pattern of

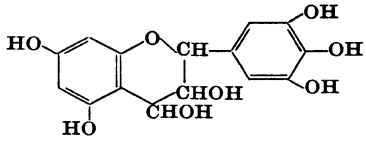
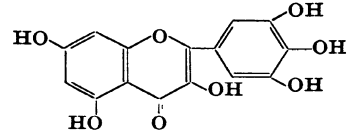
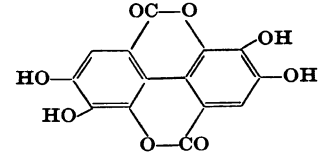
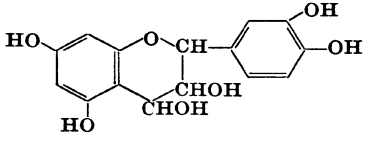
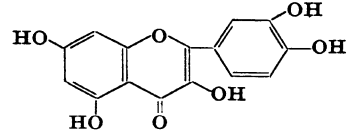
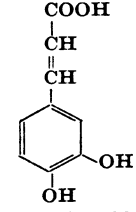
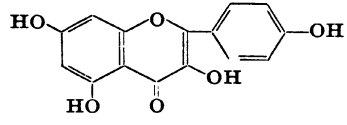
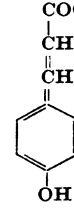


(II)
Vitexin

flavonoid distribution in the Rosaceae is the very common one of leucocyanidin, quercetin, kaempferol, caffeic and *p*-coumaric acids. Vitexin had been reported in "hawthorn" (*Crataegus oxyacantha* L. complex, Fiedler, 1955) and its presence has been confirmed in both *C. monogyna* and *C. oxyacanthoides*. It is absent from all other species of *Crataegus*, from all species of *Sorbus*, and from all other members of the Rosaceae so far examined. Kaempferol (Table I), if not completely absent, is present only in the minutest traces in *C. monogyna* and *C. oxyacanthoides*. It seems therefore that in these species (or in the original *C. oxyacantha* L.

TABLE I

Regular flavonoid constituents of plants

	Leucoanthocyanins*	Flavonols	Hydroxy acids†
Trihydroxy	 Leucodelphinidin	 Myricetin	 Ellagic acid
Dihydroxy	 Leucocyanidin	 Quercetin	 Caffeic acid
Monohydroxy		 Kaempferol	 *p*-Coumaric acid

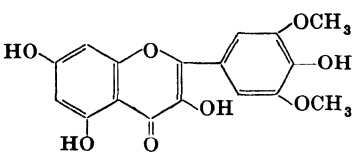
* Monomeric structures shown; polymers of these will more usually be present.

† Ferulic acid (3-*O*-methyl-ether of caffeic acid) and sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) are also widespread in plants (Bate-Smith, 1956, 1962).

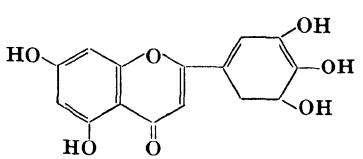
complex) kaempferol is vicariously represented by vitexin. Although, in these species, quercetin is present in quantity, the glycoflavone analogous to it, orientin, is absent. It does seem, therefore, that kaempferol alone of the flavonols is vicariously represented by the glycoflavone. This gives rise to interesting speculations about the relationship between the mono- and dihydroxy representatives of the different classes of constituents mentioned above; the route by which synthesis of kaempferol could be switched to vitexin; the physiological consequences of the substitution of vitexin for kaempferol; and the systematic significance of the so-called "carbon–carbon glycosides", which appear at present to be so randomly dispersed both systematically and as regards the classes of compounds in which they occur (Hörhammer and Wagner, 1961).

III. Transformations of Flavonoids

One of the most striking features of the distribution of flavonoid compounds is the absence of certain substitution patterns from particular classes of constituents. So far, for instance, neither the flavonol (III) corresponding to malvidin nor the flavone (IV) corresponding to del-



(III)



(IV)

phinidin have been reported (although they would have been detected had they been present). The *O*-methylated leucoanthocyanins, if they exist at all, must be exceedingly rare, and this is to be contrasted with the universal presence of *O*-methylated products and the complete absence of di- and trihydroxy substituted products arising from the breakdown of lignin. Taking into account also the non-occurrence of 3,4,5-trihydroxycinnamic acid, there are indications that certain sequences of biosynthesis are prohibited. If, as seems likely, the regular pattern in the most primitive vascular plants is that shown in Table I together with the phenolic elements included in the structure of lignin, departure from this pattern could take place (a) by the loss of steps in the biosynthesis of particular flavonoid molecules, (b) by failure to complete the synthesis and deposition of lignin, (c) by the modification of intermediates in biosynthesis (e.g. the modification leading to the formation of vitexin in lieu of kaempferol in the hawthorn) and (d) by the suppression of processes in particular tissues which the plant, taken as a whole,

has the capacity to perform. The last is particularly well seen in many herbaceous Leguminosae, the vegetative tissues of which do not contain leucoanthocyanins, which are nevertheless richly present in the testas of the seeds.

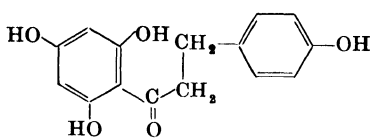
Physiological knowledge and the facts of systematic distribution are continually interacting in the elucidation of these problems. The bearing on taxonomy will come through the identification of the individual processes which particular plants can, and cannot, perform. Since "processes" tend to be envisaged as single enzymic steps or chemical reactions, for which there is no present evidence or justification, it seems better for the time being to use a noncommittal term in discussing them, and for the moment therefore I am using the term "transformations" to denote the ways in which flavonoid compounds differ in structure from the regular constituents shown in Table I.

In the present context it is important to know to what extent these transformations can be used as taxonomic guides. If the transformation is common, for instance the reduction of the flavonol to the flavone, it is unlikely in itself to be indicative of relationship between the plants exhibiting it. But if it is uncommon, for instance the shift of the B ring from C2 to C3 resulting in isoflavone formation (Fig. 1), then it may well indicate a possibility of relationship. This remains only a possibility, however. Isoflavones are at present known to occur in only a very few families. Two of these, the Rosaceae and the Leguminosae are obviously related. Others, such as the Iridaceae and the Podocarpaceae are just as obviously quite unrelated to either or to each other. The value of such coincidences at the present time can only be to draw attention to the *possibilities* of relationships which may not be apparent from the study of morphological characters alone, to strengthen the evidence of relationship which is otherwise inconclusive, or to help to elucidate the considerable number of taxonomic problems still outstanding.

A "transformation" of quite a different kind can be used as an illustration. This is the absence of a hydroxyl group in the 5-position of the flavonoid structure. This almost certainly represents an aberration in the regular process of synthesis of the A ring, so that a resorcinol residue is formed in lieu of the usual phloroglucinol residue; although Robinson (1955) suggests that the loss of hydroxyl might be due to "capture" by the double bond of a flavone. In any case, this transformation is found in only three families: in the Leguminosae, the Anacardiaceae and the Compositae. It is especially frequent in the first, representatives of practically every class of flavonoid compound possessing this feature having been isolated: anthocyanins, leucoanthocyanins, catechins, flavonols, aurones (not flavones!), chalcones and isoflavonoids (Fig. 1) in great

variety. In the Anacardiaceae the leucoanthocyanins and flavonols are the best-known representatives, and in the Compositae, tribe Heliantheae, only the aurones, chalcones and flavonones. In many instances both in the Leguminosae and the Compositae this feature is accompanied by substitution of an extra hydroxyl or methoxyl group in the 6- or 8-position (see Fig. 1). As Geissman pointed out, the chalcone and aurone structures are more probable configurations than the flavanone and flavone structures when the hydroxyl group in the 5- position is absent, so that several "transformations" are linked together as a consequence of one aberrant step in synthesis. It seems likely that many of the numerous transformations observed in the leguminous isoflavonoids are similarly linked to the absence of the 5-hydroxyl group in these constituents.

Yet another useful illustration is provided by the dihydrochalcones, of which phloretin (V) is the best-known example. As the glucoside



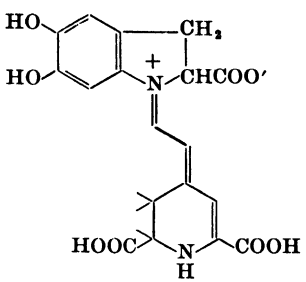
(V)
Phloretin

phloridzin, this occurs in the apple tree (*Malus sylvestris* Tourn.) and Williams (1960) has shown that alone, or together with its 3′-hydroxy derivative, it occurs in all species of *Malus*. It has not been reported from any other genus of the Rosaceae, but occurs in *Pieris japonica* in the Ericaceae, together, in some individuals, with its 4′-*O*-methylated derivative, asebogenin, and also in some specimens only of *Smilax glycyphylla* in the Liliaceae (Williams, personal communication). There is obviously some mystery attached to the irregular appearance of these substances, and it is at present unsafe to attempt to draw any conclusions as to the taxonomic significance of their distribution. Fortunately such instances of variability of occurrence of flavonoid compounds in the vegetative tissues of species are rare (this is, in fact, the best-documented case) and as stressed earlier this is one of the reasons why the flavonoid compounds are so outstanding as taxonomic guides.

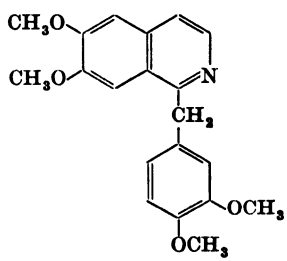
IV. Biosynthetically Related Compounds

The above examples have been chosen as cases where there is reason to suppose that the end products represent modifications of pathways of synthesis towards the regular flavonoid constituents. At least a dozen

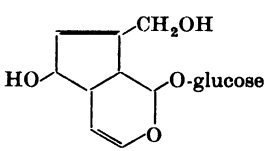
different modifications can be identified in which the "unit transformation" takes place at a point fairly far advanced in the synthetic pathway, where the 15-carbon structure of the end product has already been determined. There are several other examples where the flavonoid nature of the end product can be inferred but not strictly demonstrated. Some of these occur among the isoflavonoids and rotenoids of the Leguminosae, so ably reviewed recently by Grisebach and Ollis (1961). Obviously related to the flavonoids are the betanins (VI) (Mabry, Taylor and Turner, 1963) and the isoquinoline alkaloids (e.g. papaverine, VII), and an ingenious suggestion by Wenkert (1961) would even bring the aucubins (VIII) into a distant biosynthetic relationship with the flavonoids through his postulated *seco*-prephenic-formaldehyde (SPF) precursor (IX).



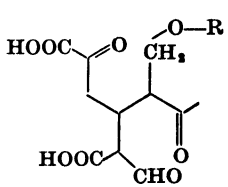
(VI)
Betanidin



(VII)
Papaverine



(VIII)
Aucubin

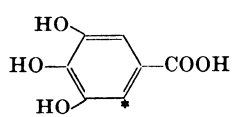


(IX)

The classes of substances represented by (VI) and (VII) are confined to very closely related taxa, and those represented by (VIII) are also significantly restricted to particular families of the dicotyledons, especially the Contortae, the Tubiflorae and the Rubiales. The common feature of the biosynthesis, actually demonstrated or postulated, of these and all the flavonoid compounds is the shikimic–prephenic pathway, but it is a particular further development of that pathway which appears, as I said at the outset, to have been developed only in the vascular plants.

The innumerable non-flavonoid phenolic compounds found in both vascular and non-vascular plants can be formed by a number of routes other

than the *shikimic*-prephenic *pathway* mentioned above. They may arise *directly* from shikimic acid, or from acetate, and it is likely that the great majority of the phenolics of non-vascular plants, many of them methylated in the aromatic ring, are formed from this last named precursor. The hydroxy- and hydroxymethoxybenzoic acids are especially interesting in this context, since in any given instance they might be formed either directly from shikimic acid or by the prephenic pathway, followed by a shortening of the three carbon side-chain. For this reason it is necessary for the time being to refrain from a consideration of the taxonomic significance of these possible flavonoid fragments, although many of them, as Ibrahim *et al.* (1962) have recently shown, may prove to be very valuable in this connection. There is, however, one exception to the application of this cautionary principle, and that is the case of hexahydroxydiphenic acid. In acid hydrolysates this appears as its dilactone, ellagic acid (Table I) and the presence of this constituent is usually indicative of the presence of "ellagitannins" (usually esters of the parent acid with glucose) in the original plant material. Ellagic acid might, however, be present as such in the material, arising by oxidative coupling of the two molecules of gallic acid (X). It is possible by a colour reaction



(X)

(* indicates point at which dimerization takes place)

with alcoholic extracts of the plant material to distinguish between these two possibilities. I wish to suggest that in vascular plants hexahydroxydiphenic acid (identified as ellagic acid in acid hydrolysates) replaces trihydroxycinnamic acid which, from considerations of symmetry, should be found in those plants containing other trihydroxy flavonoids, just as caffeic and *p*-coumaric acids are found along with the corresponding di- and monohydroxy flavonoid constituents. The distribution of ellagic acid is rather remarkable. So far, it has not been found in ferns, gymnosperms or monocotyledons, although the number and diversity of those examined is more than sufficient to have disclosed its presence, had it been at all common. In the dicotyledons it is confined to certain families where it is usually, but not always, associated with other trihydroxy flavonoid compounds. It has, in fact, the possibilities of being a remarkably accurate taxonomic guide, both as regards its presence in plants currently placed outside the groups in which it consistently occurs,

and its absence from plants in which from their taxonomic position it might be expected to occur. When, as sometimes happens, ellagic acid occurs sporadically and unexpectedly, and the alcoholic extract of the plant concerned fails to give the colour reaction, mentioned above, for ellagitannins it can be assumed that it arises from the enzymic oxidation of other aromatic constituents, and not by any biosynthetic pathway. An example of this is found in the alga *Spirogyra*. In *S. arcta* Japanese workers reported the presence of gallic acid, and in *S. majuscula* the author found ellagic acid, although the alcoholic extract did not give the colour reaction for ellagitannins.

The emergence of ellagitannins uniquely in the dicotyledons has interesting phylogenetic implications which it would be out of place to discuss here. It is worth while taking note of this example of the importance not only of the actual presence of a particular constituent in a particular plant, but of the form of combination in which it occurs in that plant. The truth of this is becoming daily more apparent in the case of the hydroxy acids, such as caffeic acid, where the type of combination—as the acid moiety of an ester with quinic acid, tartaric acid (Scarpati and Oriente, 1958a) or glucose (Harborne and Corner, 1961) or in such bimolecular combinations as rosmarinic acid (Scarpati and Oriente, 1958b)—are proving to be highly specific to different families of plants. There is increasing evidence that this is also true of the different glycosidic combinations of the flavonoid compounds, such as the "glycoflavonols" (Bate-Smith and Swain, 1960) which now (Harborne, 1962) seem likely to be difficultly hydrolysable 7-glycosides. Glycosides are not so easily dealt with on a survey basis as are the aglycones and for some time to come their value will be most evident in the analysis in fine detail of the smaller taxonomic groups (cf. Chapter 13).

V. Conclusions

I would like to conclude by returning once again to the need for chemical data to be correlated with morphological characters if they are to be of any use to the taxonomist, who must rely mainly on visual characteristics for the recognition and classification of natural forms. If the correlation could be established through a chain of causality, confidence in the usefulness of the chemical data would be correspondingly increased. So far as the flavonoid constituents are concerned, the outstanding correlation between these compounds and plant morphology is with the woody or herbaceous habit of the plant, and this is only conspicuously employed at the present time in Hutchinson's classification of the dicotyledons. It is, nevertheless, a valid taxonomic character, and

it is now possible to see beyond this character to its physiological and biochemical causation in terms of the promotion and suppression of lignin deposition in the vascular plant. There are also indications that such critical taxonomic features of the angiosperms as choripetaly/sympetaly and actinomorphy/zygomorphy may be correlated with particular flavonoid patterns in vegetative organs.

As regards indicators of promotion or suppression of wood formation, the critical constituents seem to be the leucoanthocyanins. It was, however, noted as long ago as 1954 (Bate-Smith, 1954) that flavonols predominate in woody species, while flavones and flavanones predominate in herbaceous species. Later (Bate-Smith, 1956) it was observed that caffeic and ellagic acids were commonly present in predominantly woody families, while the methoxy acids (ferulic and sinapic) were more commonly present in herbaceous families. It would seem, therefore, that the presence of leucoanthocyanins, flavonols and hydroxy acids in the leaves is associated with the uninhibited deposition of lignin whereas the presence of flavones and methoxy acids in leaves is associated with a tendency for suppression of lignification.

The reciprocal situation regarding the substitution pattern of the lignin breakdown products and that of the cinnamic acids in the leaves of herbaceous plants has already been remarked. There is another coincidence not so far mentioned in that the flavonoids found in woody tissues are, as a rule, more reduced analogues of those found in the leaves; thus when quercetin is present in the leaves, dihydroquercetin is found in the wood; morin in the leaves, dihydromorin in the wood (Carruthers, Farmer and Laidlaw, 1957) and so on. Thus in the leaves methoxy-substitution of the acids is associated with flavonols and flavones, while in the wood methoxy-substitution of the lignin elements is associated with flavanonols and flavanones. It seems possible that the dislocation of the lignification process might be connected with the absence of flavonols from what would be the wood-forming tissues, and this would imply that the flavonols are in some way connected with the lignification process.

Not enough is known at present of the flavonoids in the leaves and wood of different species, especially of hardwoods, to do more than draw attention to the conjunction of reduced flavonols with methoxylation of aromatic residues related to lignin formation. It seems worth while doing so, however, since this is a feature of the systematic distribution of flavonoid compounds and, moreover, one which is correlated with a morphological character of which use is made in plant taxonomy.

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CHAPTER 6

Biosynthetic Pathways

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I. General Observations on Biosynthetic Sequences

The morphology and physiology of a plant are largely determined by genetic factors which presumably act through enzyme systems controlling chemical syntheses. Morphology, on which classification is normally based, is likely to depend on genetic constitution in a more complex way than that aspect of physiology which is concerned with the biosynthesis of relatively simple secondary constituents such as flavonoids, terpenes, coumarins and alkaloids. Each stage of such a synthesis requires an enzyme system which is genetically controlled and which can be modified or lost as the result of genetic mutation.

Examination of the results of such mutations in terms of alterations

of the structures of secondary constituents could therefore give information on evolutionary sequences, and to this extent it could be a very useful aid to taxonomy. Since the substances are the expression of chemical reactions and it is these reactions which are changed when their catalytic enzymes are affected, I propose to discuss the subject more in terms of chemical sequences and reactions, than in terms of classes of compounds. To set the background it is necessary to consider some of the commoner biosynthetic sequences and how they may be affected by mutation. In the limited space available it is not possible to consider more than a few important illustrative examples to demonstrate the kind of reasoning involved. A number of reviews of various fundamental aspects of the subject exist (e.g. Birch, 1957, 1960, 1962; Geissman, 1962; Ollis, 1961; Lynen and Tada, 1961) and may be referred to for detail.

Some processes are clearly more chemically "probable" than others, and the chemist can express some opinions based on mechanisms and laboratory analogies, and on grounds of biological frequency, as to the "probability" of a given process. The more probable the process, and the more closely related in type to normal biochemical mechanisms of metabolism which seem to be almost universal, the more likely it is to recur independently in unrelated organisms, and the less likely it is to be significant. The ready hydroxylation of phenols, for example, almost guarantees that evolution of processes such as those involved in the hydroxylation of flavonoid pigments in certain positions will occur repeatedly and independently. Other oxidations, such as those of saturated positions in triterpene or amino-acid molecules, or the highly stereo- and structure-specific cyclizations of terpene chains, might be considered much less chemically probable and therefore more likely to be significant of a specific evolutionary development.

A further point about mutation may be noted: that it is more probable on general grounds that a stage of a biosynthetic sequence will be lost, rather than gained. This is clearly illustrated for example by genetic work on flower pigments, where delphinidin → cyanidin → pelargonidin is the usual observed sequence after mutation, involving a progressive loss of ability to hydroxylate ring B of the nucleus (cf. Birch, 1960). The chemist should, it seems to me, not only attempt to define the nature of the biosynthetic alterations, but also try to assess their trivial or fundamental nature. Only the botanist can attempt to define how far he wishes to use such information in classification. It is already clear from Australian work on Myrtaceae, particularly *Eucalyptus* (Penfold *et al.*, 1953), and from Japanese work on *Ocimum* and *Cinnamomum* species (Fujita, 1951) to mention a few examples, that "physiological forms" of species occur in which the proportions and nature of the essential oil constituents vary

widely. Genetic mutations affecting the exact nature and proportions of anthocyanins in flowers are also well known. However, despite some superficial appearances these changes are usually relatively trivial in terms of the alterations required in the *processes*. Some aspects are discussed below.

A final general point concerns experimental methods for detecting changes in constituents, not only qualitatively but quantitatively. A number of modern techniques of great power and precision now permit the use of small amounts of material. These techniques include chromatography (paper, gas–liquid or thin-layer) combined with the use of the mass-spectrometer and other physical tools. It is now for the first time possible to analyse in detail and quantitatively very small amounts of such types as lipids, alkaloids and terpenes, including quite large molecules. Variations in relative proportions as well as positive or negative occurrence can be rapidly and precisely examined. A good deal of preliminary work, however, is required on the significance of such variations: the type of material, the stage of growth, the ambient conditions, clearly all have a profound influence.

Let us now consider some important illustrative examples.

II. Phenylpropane Derivatives

The fundamental skeleton of these clearly arises from phenylalanine (I) and its congeners, derived from shikimic (II) and prephenic (III) acids (Fig. 1). Let us consider one example of a C_6–C_3 series, where genetic and

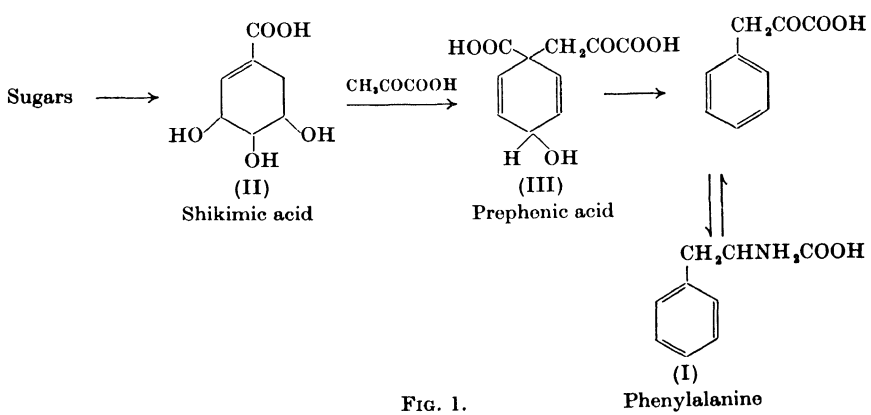


FIG. 1.

chemical considerations interact to give mutual information. The precursors of lignin are apparently the oxygenated cinnamyl alcohols (Freudenberg, 1960), probably derived by biochemical reduction of the

coenzyme-A esters of the cinnamic acids, themselves arising directly from phenylalanine or tyrosine with nuclear oxidation at some stage. In various *Cinnamomum* species a series of volatile C_6–C_3 compounds occur such as safrole (IV), eugenol (V), cinnamic aldehyde (VI), methyleugenol (VII), myristicin (VIII) and elemicin (IX). As expected, the production of safrole in *C. camphora* Sieb. was shown to parallel lignification, and was not related to terpene formation (Fujita, 1951). The most likely method for the production of substances lacking the side-chain oxygen (IV–IX) is reduction of the cinnamyl alcohol, and on the basis of modern biochemistry a process of the type shown below (Fig. 2) would be predicted.

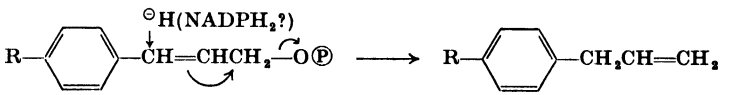
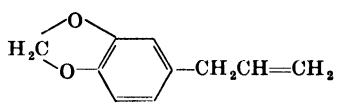
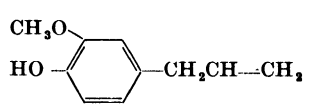


FIG. 2.

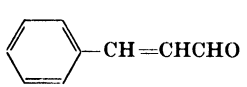
This sequence explains the predominance of the allyl rather than propenylbenzene type in such compounds and we will return to this mechanism later. Recently Kaneko (1961) has shown that [^{14}C]phenylalanine is in fact converted into anethole in *Foeniculum vulgare*.



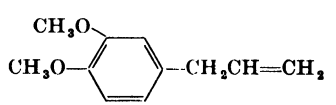
(IV)
Safrole



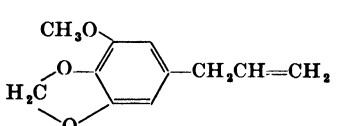
(V)
Eugenol



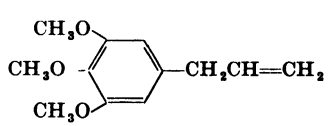
(VI)
Cinnamic aldehyde



(VII)
Methyleugenol



(VIII)
Myristicin



(IX)
Elemicin

The occurrences of C_6–C_3 compounds, taken from surveys by Fujita (1951) and Guenther (1952) are shown in Table I.

It is probable that more exact sampling methods would sharpen the distinctions between the three types of oil which are distinguishable containing: (1) Safrole with lesser amounts of eugenol, methyleugenol,

TABLE I

Distribution of phenylpropane derivatives in *Cinnamomum* species

No.	Species	Safrole	Eugenol	Methyl-eugenol	Elemicin	Cinnamic alcohol	Cinnamic aldehyde
1	*C. nominale* Hay	+					
2	*C. laubatii* F. V. Mull	+					
3	*C. parthenoxylon* Meissn.	+					
4	*C. mercadoi* Vid	+					
5	*C. pendunculatum* Presl. (var. A)	60%	3%				
6	(var. B)		+	+			
7	*C. kanakirai* Hay	+	+				
8	*C. micranthum* Hay	+		+	+		
9	*C. pedatinervium* Meissn.	50%					
10	*C. cecidodaphne* Meissn. (Type)*	+			+		
11	(var. A)			+			
12	(var. B)	20%		45%			
13	*C. massoia*	14%	79%				
14	*C. glanduliferum* (var. A)			+			
15	(var. B)	25%		45%			
16	*C. culilawam* Blume (Type)	+					
17	(var. A)	20%		60%			
18	(var. B)		76%				
19	*C. sintok* Blume (Type)	+					
20	(var. A)	40%		60%			
21	(var. B)		+				
22	*C. zeylanicum* Breyn (var. A)	+	65–95%			+	+
23	(var. B)†		10%				65–76%
24	(var. C)	+	+				+
25	*C. loureirii* Nees (var. A)		+				
26	(var. B)						+
27	*C. tamala* Nees & Eber (var. A)		+				
28	(var. B)						+
29	*C. kiamis* Nees (var. A)		10%				45–62%
30	(var. B)						+
31	*C. mindanaense* Elmer						+
32	*C. cassia* Blume						+

* Also contains myristicin.

† Also contains 3-phenylpropionaldehyde.

myristicin or elemicin; [1–12 (Table I) comprising 12 species or varieties]; (2) Eugenol or methyleugenol with lesser amounts of safrole or cinnamic aldehyde; [13–22, 25, 27 comprising 12 species or varieties]; and (3) Cinnamic aldehyde with lesser amounts of eugenol or safrole [23, 24, 26, 28, 29–32 comprising 8 species or varieties].

The chemical groups overlap in species which produce varieties ("physiological forms") which are, or have been thought to be, morphologically identical. Groups (2) and (3) are represented by forms 22–24, 25–26 and 27–28, and groups (1) and (2) by forms 5–6; in 14–15, 19–21, and 23–24, forms also occur in which the relative proportions of safrole and eugenol vary considerably.

A probable relationship between safrole and eugenol can be postulated on the basis of the work of Scribney and Kirkwood (1953) on the biogenesis of protopine, who showed that the carbon atom of the methylenedioxy group comes from the C_1-pool also responsible for *O*-methylation. The methylenedioxy group could well come directly by a one-stage oxidation from the ortho-methoxy hydroxy compounds as shown below (Fig. 3).

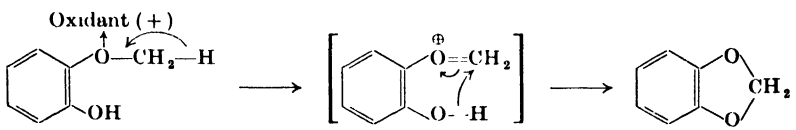


FIG. 3.

The difference between plants producing the methylenedioxy-(safrole), methoxy-hydroxy-(eugenol) and the dimethoxy-(methyleugenol) groups, would depend on competing oxidation or methylation processes which would be genetically very simply determined. In particular a mutation to eliminate the oxidation stage would be very likely to occur.

The most interesting relationship is that between on the one hand, cinnamic aldehyde or cinnamyl alcohol with an oxygenated side-chain but no oxygen in the ring, and on the other hand, safrole or eugenol with an oxygenated ring but an oxygen-free side-chain. A similar relationship exists in *Ocimum basilicum* Linn. and *O. canum* Sims where numerous varieties occur, the C_6–C_3 compounds interchanged being methyl cinnamate and methylchavicol (4-methoxyallylbenzene). It is noteworthy also that although cinnamic acid, cinnamyl alcohol and cinnamic aldehyde are not uncommon natural products, allyl and propenylbenzene have never been noted. This evidence suggests that a *para*-substituted oxygen is necessary in the ring for reduction of the side-chain to occur, in agreement with the mechanism cited above (p. 144), the mesomeric effect of the oxygen greatly facilitating ionisation of the phosphate (or similar)

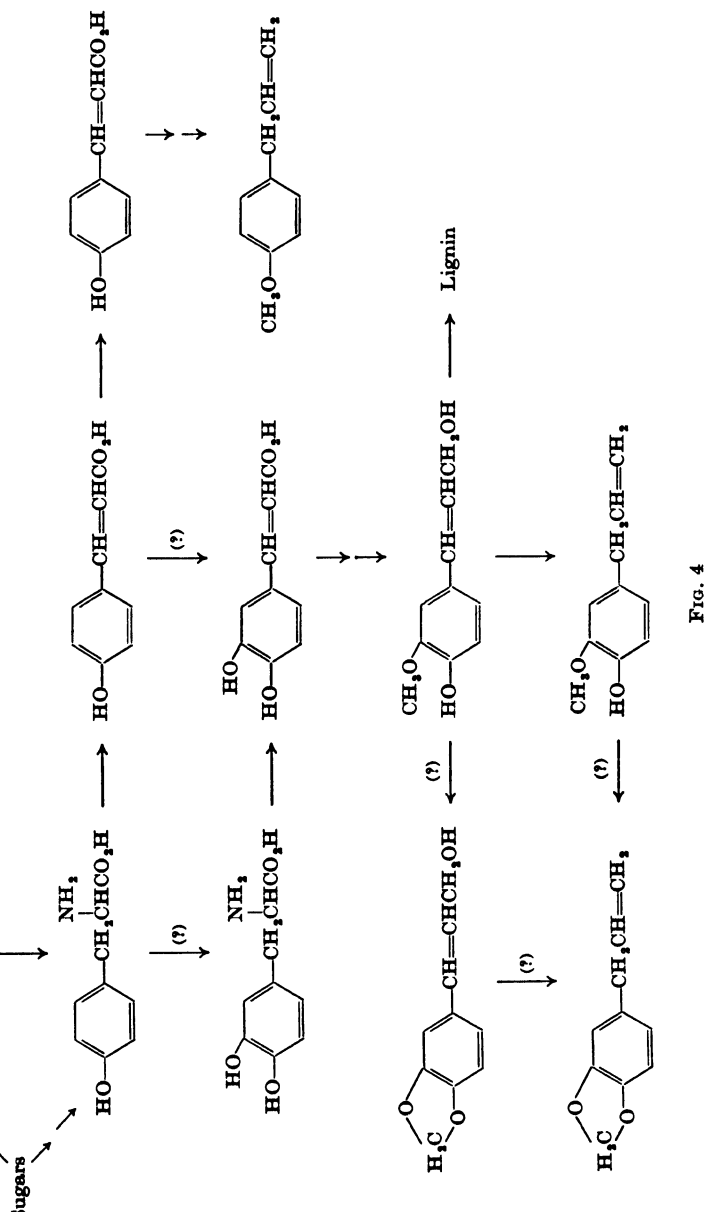


FIG. 4

group of the side chain. A model process based on this mechanism has been carried out in the laboratory in the conversion of methylenedioxycinnamyl alcohol into a mixture of safrole and isosafrole. Lack of the *para*-oxygen would be sufficient to prevent reduction. A schematic representation of the major relationships in the phenylpropane group of compounds would then be as shown (Fig. 4). The exact sequences are at present necessarily vague: only biochemical experiment can decide, for example whether the methylenedioxy ring is formed at the eugenol stage, or in a precursor.

In some *Melaleuca* species an interchangeability is found between allyl- and propenylbenzenes: in this case a further genetically determined simple step must involve the migration of the double bond into conjugation with the ring.

Considerations of this kind are important in assessing genetic differences: in particular it is possible to note that the following reactions among others involve an *additional* chemical process: (1) oxidation, in the case of conversion of eugenol into safrole; (2) methylation, in the conversion of eugenol into methyleugenol; (3) reduction, in the removal of the side-chain hydroxyl group from cinnamyl alcohols.

The accumulation of cinnamyl alcohol, aldehyde or acid, indicates, to some extent, the *loss* of ability to introduce *para*-oxygen into the ring. From this information the tendency of the plants to diverge from a common source (presumably the eugenol-producers) can at least partially be assessed.

III. Flavonoid Pigments and Related Compounds

A. Anthocyanins, Flavones and Isoflavones

The skeleton of the flavonoid and anthocyanin pigments arises from a cinnamic acid unit and three "acetate" units (biochemically the substituted cinnamoyl coenzyme-A and malonyl coenzyme-A) (Birch and Donovan, 1953; Underhill, Watkin and Neish, 1957; Grisebach, 1957).

The genetics of flower pigment variation has been discussed previously (Birch, 1960; Harborne, 1962), and cannot be considered here in detail. Apart from methylation or the addition of sugars to hydroxyl groups, the evidence is chiefly concerned with variations in the state of oxidation of the rings, which, as already noted, embodies a type of process likely to be independently evolved with some frequency. Some general points of interest emerge, however. It has been clear for some time that in anthocyanin biosynthesis the route flavanones → flavanonols → flavenols → anthocyanins is probably the direct one (route (a), Fig. 5) (cf.

Birch, 1960). The 3-hydroxyl group is probably introduced as the result of an oxidation of the enol-form of the flavanone, presumably with formation of a cation as shown below. The production of flavones and isoflavones could proceed directly through such a cation, providing it had the correct stereochemistry when attached to the enzyme surface; in one case (route (b), Fig. 5) there is trans-elimination of a proton, in the other case (route (c), Fig. 5) migration and rear-attack of the cation by the 2-substituted aryl ring. Relatively small modifications of the enzyme might result in any one of the three pathways (a)–(c); the same cation would be attacked by water to attach an hydroxyl leading finally to the anthocyanins. The Leguminoseae are the major groups of plants which seem to have "learned" both how to transpose the aromatic ring and also how to introduce an ortho hydroxyl into this ring.

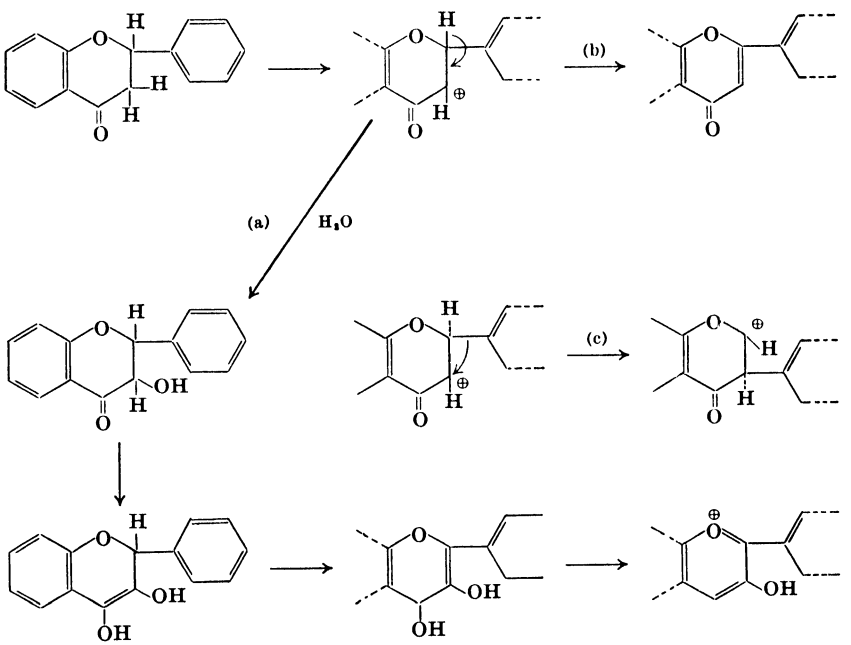


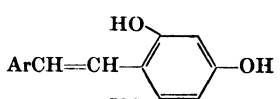
FIG. 5.

It is of greater interest to consider more unusual modifications of the skeleton due to genetic variations which could produce enzymes capable of: alternative ring-closures; alterations of synthetic stages involving different ratios of cinnamic acid and acetate units; additions of synthetic stages involving other biosynthetic units; replacement of the cinnamic acid by other acid units; and oxidative dimerizations.

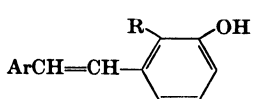
B. Alternative Ring-closures: Stilbenes

The enzyme-bound intermediate in flavonoid synthesis is probably the coenzyme-A ester of (X) (Birch and Donovan, 1953; Birch, 1962) which ring-closes to the chalcone (XI). An alternative ring-closure of a β-polyketo chain common in mould products (Birch, 1957), mechanistically readily acceptable as an aldol-condensation, would lead instead to (XII, R = COOH), the precursor of the plant stilbenes, e.g. (XII, R = H) (Birch and Donovan, 1953). Despite the difference between the two series, we would not expect any great difficulty in modifying the enzyme systems involved. In fact, in most pine heartwoods, flavonoids and stilbenes occur together (Lindstedt and Misiorny, 1957), as they do in some *Eucalyptus* (Hillis and Hasegawa, 1962), and in *Hydrangea*. These are probably cases of parallel but independent evolution. It is notable also that *Eucalyptus sideroxylon* A. Cunn (Benth.) occurs in two varieties, one containing quercetin, the other biogenetically related stilbenes (Hillis and Hasegawa, 1962).

$$ArCH{=}CHCOCH_2COCH_2COCH_2CO_2H$$
(X)



(XI)

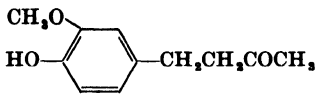


(XII)

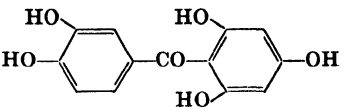
C. Cinnamic Acid and Acetate Units in Different Ratios

Many natural products are known the biosynthesis of which appears to involve a "starter-unit" consisting of a cinnamic acid with the addition of greater or lesser numbers of acetic acid units than those mentioned above. Examples are zingerone (XIII), maclurin (XIV), piperic acid (XV), kawain (XVI), and cortisalin (XVII), the analysis of whose structures into such biosynthetic "units" is fairly obvious. Other variants can be noted: shogaol (XVIII) probably involves the union of a fatty acid chain (itself acetate-derived) and a cinnamic acid, and curcumin (XIX) involves two cinnamic acid and one acetate units. Since flavonoids are far commoner in occurrence than such compounds, these unusual structures may well mark alternative methods for the disposal of products of interrupted flavonoid biosynthesis. To judge by these diverse types encountered, however, there seems to be little biochemical difficulty in adding "acetate" (malonyl coenzyme-A) units to almost any

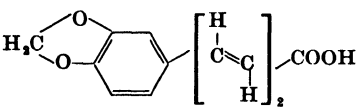
acyl coenzyme-A. The reduction, dehydration and other reactions necessary for the elaboration of the final products are also of expectable type.



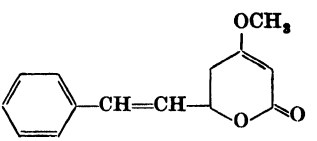
(XIII)
Zingerone



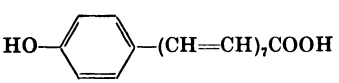
(XIV)
Maclurin



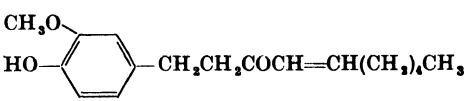
(XV)
Piperic acid



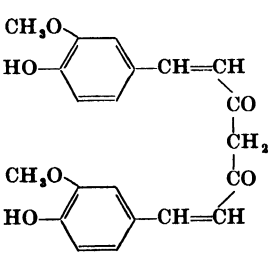
(XVI)
Kawain



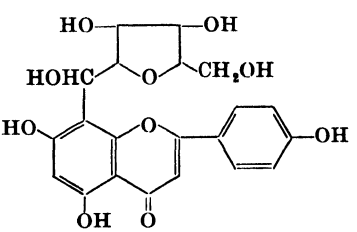
(XVII)
Cortisalin



(XVIII)
Shogaol



(XIX)
Curcumin



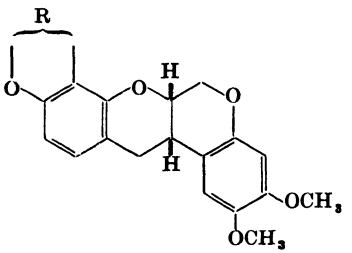
(XX)
Vitexin

D. Addition of C_1 and Terpene Units

Methyl groups (from methionine) and terpene units (from the corresponding C_{5n} alcohol pyrophosphate) are frequently encountered on both oxygen and nitrogen. The alkylation of aromatic rings by such units, particularly in the coumarins and flavonoids, occurs apparently less commonly, but is nevertheless also well known. It is particularly frequent in some families: Rutaceae species, for example, frequently contain coumarins and alkaloids with isopentenyl groups or the derived simple

furan ring. *C*-Methylation of flavonoids and related compounds is particularly notable in some pines and ferns.

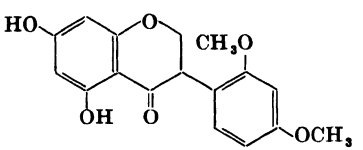
The sugar units found in glycosides appear to be capable of addition to phenolic groups in almost all plants. On occasion the sugar reacts with the carbon of a ring instead of oxygen, e.g. to give vitexin (XX), a mechanistically acceptable variant of the normal process.



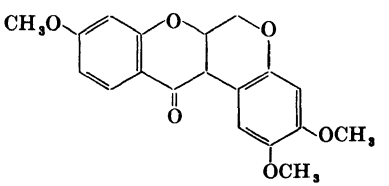
(XXIa) Rotenone R = $H_2C{=}C(CH_3){-}CH{-}CH_2$

(XXIb) Deguelin R = $(CH_3)_2C{-}CH{=}CH$

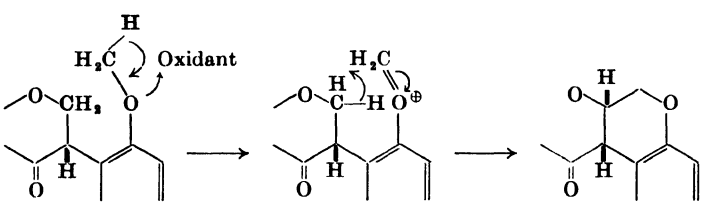
(XXIc) Elliptone R = $CH{=}CH$



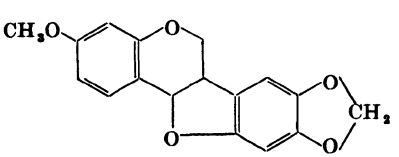
(XXIV) Homoferreirin



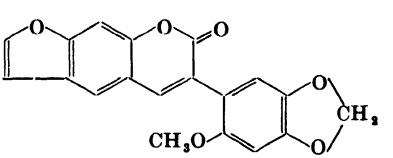
(XXV) Munduserone



(XXII) (XXIII)



(XXVI) Pterocarpin



(XXVII) Pachyrrhizin

The numbers, positions and modifications of such "extra" units vary in a manner at least partially interpretable in terms of the reactions involved. For example, a compound containing two *C*-methyl groups clearly requires an extra stage compared with a substance containing only one. The first introduced C-linked group in the case of the isopentenyl

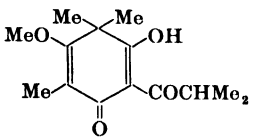
(isoprene) unit is almost certainly the unsubstituted $(CH_3)_2C{=}CHCH_2{-}$, so that modifications of such a group by oxidation and ring-closure involve extra biosynthetic stages. Merely to illustrate some complete sequences let us look at rotenone (XXIa) and related substances (cf. Grisebach and Ollis, 1960).

Rotenone (XXIa), deguelin (XXIb) and elliptone (XXIc) all appear biogenetically to be derivatives of isoflavones, e.g. (XXIV), with an extra carbon to form a second heterocyclic ring and an introduced, cyclized and oxidized isopentenyl unit (XXIa) and (XXIb), to form a third, the C_3 side-chain being eliminated in (XXIc) to give an unsubstituted furan ring. The exact *sequence* of reactions producing these substances is not entirely clear, but the types of reaction involved are fairly certain and involve a series of extra stages based on an initial flavonoid-type biosynthesis. These are (possibly roughly in order): (*i*) synthesis of a flavanone from a cinnamic acid and three "acetate" units; (*ii*) establishment of the oxygenation pattern (particularly introduction of an ortho-oxygen (2′) into ring B) and this does not necessarily precede the next stage but it is notable that many isoflavone derivatives possess it, whereas flavones do not; (*iii*) migration of the ring-B from position 2- to position 3- as the result of an oxidation reaction; (*iv*) introduction of the requisite C_1-units (2′-OMe) and C_5-unit (in position 8-); (*v*) oxidative ring-closures to complete the main nucleus (e.g. of the type (XXII)→(XXIII) (which agrees with the presence of a *cis*-ring junction) together with mechanistically obvious reactions to generate the furan or pyran rings. We may note that examples of many of the postulated intermediate stages, or obvious alteration products, are known in Nature: isoflavanones such as homoferreirin (XXIV), and compounds with one extra heterocyclic ring such as munduserone (XXV). With such ideas in mind it is possible to suggest, and ultimately to test, the points of departure from the main biosynthetic schemes of these and many related compounds such as pterocarpin (XXVI) and pachyrrhizin (XXVII). The absolute configurations of all these substances, so far as they are known, agree with a common type of intermediate.

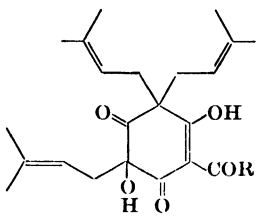
E. Replacement of Cinnamic Acid by Other Acid Units

A number of acyl phloroglucinol derivatives are fairly widespread in plants although in small quantities, many containing "introduced" methyl groups such as tasmanone (XXVIII) or isopentenyl groups such as humulone (XXIX). It is particularly striking that hops, which contain (XXIX) also contain flavonoid pigments with introduced isopentenyl groups. The former compounds although not directly on the biosynthetic

route involving flavonoids and their congeners, obviously arise by biochemically parallel ones.

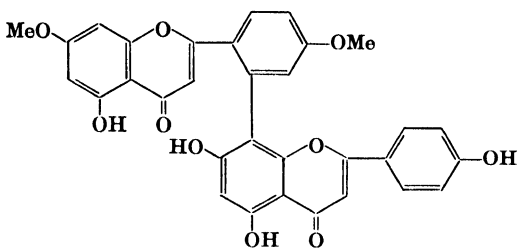


(XXVIII)
Tasmanone



(XXIX)
Humulone

R = $CHMe_2$, CHMeEt etc.



(XXX)
Ginkgetin

F. Oxidative Dimerization of Flavonoids

In recent years a number of dimeric flavonoids, e.g. ginkgetin (XXX) have been found which are the result of a specific oxidative dimerization. Their taxonomic distribution has been discussed by Baker and Ollis (1961); they seem to be almost entirely restricted to the leaves of Gymnosperms. In particular they are absent in Pinaceae but present in all other members of the Coniferales, and particular types seem to have restricted distributions. They clearly may well be useful both in classification and when considering phylogeny.

IV. Terpenes

Terpenes are now known to arise from the isomeric isopentenyl pyrophosphates (XXXI) and (XXXII) (Fig. 6) the number of C_5-units being 2, 3, 4, 6, etc., up to several thousand in compounds like rubber (XXXIII). Various cyclization and oxidation processes occur to give the complex molecular skeletons of sesqui- (C_{15}), di- (C_{20}) and tri- (C_{30}) terpenes, and

the biochemical mechanisms of such processes are now becoming understood in some detail. We can only select a few examples illustrative of different aspects of the subject.

Terpenes of different molecular size appear to be "interchangeable" according to the genetic constitution of a plant, as would be expected in view of the common precursors. *Cryptostegia grandiflora* and *C. madagascariensis* produce rubber (XXXIII) and lupeol (XXXIV) respectively.

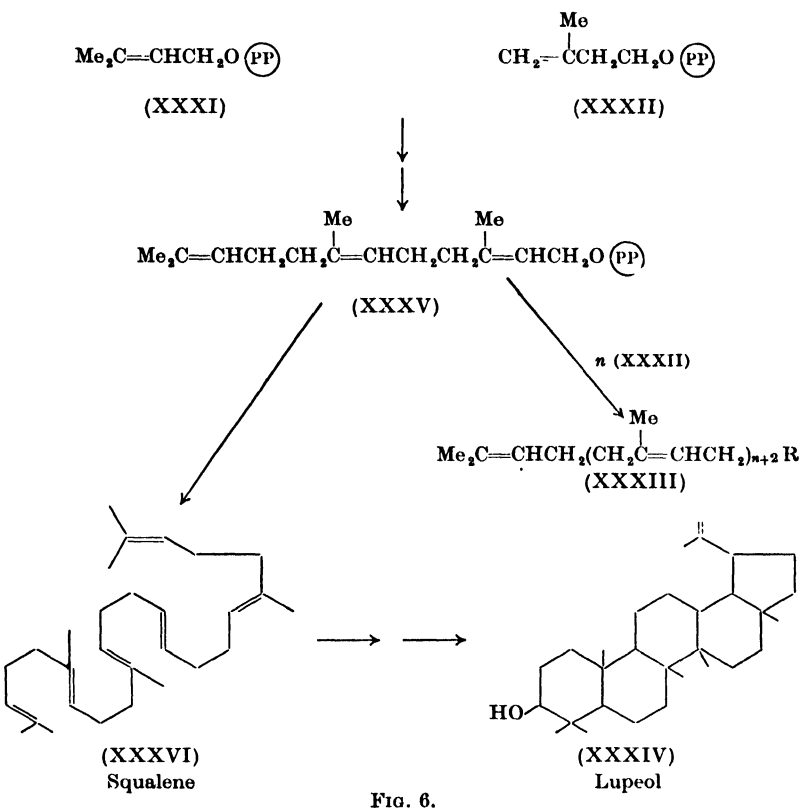


FIG. 6.

Inter-specific hybrids form rubber, so that polymerization is the dominant process when the requisite enzymes are present. The F_2 generation segregates in a manner which demonstrates a simple Mendelian recessive character for the gene producing lupeol (Bonner, 1949). The largest common precursor here must be farnesyl pyrophosphate (XXXV) which is known to be a precursor of both the triterpenes and the steroids (e.g. Lynen, and Henning 1960). The rate-determining process in lupeol formation is probably dimerization to squalene (XXXVI); the enzymes to

produce this are probably present in the hybrid, but the reaction must be slow compared with the competing polymerization.

A. Some Monoterpenes

Owing to the complex composition of most terpenoid oils little evidence of inter-relationships can be obtained merely by surveying the occurrences of such mixtures. Three varieties of *Eucalyptus dives* Hook contain, "interchangeably", (+)-piperitone (XXXVII), (−)-α-phellandrene (XXXVIII) and cineol (XXXIX). On the basis of present knowledge, the origin of these compounds can be postulated as shown below and the difference between the trees probably depends on a single step in the pathways outlined. Owing to the operation of "feed-back" mechanisms, it does not necessarily follow that the immediate precursor of a genetically restricted stage is the substance which accumulates. In the present case, guesses can be hazarded that the stage leading to α-phellandrene is probably blockage of the oxidation system for piperitol (XL), which is occasionally present and which is known to dehydrate to α-phellandrene of the correct optical configuration. Piperitenone (XLI), though not known as a constituent of Eucalyptus oils, is a constituent of some other oils containing piperitone (XXXVII).

Relative and absolute configurations, which are now known in relation to optical rotations in the monoterpene series (e.g. Birch, 1950), can be used as markers in other cases also. Unfortunately data in the literature for examining such relations are not very reliable owing to frequent lack of purity of the substances examined. However, in order to see what indications there are of sequential conversions, pairs of compounds of the same sign of rotation, implying *in these cases* the same configurations, have been examined. The information, from Fujita (1951) and Guenther (1952), involves 36 oils. The results are shown in Table II, the biosynthetic reactions being shown in Fig. 7.

TABLE II

	A	B	C
Limonene (XLII) and α-terpineol (XLIII)	3	1	1
Limonene and α-pinene (XLIV)	11	6	3
α-Terpineol and α-pinene	4	2	3
α-Pinene and camphor (XLV, borneol)	16	3	0
α-Pinene and camphene (XLVI)	10	1	0
Limonene and camphor	2	3	2

A = Same sign. B = Opposite sign. C = One or other (±).

Borneol and camphor show complete correlation and are recorded together. Despite the small number of cases there is very clear evidence of frequent sequential conversion, or at any rate of identical stereochemistry in the first cyclization stage of geranyl pyrophosphate (XLVII) to the cation (XLVIII). However, there seems to be only a random relationship between the configurations of the series above and those of the α-phellandrene–menthone–piperitone series, in line with the view that the initial cyclization in the latter series (cf. production of piperitone (XXXVII)) is mechanistically different.

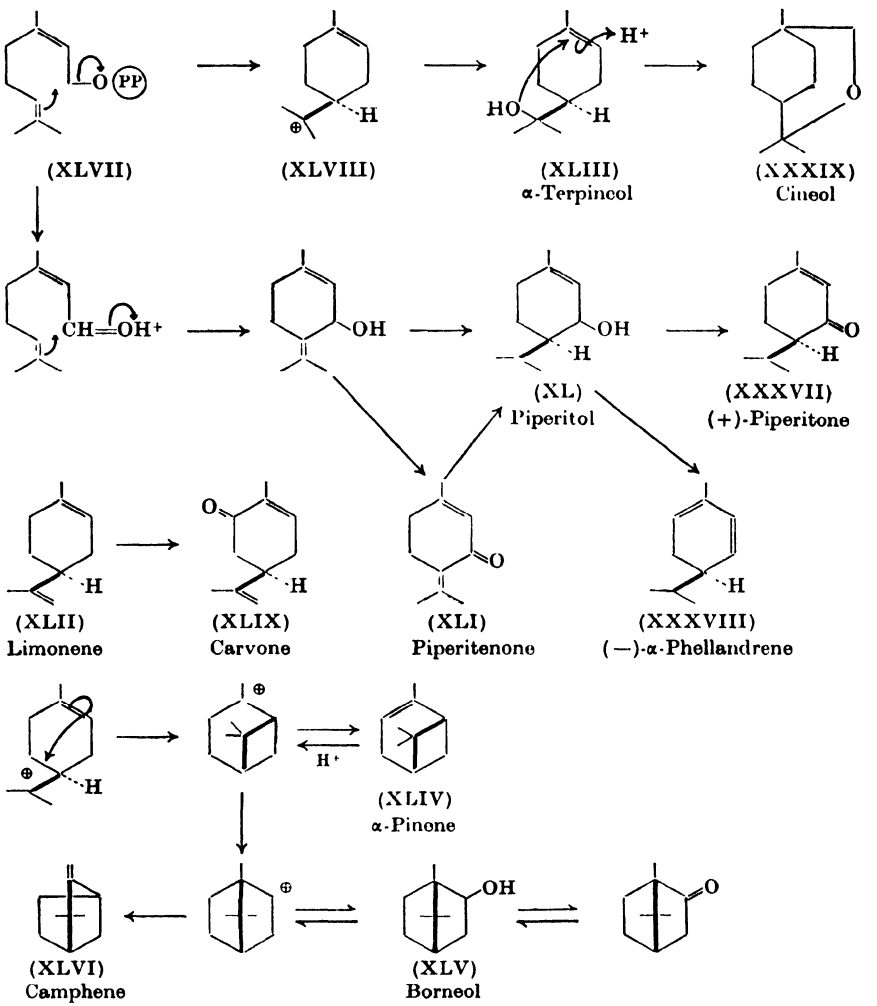


FIG. 7.

There are several possible explanations for the discrepancies in Table II. They may be genuine, or due to errors because of impurity. If genuine they may imply initial cyclization in some cases to a (±)-compound and continuation of the biosynthetic sequence from some subsequent stage utilizing only one stereoisomer, the other being left to accumulate. Clearly it would be worth while to re-examine the question, using modern techniques, with the determination not only of rotation but of exact degree of racemization.

A more clear-cut case concerns the relationship between carvone (XLIX) and limonene (XLII). The latter occurs frequently without the former, but almost invariably carvone is accompanied by limonene and is presumably formed from it by a stereospecific allylic oxidation of the type shown (Fig. 7). This assumption is supported by the fact that the absolute configurations of the two compounds occurring together are invariably identical.

B. Some Diterpenes

The diterpene series is another in which directions of transformations can now be predicted with some confidence. The sequence shown in Fig. 8 is based in all cases on the same kind of initial concerted cyclization to a bicyclic intermediate, with further cyclizations to tri- and tetracyclic sybstances, together with some characteristic off-shoots to several well-known diterpenes: manoöl (L), rosenolactone (LI) and the parent of the abietic acid group (LII). It is remarkable that almost all known diterpenes are recognizable variants of the scheme; sesquiterpenes, despite their smaller molecules show far wider variations in skeletal structures. A perhaps dangerous speculation on the reason for such diterpene relations is that they represent alternative methods of disposal of arrested intermediates to the important plant hormone gibberellic acid (LIII). This arises apparently from (−)-kaurene (LIV) (Grove, unpublished), and mutations leading to production of substances which are not injurious or positively useful, could cause either accumulation of intermediate, or development of further processes based on such intermediates. The divergence in the case of many of the diterpenes, including phyllocladene (LV) which has obvious structural relations to gibberellic acid, must be initiated by inversion of the stereochemistry of the initial ring-closure which would in any case inhibit the formation of gibberellic acid. It is possible that the diterpenes may be only one of a series of such cases; that is, if a function can be discerned for one substance or reaction, then biogenetically related substances, or compounds produced by the same reaction on different substrates may be encountered. An example

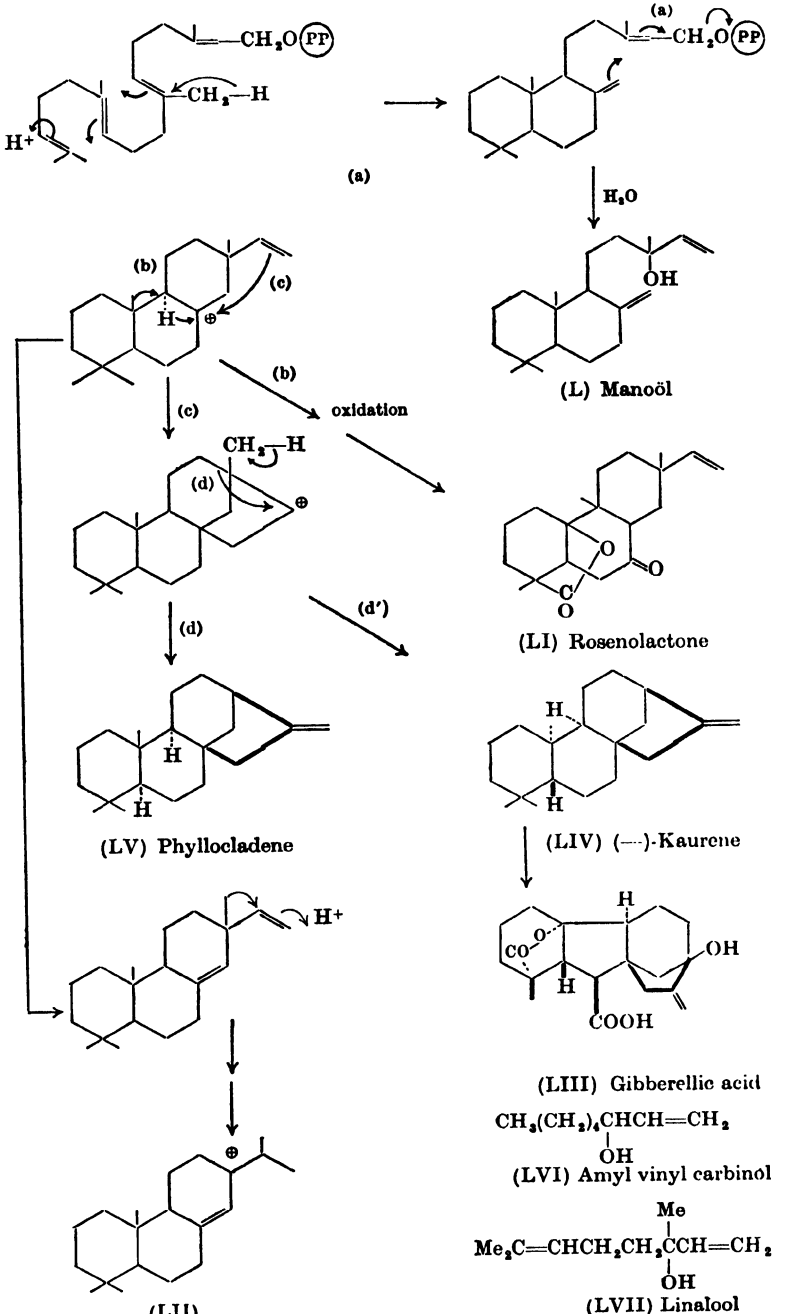

(a)
(b)
(c)
(d)
(d')
oxidation
H₂O
(L) Manoöl
(LI) Rosenolactone
(LIV) (—)-Kaurene
(LV) Phyllocladene
(LII)
(LIII) Gibberellic acid
CH₃(CH₂)₄CHCH=CH₂
OH
(LVI) Amyl vinyl carbinol
Me
Me₂C=CHCH₂CH₂CCH=CH₂
OH
(LVII) Linalool

FIG. 8.

in favour of the latter expectation may be the presence of amyl vinyl carbinol (LVI) in some lavender oils, together with linalool (LVII); the former could be produced from fatty acid derivatives by enzymes (perhaps modified) intended to produce the latter. The vinyl carbinol structure in natural *n*-alkane derivatives is very rare, and it seems more than coincidence that it should be found in two compounds in the one oil.

C. Some Triterpenes

Recent work on the biosynthesis of the triterpene lanosterol (e.g. Bloch, 1958; Lynen and Henning, 1960; Cornforth and Popjak, 1960), and theoretical considerations on the stereochemistry of ring-closures and methyl migrations (e.g. Ruzicka, 1958), have led to a considerable understanding of the biogenesis of triterpenes. A number of points emerge. Cyclization involves a concerted series of reactions, and the stereochemistry of the product depends on the type of folding of the original squalene chain, presumably on an enzyme surface (see Fig. 9). Migrations of angular methyl groups occur, usually stimulated by the positive charge remaining after the original ring-closure. It is therefore possible to detect the biogenetic relations between substances of different stereochemistry, and even different skeletons, in terms of the changes in the processes involved. Furthermore, since squalene (LVIII) is probably the universal precursor of this group of compounds, the only biogenetically necessary oxygen, as far as production of the skeleton is concerned, is that involved, as an equivalent of an oxonium cation, in initiating the cyclization. This oxygen is usually present as a 3-β-hydroxyl group. Other oxygens which are frequently encountered must be introduced as the result of specific oxidation processes. One obvious relationship is between α-elemolic acid (LIX) and masticadienoic acid (LX) both derivable from butyrospermol (LXI) generated from squalene (LVIII) as shown in Fig. 9.

The highly oxidized substance limonin (LXII), the bitter substance of citrus, probably arises initially by the same steric folding of the chain but methyl migration stops after only one has moved. Oxidations then follow in what is at present a largely unpredictable order. The substance flindissol (LXIII) supplies one missing link in the same stereochemical series in that it contains the potential furan ring with the side-chain still intact. It is notable that flindissol occurs in *Flindersia* related to Rutaceae and Meliaceae which also produce compounds containing a furan ring.

No survey of occurrences of triterpenes on such a biosynthetic basis seems to have been made. It might be very revealing of phylogenetic relations.

Proceeding from triterpenes to steroids, another interesting possibility

concerns the "extra" C_1 and C_2 attached to the side-chain of ergosterol and stigmasterol, the latter found only in higher plants. The first is due to the addition of a CH_3 from methionine (Alexander, Gold and Schwenk,

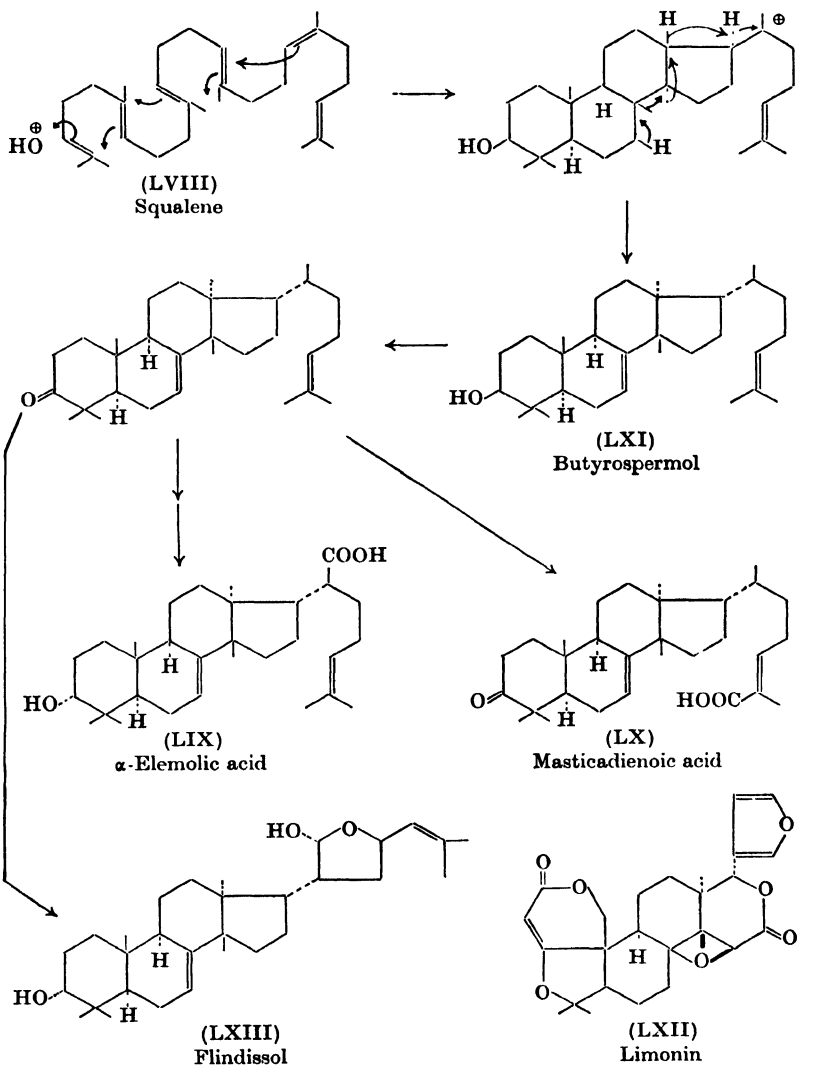


FIG. 9.

1957) and we venture to suggest that the ethyl group involves a second CH_3 unit from the same source. The mechanism is possible as shown in Fig. 10. Therefore, compared with yeasts, the higher plants in this case

have apparently evolved an extra stage, which is mechanistically an extension of the first one. A survey of occurrences in this field might in consequence give some information on evolutionary relationships.

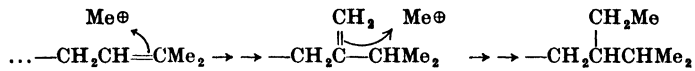


FIG. 10.

V. Alkaloids

The great variety of structures encountered among alkaloids and the frequent ease of isolation due to basicity makes them potentially attractive taxonomic markers. Surveys of structural relations (e.g. Robinson, 1955) and biosynthesis (e.g. Battersby, 1961) have been published, and the field is much too complex to outline more than a few examples. One fact which emerges from the biosynthetic work is that despite the vast numbers of compounds, many superficially very unlike, only a comparatively few precursors are involved, such as phenylalanine, tryptophan, "acetate" units, terpene units, methionine, and a limited number of other amino acids such as ornithine. The *types* of reactions involved are also not very numerous, so far as at present known, comprising principally of acylations, aldol-condensations, *C*-methylations, and most complex of all, specific oxidations often directly connected with the production of new ring-systems. Phenol oxidations have recently been shown to play a particularly important part in some instances (e.g. Barton and Cohen, 1957). Many of the earlier theories are at present receiving considerable support from ^{14}C-tracer experiments (Battersby, 1961). It is clear therefore that considerable light may be shed on phylogenetic relations by a study of alkaloids but not merely by studying their *structures*. It is again necessary to consider routes and reactions and how these are altered in specific cases.

A. Morphine Group

As one now well-authenticated example of complex transformations let us consider the geneses of the skeleton of morphine (LXIII, Fig. 11). This is outlined in Fig. 11, the reactions emphasising the relationship to the benzylisoquinoline series, such as papaverine (LXIV) also found in *Papaver somniferum*. Fairly obvious reactions lead to other accompanying alkaloids such as thebaine (LXV). The labelling pattern shown in (LXIII) is that found after feeding [α-^{14}C]tyrosine to the plant. Many other variants of transformation of the benzylisoquinoline nucleus are

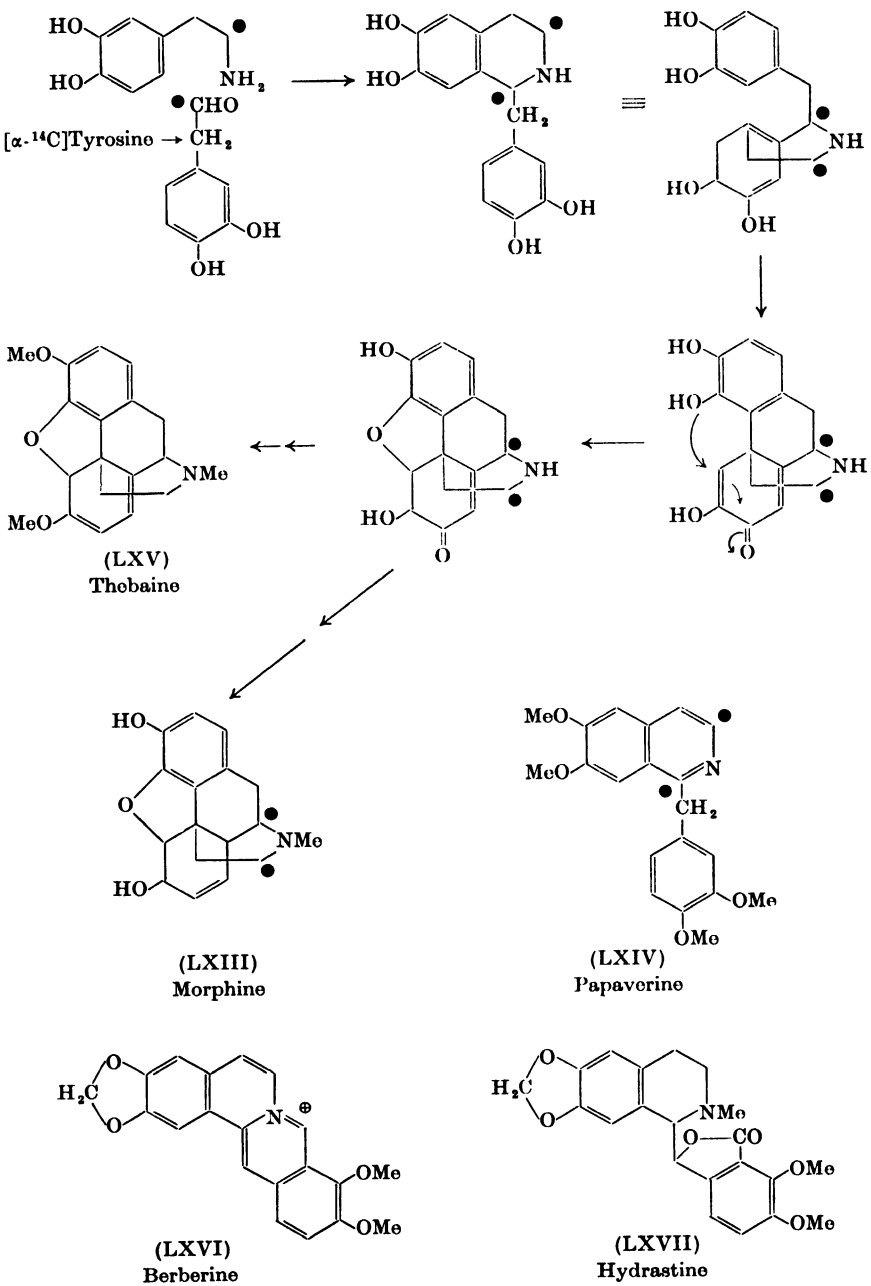
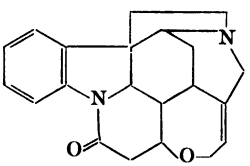

[α-14C]Tyrosine
(LXV)
Thebaine
(LXIII)
Morphine
(LXIV)
Papaverine
(LXVI)
Berberine
(LXVII)
Hydrastine

FIG. 11.

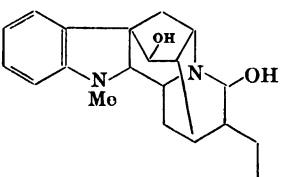
known, for example addition of an extra carbon atom in the nucleus leads to the berberine group [e.g. berberine (LXVI) itself a parent-type for other variations, such as hydrastine (LXVII)]. Information on the exact sequences involved, which may be expected shortly from biochemical work, will undoubtedly assist in defining possible evolutionary sequences.

B. Indole Group

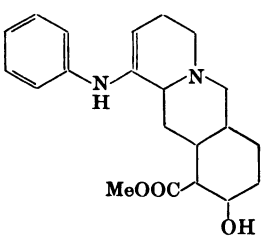
A very large group, which is currently revealing an enormous range of ring structures is based on the addition of complex ring-systems to the tryptamine nucleus derived from tryptophan. The ultimate source of the



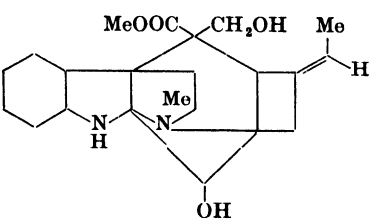
(LXVIII)
Strychnine



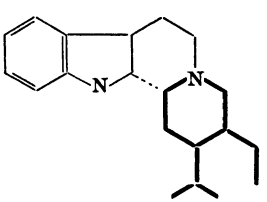
(LXIX)
Ajmaline



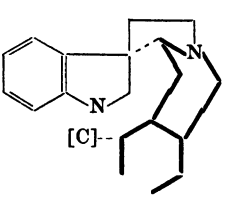
(LXXI)
Yohimbine



(LXX)
Echitamine



(LXXII)



(LXXIII)

carbon atoms added is not yet known. There are a number of alternative theories: phenylalanine or congeners (Woodward, 1948), prephenic acid (Wenkert and Bringi, 1959) or terpene units (Thomas, 1961).

Whatever the source, the formulae of the substances, including even

strychnine (LXVIII) can be built up on paper from the same branched skeleton with minor variations, the various ring-closures being due in part to specific oxidations followed by other specific reactions many of which can be explained in acceptable mechanistic terms. Merely to illustrate the diversity the structures of ajmaline (LXIX), echitamine (LXX) and yohimbine (LXXI) are shown. Some *Strychnos* species produce the yohimbine family based on the yohimbine-type precursor (LXXII), others give strychnine relatives based on (LXXIII). The diversity of structures clearly provides a rich field for speculation and investigation as to the exact stages of sequences where branching occurs leading to the different alkaloids. Such work should ultimately provide considerable genetic information related to the capability of a plant to perform a specific biosynthetic process.

VI. Conclusions

An enormous amount of exact chemical work requires to be done using modern techniques, for the analysis of as many as possible of the constituents of a given plant. Chemists in the past have been interested in natural molecules mainly as structural problems. With the revelation of so many closely interwoven biosynthetic routes it is clear that not very much phylogenetic information can be obtained from a few fortuitously isolated substances. In the end the most illuminating information is likely to come from studies of the enzyme systems involved, not only their presence or absence, but the alterations in their substrate specificities and catalytic activities. The elegant work of Eglinton *et al.* (1962) on plant waxes is an example of what can be done experimentally, and it is already becoming necessary to consider the distribution of alkanes in terms of enzymic specificities.

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CHAPTER 7

Intrinsic and Extrinsic Factors Affecting the Production of Secondary Plant Products

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I. Introduction

For a proper discussion of chemical taxonomy it is important to know how far intrinsic and extrinsic factors may influence the production and the content of chemical substances in plants. Only if it is clearly known whether a substance is a genetically fixed constituent, or whether its presence is due to the influence of extrinsic factors, can its role in regard to chemical taxonomy be settled. The subject is so wide that it will only be possible to deal with a few examples of the influence of each factor.

The production of the so-called primary products of plant metabolism, for example, starch and proteins, will only be treated in a limited manner. The main field of inquiry will be the group of the so-called secondary plant products, especially those which are responsible for the medicinal or industrial properties of plants such as mucilages and gums, glycosides, tannins, alkaloids, essential oils, pectins, and so on. These groups fortunately also represent the most useful substances for chemical taxonomy.

When speaking of the "production" of natural substances, one must

remember that the biochemical mechanisms underlying such production are insufficiently known. Even for those which are now more or less understood, the influences of intrinsic and extrinsic factors on the individual steps in the biosynthesis have hardly been investigated.

II. Problems of Analysis and Interpretation

A. Reasons for Conflicting Results

If one compares the results of many different investigations, conflicting or even grossly contradictory findings appear. These contradictions can be due to many different causes: to difficulties in the planning of the investigation or in the analytical methods used; to an inherent variability of the substances under investigation; to insufficient consideration of statistical requirements; or to genetical variability of *individual* plants and so forth.

Some discrepancies can also arise from differences in the time of sampling. The analytical results obtained are always a measure of the balance of two processes, i.e. of formation and breakdown. The latter phenomenon may be due to actual metabolism of the substance by the plant, or to a loss caused by evaporation (e.g. essential oils), or to polymerization, or other processes.

B. Analytical Methods

When considering the errors originating from the analytical methods used one should be aware that most of them are not absolute, and strictly comparable results can only be obtained when the methods used are exactly the same. Hence, it is absolutely necessary to always indicate the method of analysis used.

Very serious consideration should also always be given to the *base* to which analytical results are referred. This must be chosen according to the problem under investigation. For example in research on diurnal variations the dry weight varies so considerably itself during a 24-hour period that it is of little use as a reference. In this case it is better, therefore, to use the crude fibre, or calcium content, or the surface area of the leaves, since these show hardly any diurnal variation. However, although crude fibre is a good base for investigations on diurnal variations, it would not be suitable for research on seasonal or ontogenetic variations, because it, itself, changes during such periods. In many investigations which are carried out with regard to the production of foods or drugs dry weight may be quite suitable as a base of reference. However, one should be wary of deriving physiological conclusions from such investigations. Another suitable reference base is the "absolute" content,

calculated with reference to the number of organs (leaves, fruits, etc.) of the plant in question.

Other Sources of Error

Due consideration should always be given to the ontogenetic state of the plant. As will be shown later, ontogenetic variations in the amount of substances present are usually larger than any others observed.

Investigations carried out with plants grown in the open will be influenced by ecological factors such as irradiation and temperature. The results of investigations performed on plants grown under defined conditions in a phytotron may therefore give more reliable results. But even in such experiments intrinsic factors, such as the state of nutrition, may influence the effect of a deliberately applied extrinsic factor, as for instance the effect of certain wave-lengths of light on growth (Kandeler, 1960).

Due to the sources of error mentioned above one should always keep in mind that absolute results are very difficult to obtain even when the same clone of a given species is compared from one season to another.

III. Intrinsic Factors

A. Genetical Factors

Chemical non-uniformity in a given taxon is mainly quantitative. In other words the various different substances present in a plant will differ in quantity, but all will be present in all "clines" or individuals of the species, even if only in traces. Reports of qualitative variation, characterized by the fact that one or more substances are *completely* absent in one or more subdivisions of the taxon, and which is, therefore, of interest to chemical taxonomy should always be regarded with suspicion. One should remember that today, newer analytical methods such as paper chromatography, thin layer chromatography, gas chromatography, i.r. spectrophotometry have lowered the limits of detection to 1/1000 or less of that of classical methods. Therefore, substances which formerly had been considered to be absent in certain infra-specific groups (e.g. nicotine in the so-called nicotine-free tobacco, ergot alkaloids in several ergot drugs (Stoll, 1942)) may now be found to be present in every individual, at least at certain stages of development. This point is most important and infra-specific groups previously established on the "absence" of one or more substances should be thoroughly re-investigated.

B. Diurnal Variations

The regular diurnal change of light and darkness gives rise to a variation in the metabolism of the vegetative aerial parts of photosynthetic plants. How far subterranean and reproductive organs undergo a similar

variation in metabolism is not sufficiently clear. For secondary plant products diurnal variations have been investigated by several authors, but in spite of this the problem is so far only partially resolved. The effect of radiation is also important and will be treated later.

1. Plants containing glycosides

For glycosides, the problem can be illustrated by the example of changes in digitoxin and gitoxin in digitalis leaves. Based on physiological assays, Dafert (1921) and Boshart (1936–7) found a maximal content towards the end of the afternoon, whereas Dare and Nelson (1952) found a maximum at midday and a minimum at midnight. Court and Allemann (1942) came to the same conclusion for the leaves of *Digitalis lanata* Ehrh., basing their results on fresh-weight. On the other hand, Fuchs, Soos and Kabert (1951), Neuwald (1950) and Tattje (1957) found no significant differences for periods of 24 hours either in total glycosides or in the ratio between digitoxin and gitoxin. Among plants with flavonoid glycosides, Nöll (1955) showed that in *Fagopyrum esculentum* and *F. tataricum* a significant and regular increase occurred in the content of rutin, as well as in total flavonoids, during the day and a corresponding decrease during the night, when the results were calculated with reference to the crude fibre, although calculated with reference to dry weight irregularities appeared. The degree of increase varied from 5·3–13·7%.

2. Plants with alkaloids

Hemberg and Flück (1953) found significant differences during 24 hours in the alkaloidal content of the leaves of *Datura stramonium*, referred to crude fibre, the maximum occurring in the leaves at 07.00 hr and the minimum at 23.00 hr, whereas in the roots the maximum and the minimum occurred at the opposite times. The differences were 22% for the leaves, and 24·5% for the roots. No clear relation was found with total proteins or with the different nitrogen-containing fractions. Ploski (1926) had reported similar observations for the leaves of a variety of the same plant. Later Flück and Hegglin (1958) found a similar behaviour for the leaves of *Datura innoxia*; but the reciprocal relation between the contents in the roots and leaves was not so clear cut. The proportion of scopolamine was found to vary irregularly, although the changes were rather small.

3. Plants with essential oils

The greatest number of investigations on diurnal variation have been carried out on plants containing essential oils. Essential oils, secreted in glandular hairs, or in internal glands, are not apparently metabolized once formed, and one could expect a continuous accumulation if secretion

continued. However, evaporation and resinification here play a role, being especially important in glands situated on the surface of the plant. Among the many investigations noted, those carried out by Schib (1958a, b) are worth considering, because this worker tried to avoid sources of error as far as possible, using crude fibre as reference base and taking into consideration the age of the leaves, etc. With the leaves of *Salvia officinalis* the maximum content of oil (34–45%) occurred on four different days in the early afternoon, and the minimum between midnight and the early morning. In the leaves of *Pinus silvestris* no significant variations appeared. The difference between the behaviour of the leaves of salvia and those of the pine is not conflicting, because in the former the essential oil is present in the glandular hairs covered only by a very thin lipophilic cuticle, whereas in the pine the excretory glands are protected by a thick-walled epidermis and an even thicker-walled fibrous hypodermis. Evaporation and resinification are therefore easily possible in salvia but are strongly hindered in Pinus. The results with salvia confirm earlier observations of Tucakov (1952). In peppermint (*Mentha piperita*) Hecht, Himmelbauer and Munich (1936) found that the variations of the content of essential oil were irregular and rather small, whilst Tschirikow (1952) found higher yields during the night, based on dry weight. With *Matricaria chamomilla*, Rombaux and Laruelle (1960) demonstrated two maxima in oil content, one in the early morning and the second late in the afternoon. In the oil of the same species Blazek and Hubik (1952) and Michaluk and Oswiecinska (1956) detected the maxima of the percentage of azulenogenic substances at noon. Very great variations in the content of essential oil in the rhizomes of *Acorus calamus* (up to 50%) have been found by Koslowski (1956), whereas in the leaves the differences were reported to be small. It seems really astonishing that so great diurnal variations should occur in secretory cells, and confirmation of this observation is highly desirable.

The occurrence of diurnal variations are undoubtedly proven for *Salvia*, but although such changes may occur in other plants, they do so without clear regularity. Only a few results deal with the determination of the changes in one individual substance in a complex mixture such as the essential oils, total alkaloids, total glycosides. However, in no case during a diurnal cycle has a substance been observed to disappear and reappear again completely and this is true of the effect of all extrinsic and intrinsic factors.

4. Long- and short-day plants

During the discussion on the diurnal variation above, we did not consider variations in the length of day. The problem of long- and short-day

plants will not be treated here. I would only like to mention that no case has been reported in which a substance normally found in the species concerned disappeared, or a new substance appeared, when a plant was grown either in a long- or short-day régime. Certain quantitative changes in carbohydrates and mineral nutrients (N, P, K, etc.), however, have been noted. Such variations may be different in the different organs of the plant concerned (see, for example, Smatok, 1956).

C. Ontogenetic and Seasonal Variations

Although partly conflicting results have been found for diurnal variation, of secondary plant products, there is general agreement that the concentration of these compounds changes during the course of the year or the growing season.

1. Translocation of plant-products

Before going into detail of seasonal variation we should consider the effect of translocation on such changes. First of all it should be noted that secondary plant-products, for example alkaloids, can be translocated in plants. This has been widely shown for solanaceous alkaloids, the tropane nucleus being synthesized and esterified to hyoscyamine in the roots and the alkaloids then transported to the leaves and other aerial organs of the plant. For lipophilic substances, such as essential oils and resins, however, transport over large distances cannot be expected, and in fact no investigations have shown such a translocation. Polymeric carbohydrates, such as starch, mucilages, gums, also cannot be expected to migrate, except after depolymerization to oligo- or monosaccharides.

2. Reasons for ontogenetic variations

An increase or decrease of secondary plant products can take place in four possible ways; the compound could be metabolized such as probably happens for several alkaloids; the compound could be translocated to other organs as discussed above; the ratio between the substance and other compounds present in the organ under investigation could change; finally, the compound can be lost by evaporation or resinification and so on. In many cases, the concentration of the compound may be affected in more than one way.

3. Examples of ontogenetic variations

(a) *Vegetative organs.* Among vegetative organs, the leaves of solanaceous plants which contain tropane alkaloids (especially those of the

genera *Atropa*, *Datura* and *Nicotiana*) have been studied most extensively. However, species containing other classes of alkaloids, seem to behave similarly.

In the leaves of *Atropa belladonna* Kuhn and Schäfer (1939) found a large increase in total alkaloids (0·6%–0·8%) occurred from the onset of growth (March) to the just visible formation of the flowers (June). A sharp decrease then took place during June and July and this was followed by an even greater increase from July to September. Unfortunately, the authors did not indicate whether the leaves were always of the same physiological age and it is likely that the second increase occurred only in newly formed leaves, since *Atropa belladonna* forms new ones throughout the whole season. Later authors (Hegnauer, 1950; Nisoli and Flück, 1954; Flück, unpublished) confirmed that alkaloids increased in leaves of belladona and *Datura stramonium* during the early part of the season but did not observe a second increase later on, except in new leaves. These findings show that the production of alkaloids takes place mainly in periods of intensive growth, a finding which has also been shown to be true of other secondary plant products. Mothes (1957) has expressed this very clearly by stating that, at least in vegetative organs, most of these compounds are formed by secondary reactions during periods of intensive metabolism.

However, this is not always the case, for it depends on the localization and on the site at which such compounds are produced. Thus in the leaves of *Veratrum album*, Hegi (1956) and Jaspersen-Schib and Flück (1960) noted a constant decrease in the percentage of total alkaloids during growth. The buds and young unopened leaves were found to contain about 1% (a slight loss being observed in the latter) whereas the fully developed leaves only contained 0·2%. Here then, growth is accompanied by a marked decrease in the percentage of total alkaloids. Unfortunately we do not know where the alkaloids are produced in this plant but it is highly probable that they are stored in the subterranean organs and in the buds during the cold season. Leaf development in the spring then gives rise to an observed dilution of the alkaloids.

(b) *Generative organs.* It is well known that generative organs, especially seeds, often contain large amounts of alkaloids and other secondary plant products such as mucilage (linseed), glycosides (seeds of *Digitalis* and *Brassica* species, and related genera, etc.), and that in the case of mustard (*Sinapis alba*), for example, the seeds contain much more of these compounds than do other parts of the plant. There are, however, plants in which the seeds are free from certain secondary plant products. The classical example of this type is *Papaver sommniferum* in which all organs, up to the placenta, contain latex-tubes with opium alkaloids,

especially morphine. The only organs which are free of alkaloids are the seeds. Almost immediately after germination, however, latex-tubes appear and alkaloids are found to be present (Miram and Pfeifer 1959, 1960; Heydenreich, Miram and Pfeifer, 1961; Pfeifer and Heydenreich, 1962). Similar conditions exist in other taxonomic groups. For example, in the Labiatae, where all aerial organs, except the fruits, bear glandular hairs which contain essential oil. Here also changes take place on germination, and the cotyledons have glandular hairs in which essential oil are formed (Frey-Wyssling and Blank, 1940).

Up to now we have only discussed changes in groups of substances such as alkaloids and essential oils. The ratios between the individual components of such groups may also vary during the season. To mention only two examples. The ratio of scopolamine to hyoscyamine decreases during growth in young plants of *Datura stramonium* and, as will be seen later, this change is little affected by extrinsic factors.

The percentage of both the carbonyl fraction and the total quantity of essential oil in the fruits of four umbelliferous species, *Anethum graveolens*, *Carum carvi*, *Oenanthe aquatica* and *Cicuta virosa*, was found to increase during development, whereas the amount of non-carbonyls (e.g. phellandrene) decreased (Leuwendijks, 1958). Leuwendijk also proved quite conclusively that the absolute quantity as well as percentage of oil was greatest at the moment when the fruits were fully ripe and that after this no further formation of essential oil takes place. This is in contrast to several authors (Kofler, 1936; Steiner and Hochhausen, 1952) who believed that a considerable increase in essential oil content occurred after the fruits had been harvested.

Several workers (Kuhn and Schäfer, 1939; Nisoli and Flück, 1954; Sievers, 1921; Hegnauer, 1951) have shown, at least for species with tropane alkaloids, that a correlation exists between the onset of flowering and the maximum content of secondary plant products. At flower development, a clear break can be noted in the rate of the increase of alkaloids. If the flowers, or even the young fruits are removed, this break is hardly noticeable, and the production of alkaloids continues nearly at the same rate as before. Hegnauer (1951) believes, that by delaying the beginning of the reproductive cycle the plant continues vegetative growth, and as we have already seen, strong vegetative growth is usually accompanied by intensive formation of secondary substances.

One final intrinsic factor which can be mentioned is the change which takes place on continued harvesting. For example, in herbaceous plants like peppermint where a second or even a third crop can be taken, the later crops are poorer in essential oil. The growth of second shoots of peppermint (and similar plants) is always less intensive than the first

one, and it is possible, therefore, that the parts of the plant remaining after the first cut are poorer in primary metabolites, which are the starting materials for the formation of secondary products.

The last remarks indicate that the plant must be in a good state of nutrition, if the production of secondary products is to be a high one. As an example, Cromwell (1937) demonstrated that with *Atropa belladonna* increasing doses of potassium nitrate provoked a greater production of alkaloids only if the plant had a sufficient supply of carbohydrates, and that a dearth of such products led to a decrease in alkaloids. We will return to these results when we come to deal with the influence of light.

The influence of ontogenesis on the production of secondary plant-products, may be summarized as follows. (1) Seasonal or ontogenetic variations in secondary substances can be very considerable, sometimes approaching values higher than fivefold. (2) The most intensive formation of secondary plant-products occurs mainly during periods of intensive growth. (3) No case is known in which a substance either freshly appears in, or disappears completely from, an organ.

III. Extrinsic Factors

The most important extrinsic factors affecting the formation of secondary plant-products are climate and soil (Flück, 1954, 1955a,b, 1961). From the viewpoint of chemical taxonomy one has to remember that most plants which have been examined, are grown in a natural habitat and only in a few cases have been grown under defined experimental conditions in a phytotron. Obviously, then, the effects of extrinsic factors are usually of importance. We will first consider the influence of the individual factors, beginning with the soil, and later take up the problems of interaction between various types of soil and climate.

A. Soil

The main three characteristics of soil which affect the formation of plant products are the physical, the chemical and the microbiological factors. Among these, hardly any investigations concerning microbiological factors have been carried out. The different physical factors (humidity, temperature, particle size) and chemical factors [pH, availability of major (N, P, K, Ca) and trace elements] may interact with one another in their effect on the formation of secondary plant products; however again very little is known about such interactions.

1. Individual factors

(a) *Physical factors.* Although the physical factors of the soil must

affect the production of secondary plant products, very little is known about them. One important factor is particle size, and it seems to have some influence on the content of mucilage in *Althaea officinalis* (Dafert and Fuchsgelb, 1930). This species was shown to yield roots with a higher mucilage content (as measured by viscosity) on soils with large particles (sand) than on soils with smaller ones (clay). Since mucilage has a high capacity for holding water, these effects may be related to the water holding capacity of these soils, soils with greater particle size having a lower capacity for retention of water, and thus give plants having the capacity to retain more.

The influence of the moisture in the soil is rather difficult to investigate because the mineral substances, essential for growth, affect its uptake. Differences in water régimes using quartz sand were shown to have no significant influence on the percentage of essential oil in old dry leaves of peppermint, but caused a slight increase in the fresh leaves (Birkeli, 1948). However, the quantity of oil produced per unit area was increased significantly, due to the larger number of leaves formed with higher amounts of water. Dafert and Fuchsgelb (1930) found a lower mucilage content in *Althaea officinalis* grown on soils with higher moisture, than on drier soils. Using a phytotron, Winters, Loustalot and Childers (1947a, b) found that increased watering caused a decrease in the total alkaloids, as well as in the percentage of quinine, formed in the stem of young plants of *Cinchona ledgeriana*. In the roots of the same plants the reverse was true. This result indicates clearly that in such experiments conclusions can only be made for the individual organs under investigation.

(*b*) *Chemical factors.* Before dealing with the influence of individual chemical elements, the pH of the soil which is often a limiting factor for the presence of a plant species must be considered. Within limits, typical for the species concerned, the pH of the soil may vary only about 1·5–2 units (for example, *Chrysanthemum cinerariaefolium*, 5·9–8·1, *Majorana hortensis* 5·6–6·4, and *Datura stramonium* 6·0–8·2; Flück, unpublished). However, no significant effect on the content of total alkaloids could be detected in *Datura stramonium* when grown in soils having a pH between 6·4 and 7·8, although the highest and the lowest pH-values did produce slight decreases in the quantity of alkaloids. Birkeli (1948), in experiments using quartz sand showed a clear effect of pH between 4·2 and 7·2 on the growth of *Mentha piperita*. However, no significant difference was found in the essential oil content of the plants. Earlier Mothes (1928) had found similarly no influence of pH on the nicotine content of tobacco leaf.

Much research has been carried out on the influence of individual nutrients, or on combinations of them. Most work has been conducted by adding varying quantities of an element, or a special form of this

element (e.g. nitrogen in the form of ammonia or of nitrate) to natural soils, or to quartz sand or to water cultures. Experiments with soil have usually yielded higher variation in content than those using sand or water cultures. This probably is due to the fact that the plant can only fully profit from the added element when other, mainly unknown, substances, or physical properties, which are present in soil, stimulate its assimilation.

The nutrients which have been most investigated are N, P, K, and to a minor extent Ca. One can only expect a direct correlation between supply of any of these and the production of a secondary plant product, in the case of nitrogen and the alkaloids. This has been shown to be true for many species. For example *Atropa belladonna* (Cromwell, 1937; Chevalier, 1910; de Como, 1941; Gstirner, 1950; Schermeister, Crane and Voigt, 1960a), *Datura stramonium* and *D. innoxia* (Boshart, 1931; de Graaff, 1928; Haller, 1946; Nisoli and Flück, 1954), *Nicotiana tabacum* (Huter, 1947), *Papaver somniferum* (Annett, 1920), were all shown to give increases in the alkaloid content when fed with nitrogen. Several authors (Gstirner, 1950; Schermeister *et al.*, 1960b) have also shown that nitrogen in the form of ammonia gives a better result than in form of nitrate; the best results being obtained with ammonium nitrate. In many of the papers mentioned it appears that there are optimal concentrations of nitrogen, above which a decrease in alkaloids occurs, probably due to some toxic action (de Graaff, 1928). In the case of *Lobelia inflata*, however, addition of nitrogen considerably lowers the content of total alkaloids (Mascré and Genot, 1932, 1933; Esdorn, 1940; Bärner, 1941). This rather controversial behaviour needs further investigations.

In the case of phosphorus controversial findings have been reported, even with the same plant. It appears that a certain amount of the element must be available, but that increases over such amounts can either increase or decrease the production of alkaloids (Gstirner, 1950). In *Lobelia inflata* (Esdorn, 1940), *Punica granatum* (Maurin, 1925) and *Chelidonium majus* (Boshart, 1941) only increases were detected.

Conflicting results have also been reported for potassium (e.g. *Punica granatum* (Maurin, 1928); *Datura innoxia* (Nisoli and Flück, 1954). Gstirner (1950) found potassium had no effect on the alkaloid content of the leaves, but led to an increase of these compounds in the roots of *Atropa belladonna*. *Lobelia inflata* (Esdorn, 1940; Bärner, 1941) on the other hand responded with clear increases even with rather high doses of potassium.

With trace elements several investigations (e.g. Haller, 1946) have shown no significant effect of B, Zn, Cu and Co on the formation of alkaloids. Obviously more research on the influence of these elements is required before any firm conclusions can be drawn.

For plants containing glycosides the results of most investigations on the influence of mineral nutrition are even more conflicting. This is not surprising because the aglycones of the glycosides can belong to so many different chemical groups (e.g. phenols, alcohols, isothiocyanates, etc.). For reason of space therefore, these compounds will not be dealt with except to emphasize that nutrients should be present in sufficient quantities to allow a good rate of growth.

Plants with essential oils have been better investigated as they are cultivated in great quantities, because of their use in pharmacy and perfumery. Although nitrogen both in sufficient and greater amounts yielded vigorous plants of peppermint, Birkeli (1948) could not find a significant increase in essential oils. Schratz and Wiemann (1949), on the other hand using five different amounts of nitrogen found increases of up to 80% with the higher quantities. Similar results were found by Springer (1937) and Brückner (1953) in plants grown on normal soils, but Schlemmer and Springer (1939) noted a decrease when N was added, and an increase with P and K. *Carum carvi* (Potlog, 1938) and *Matricaria chamomilla* (Dafert and Rudolf, 1925; Mayer, 1942) both showed increases as well as decreases in oil content when manured with nitrogen. *Thymus vulgaris*, *Ocimum basilicum*, *Satureja hortensis*, and *Foeniculum vulgare* (Weichan, 1948) showed no significant variation in oil content with low or high quantities of nitrogen. On the other hand *Majorana hortensis* yielded the highest oil content with high doses of nitrogen, whilst *Melissa officinalis* required a balanced combination of N, P and K for a high production of essential oils (Weichan, 1948). Schröder (1959) found lower contents of essential oil in poorly manured plants of *Majorana hortensis* grown on quartz sand, and similarly, on natural soil, rich manuring produced higher concentrations of oil. *Inula helenium* also produced the greatest contents of essential oil in the roots on addition of a full NPK-manure and in this case manganese was also shown to be important (Buiko, 1959).

From the papers mentioned above it is obvious that nutrients must be supplied in the right proportion, and in quantities promoting a good and intensive growth if a high production of oil is to be obtained. The composition of the oils was only investigated in a few cases, and shown to be hardly affected by different supplies of nutrients.

2. Influence of natural soils

The effect of natural soils can only be studied if the soils are situated in the same climate. For some years a research field in the neighbourhood of Zürich had been at our disposal. On this field within a maximal distance of about 100 m, nine different soils from various regions of Switzerland

had been established. Plants cultivated on these soils were therefore grown under the same climate (Wüst, 1940; Hoffmann, 1949). The soils show a wide variation with regard to chemical and physical properties. The nitrogen content was low in four soils, moderate in four others and high in the last; the phosphorus was slightly low in two, and normal in the rest; potassium poor in one and moderate in the rest; calcium poor in four and rich in the other five. The pH varied from 6·6 to 7·4. Particles smaller than 2 mm varied from 38·6% to 95·6%. The average temperature determined by the method of Pallmann, Eichenberger and Hasler (1940) varied for the different soils for the period from July to October from 17·3° to 20·8° at 5 cm depth and from 16·1° to 18·4° at 25 cm depth. Plants were grown for 2 to 4 years. In *Atropa belladonna* and in *Lobelia inflata* the highest contents of alkaloids were obtained in the soils rich in N and P. As far as lobelia is concerned these results conflict with those obtained by Esdorn (1940) and by Bärner (1939) on manured soils. However in these latter investigations, N was given in large doses, while in the natural soils the concentration of N was much lower. With belladonna, and to a minor extent also with lobelia, soils rich in potassium produced plants with lower alkaloidal contents.

In plants with essential oil, such as peppermint, the percentage of oil more or less paralleled the percentage of nitrogen, while in *Pimpinella maior*, *Artemisia laxa* and *Peucedanum ostruthium* the contents of essential oil were not affected proportional to the nitrogen content of the soil. It may be noted that no significant difference was found between the soil factors and the proportion of free and esterified menthol in the oil of peppermint.

In *Althaea officinalis* it is interesting that the viscosity of the aqueous extracts of the roots (taken as a measure of the content of mucilage) varied from 1·5 to 4·0 centipoises on the different soils, while in the leaves the viscosity was nearly unaffected. Finally, the content of tannin, and of arbutin in the leaves of *Bergenia delavayi* varied within the widest limits among all plants examined for their secondary products (up to 180%), while in other species the variation was mainly in the range of 20–50%.

B. Climate

The previously mentioned reservations with regard to variation due to intrinsic and other extrinsic factors, should be kept in mind when considering investigations on the effect of climatic factors upon the formation of secondary substances. Investigations have been made on the effect of individual factors (irradiation, humidity, etc.) and on the

effect of different types of climates (arid, humid, alpine, arctic, tropic, etc.). The effects of individual factors will be discussed first and then the effects of the different types of climate.

1. Individual factors

(*a*) *Radiation.* As a general rule Lundegardh (1957) states that within the range of irradiation from u.v. (200 mμ) to i.r. (> 760 mμ) the greatest production of natural substances is due to visible light (400–760 mμ), the maximum being effected in the red range (660–680 mμ). Ultra-violet and i.r. radiation have only little effect on the photosynthesis. Light of wave-length shorter than 600 mμ prevents or reduces growth as shown by the fact that alpine plants are generally shorter (Bonnier, 1920, and other workers). As James (1950) has pointed out, the influence of light on the formation of secondary products is only rarely a direct one, especially where the products are formed in roots or in organs covered by many layers of tissue. Its influence is rather on general metabolism but, as the secondary plant products are in effect side-products of such metabolism, any change in the latter can also affect the production of secondary substances. Kandeler (1960) has studied the problem effect of different wave-lengths on the formation of anthocyanins in *Brassica nigra* and *B. juncea* and found that the far-red was the most effective.

Fischer and Thiele (1929) showed that an increase of the production of solanine in potatoes (*Solanum tuberosum*) is caused by a wide range of wave-lengths, whereas Conner (1937) found a higher production with short wave-lengths (300 mμ) and a lower one with visible light. Most of the investigations, however, deal with daylight. Light, of course, has been considered in connection with diurnal variation, discussed previously. The effect of light is probably the greatest, because its intensity varies during a diurnal cycle many times more than the other climatic factors. From experiments based upon the cultivation of plants in full sunlight and partial shade, it appears that higher percentages of all groups of medicinally active plant substances are produced with greater amounts of light. To give a few examples, the production of alkaloids in *Atropa belladonna* (Stillings and Laurie, 1943; Flück, unpublished), *Datura stramonium* (Flück, unpublished) and *Cinchona ledgeriana* (Winters and Loustalot, 1948) grown in full sunshine showed the highest contents of alkaloids when compared with plants of the same variety grown in the shade. For tobacco, controversial results are reported (James, 1950; Stutzer and Goy, 1913; and others).

Several investigations on etiolated alkaloid-containing plants have been carried out and such plants usually were shown to contain a higher percentage of alkaloids (Ripert, 1921; Weevers, 1929). This could be due

to a conversion of other substances such as carbohydrates or proteins (Sabalitschka and Jungermann, 1926).

The behaviour of plants with glycosides to different amounts of light is a variable one, probably again due to the great chemical differences in the aglycones. Light is essential for the formation of anthocyanins (Lundegardh, 1957; Kandeler, 1960) since the red pigment of peaches is only produced if the fruit receives direct sunshine.

In species containing essential oil, the highest concentration had been found in plants grown in the sun (Bode, 1940; Schratz and Spaning, 1943). However on very hot days, shaded plants may have a higher content, probably due to greater evaporation or perhaps resinification in the exposed plants (Bode, 1940). The higher content of oil in plants grown in full light can be explained by the fact that such plants have 1·5 to 3 times more glandular hairs than do shadowed plants (Bode, 1940; Schratz and Spaning, 1943; Koelle, 1953; Bedaux, 1952; Hegnauer, 1954). Bedaux (1952) demonstrated that the number of glandular hairs depended not only on the high intensity, but also on the quantity of light received. For *Achillea millefolium* Stahl (1952) demonstrated a higher content of proazulene in sun-grown plants.

(*b*) *Temperature.* Temperature is very important for the production of secondary plant substances, and sometimes becomes the limiting factor for plant life. However, individual species are able to exist in very different ranges of temperature. Although in physical chemistry the temperature quotient Q_{10} (normally 2) is valid over very wide ranges, this is not so with biological reactions which normally take place only between about 0° and 45°. Within these limits the temperature quotient varies most widely at the lower and upper limits, starting from zero and ending again at zero (Lundegardh, 1957). Furthermore, one has to bear in mind that secondary plants substances are produced by a sequence of reactions, each of them having its own optimum of temperature, and hence the optimal temperature for the formation of the final substance is the result of the optima of the individual reactions.

Only a few results will be mentioned. In *Cinchona pubescens* grown at three ranges of temperature (15·5–21·1; 18·3–23·9; and 21·1°) the contents of both total alkaloids and of quinine rose regularly with the rise of temperature, while in *C. ledgeriana* no consistent relation was observed (Winters *et al.*, 1947). These two closely related species therefore reacted in a different manner. For *Nicotiana rustica* a mean temperature of 20° gave a higher nicotine content than temperatures of 11–12°, and of 30° (Mothes and Engelbrecht, 1958).

It has been stated that anthocyanins are produced in higher amounts at lower temperatures (Gassner and Straib, 1930; Zanker, 1930). With

red cabbage *Brassica oleracea*, however, Frey-Wyssling and Blank (1943) found an increase in anthocyanins as the temperature was raised from 10°–30°, higher temperatures still bringing about a decrease. Blank and Luedi (1953) have suggested that actual extraction of the anthocyanin pigments is necessary for proper evaluation, since the intensity of the colour of the plants depends also on conditions other than that of the puantity of pigment present.

For plants with essential oils we have already mentioned that the formation is enhanced at higher temperatures (within reasonable limits) (Bode, 1940; Schib 1958a). This has been also shown by Paech (1942) with *Asarum europaeum*. In plants which had been grown at a low temperature until cell growth was completed, exposure to a higher temperature induced formation of new excretory cells with additional essential oil.

Several authors (Ivanov, 1932; Schmalfuss, 1937) have shown that at low temperatures plants produce oils containing fatty acids with a higher content of double bonds that at higher temperatures. Ivanov (1927) indicated that an increase of latitude also raised the degree of unsaturation.

(*c*) *Water*. Water occurs in the form of rainfall, dew and humidity as well as soil-moisture. Since these forms are more or less dependent on each other, and on temperature, complicated interactions must be expected.

In conditions of low relative humidity, Koelle (1953) demonstrated that the density of glandular hairs of *Majorana hortensis* increased, yielding plants with a higher content of essential oil. Boshart (1942) in experiments at nine centres during three years concluded that both low and very high rainfall caused a reduction in the essential oil in peppermint. Unfortunately the density of glandular hairs was not determined. Many other investigations have been carried out on open land with plants containing essential oils, among which those of Rovesti (1953) on the oil content and of several of its constituents (camphene, camphor and cineol) of *Meriandra bengalensis* should be noted. The results showed that the percentage of oil (0·45–1·25%) and its content of camphene varied directly with rainfall and vapour pressure, while the content of camphor (6–70%) varied inversely.

Rain, and even dew, can produce serious losses of water soluble substances from the aerial parts of the plants. Mothes (1938), Sandfort (1940), Flosdorf and Palmer (1949) and other authors, found considerable loss of alkaloids, glycosides and even of essential oils. For water-soluble substances, loss through the epidermis must be expected and this could explain the low content of certain drugs found when plants are collected after rainy days.

2. Special types of climates

As we have seen, each individual climatic factor can affect to a greater or lesser extent the content of secondary plant products. Climates with extreme properties, such as those of mountainous regions of arid zones, or of the arctic or tropics can therefore be expected to greatly affect the synthesis of such products. Investigations on the influence of extreme climates can be performed by two ways: (1) by collecting samples of wild plants in different regions and (2) by cultivation of plants on the same soil in the different climates to be explored. In both cases the number of plants examined must meet with statistical requirements, and care must be taken to collect the plants at the same time of day and at the same stage of development. Furthermore, samples should be collected or cultivated, not only from two places with the greatest climatic difference, but also from intermediate centres. Only the influence of alpine climate in comparison with the lowland climate will be discussed.

The average variation in climate from the lowland to alpine regions are as follows: decrease of about 0·5° per 100 m of altitude; increase of about 1% radiation (g cal/cm^2 per min) per 100 m; decrease in atmospheric pressure; chemical composition of the air is practically unchanged up to 2500 m, with regard to O_2 and to CO_2; decrease in relative humidity, about 25% per 1000 m; cloudiness and rain increase, very much dependent on local conditions; wind, dependent on local conditions. Of these factors only temperature may be reduced during the night to such a level that the threshold of many metabolic processes is reached. The other factors even at the extreme still suffice for plant life at least below the snowline. Details of the behaviour of general metabolic processes in an alpine climate have been given by Lundegardh (1957) and Schröter (1926). The most detailed study on the effect of this climate on secondary plant products has been made by Flück and his collaborators (Meyer, 1936; Bänninger, 1939; Meier, 1940; Eyman, 1945; Engi, 1946) in the valley of Arosa, having research stations at 600, 940, 1250, 1460, 1840, and 2600 m. The climates at the six stations followed the general rules mentioned above with one exception: at 940 m, due to orographic conditions, the temperature was about one degree higher than at the lowest station. All species investigated showed healthygrowth at all stations.

The results obtained show the different behaviour of the individual species. In *Aconitum napellus* and *Lobelia inflata* the content of alkaloids decreased about 15–20% from the lowest to highest stations. Similarly *Thymus vulgaris*, *Mentha piperita*, *Peucedanum ostruthium* and *Achillea millefolium* showed a clear decrease (with an increase at 940 m) in the content of essential oil. In *Achillea moschata*, however, an optimal content occurred between 1250 to 1440 m, whilst with *Artemisia laxa*

and *A. spicata* there was no direct relation between oil content and altitude.

In the two alpine species of *Artemisia*, the content of bitter principles (related to azulenes) decreased regularly with increase of altitude. On the other hand, the bitter principles (glycosides) of gentian root *Gentiana lutea* increased with altitude.

In no case was the variation between the lowest and the highest value for any product greater than 50%. Unfortunately at the time when these investigations were carried out micro-methods for the detailed analysis of individual constituents of any of the groups of compounds examined were not available, so that almost nothing can be said as to whether any individual substance ceased to be formed at a higher altitude.

As mentioned above investigations with naturally grown plants at different altitudes are subject to effect of soil, and of genetic and ontogenetic variability. One example in which the ontogenetic states had been clearly considered is that of *Veratrum album* which showed a decrease in alkaloid at higher altitudes (Jaspersen-Schib and Flück, 1958).

In conclusion it can be again stressed, that no one has observed a substance disappearing, or a new substance appearing by change in any of the extrinsic factors. However, it must be stressed that most of the investigations reported were carried out before the new chromatographic methods were available. The time has now come to re-investigate many of these problems using such methods, and the results should be of great benefit to chemical taxonomy.

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CHAPTER 8

The Distribution of Alkanes

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I. Introduction

Alkanes are very widely distributed in both the plant and animal kingdoms and, as constituents of petroleum, they have an essential and formative role in our civilization. In plants they are most abundant in the cuticle waxes which act as protective coatings on leaves and stems. The alkane fraction of such waxes is commonly a mixture of hydrocarbons

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of very similar chemical and physical properties. Classical procedures of organic chemistry have proved to be quite inadequate for the total separation and analysis of such mixtures and it has only been during the last 5–10 years that methods of sufficient power, such as gas chromatography and mass spectroscopy, have emerged. Alkane fractions can now be analysed quickly and quantitatively, and our knowledge of their distribution in plants should advance rapidly.

This chapter is restricted to the completely saturated, paraffinic, hydrocarbons C_nH_{2n+2}, both straight, unbranched n-alkanes, and branched alkanes. The term, iso-alkanes, has been used to describe branched alkanes generally, but now is more commonly applied to terminally branched hydrocarbons; anteiso-alkanes possess a methyl substituent on the next but one carbon to the end of the chain (Fig. 1).

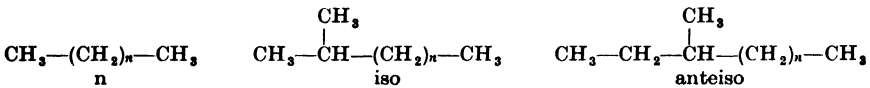


FIG. 1. Alkane isomers

Alkanes containing an odd number of carbon atoms predominate in nearly all species of plants.

II. Historical

The literature prior to 1934 was fully reviewed by Chibnall *et al.* (1934a, b), who showed that almost all the previous claims for the isolation of pure individual alkanes were erroneous. These reports had been based on ordinary melting-point determinations and micro-analytical figures, whereas Chibnall and his co-workers had found it essential to determine melting and transition points precisely, and to employ X-ray powder diagrams which indicated both the degree of purity of the sample and the approximate chain lengths in the crystal lattice. Thus, the even-carbon-number C_{30} alkane, triacontane, had been "identified" nineteen times in the literature and in every case the sample was subsequently shown to be a mixture of odd number n-alkanes, largely the ubiquitous C_{29} and C_{31} compounds. Chibnall *et al.* concluded that the hydrocarbon fractions of plant and insect waxes are mixtures of the odd carbon number n-alkanes ranging from C_{25} to C_{37}. They were unable to detect any of the even-numbered members, even after repeated fractional crystallization.

Wanless, King and Ritter (1955) appear to be the first workers to demonstrate conclusively the natural occurrence of even carbon number alkanes, although Schuette and Kahn (1953) did make a prior claim based

on X-ray measurements. Wanless and his colleagues used the mass spectrometric method to examine partially separated alkane fractions derived by column chromatography of pyrethrum cuticle wax, and showed that these fractions contained up to 7% of the C_{28} and C_{30} n-alkanes. Subsequent mass spectrometric and gas–liquid chromatographic studies have established that the even-numbered alkanes are common minor constituents of plant waxes. The same methods have led to the detection of iso-alkanes in tobacco wax (Carruthers and Johnstone, 1959), while very recently the natural occurrence of the first really long-chain n-alkane, $C_{62}H_{126}$, has been claimed (Kranz *et al.*, 1961).

Branched alkanes other than iso- or cyclo-alkanes, and simple, non-terpenoid alkenes have not as yet been detected in plant waxes. However, the number of plant species examined is still minute in comparison with the vast number awaiting study and new types of alkane are sure to be encountered. Surveys of the type reported by Eglinton *et al.* (1962a,b) should assist in achieving an overall picture of the distribution of alkanes in plant waxes. For a review of the distribution of all types of naturally occurring hydrocarbons see Gerarde and Gerarde, 1961.

III. Plant Waxes

A brief survey of the state of knowledge with regard to plant waxes seems both opportune and relevant. Useful reviews have been published by Martin and Batt (1958), Martin (1960, 1961), Kreger and Schamhart (1956), Kreger (1958a,b) and Warth (1957, 1960).

Thin, waxy layers coat the stem, leaf, flower and fruit of most plants, sometimes imparting a bluish-white cast to the surfaces on which they occur. In addition wax platelets are found embedded within the cutinized epidermal layer. Chemically speaking, the term wax refers to an ester of a higher fatty acid and a higher aliphatic alcohol, but in the present context it applies to all substances of "waxy" character isolated from the plant. Plant waxes may constitute anything from a fraction of a per cent to several per cent of the dry weight of a plant.

A. Morphology and Mode of Formation

The elegant microscope studies of De Bary (1871) established that the wax coat varies greatly in quantity and structure from one species to another but that in most cases the covering takes the form of myriads of minute plates or rods approximately 10 μ or less in length. Subsequent examination with the electron microscope (Schieferstein and Loomis, 1956, 1959; Scott *et al.*, 1958, 1960; Juniper, 1959a,b, Junniper and Burras

1962) and by the X-ray diffraction procedure (Kreger and Schamhart, 1956; Kreger, 1958a,b) has revealed much fascinating detail. Although there is some controversy, it seems fairly generally accepted that the wax originates in the epidermal cells as oily droplets that reach the surface of the plant via minute canals (plasmodesmata) penetrating the thickened cell wall—the cuticular layer. The "pores" or openings of these canals have been located very recently by Hall and Donaldson (1962) and shown to have a mean diameter of about 6 mμ. It would seem that the wax crystallizes as it emerges from the pores, for the rods and plates give well defined X-ray diffraction photographs (Kreger, 1948, 1958a,b). A solvent such as the n-tridecane employed by the "Stink Bug" (Blum *et al.*, 1960), may maintain the wax in liquid form prior to its emergence from the pores; indeed, Roberts *et al.* (1959) refer to a non-waxy or oily component in the cuticle. The wax distributions on the upper and lower surfaces of leaves differ (Martin and Batt, 1958).

B. The Role and Composition of Plant Waxes

The natural protective covering of the leaves of the higher plants consists of the non-living cuticle and its waxy coating. The waxy coating undoubtedly assists in controlling the water-balance of the plant, especially under excessively moist or dry conditions (Hall and Jones, 1961). The wettability of the leaf surface and hence the efficacy of agricultural sprays, is related to the nature, morphology, and extent of the coating (Juniper, 1959a,b; Dewey *et al.*, 1962). Further, the waxy layer seems to contain substances which inhibit bacterial, fungal and insect attack (Martin and Batt, 1958), while in one case, that of the insectivorous "Pitcher Plant" (*Nepenthes*), the waxy interior of the pitcher effectively helps to trap insects (Juniper and Burras, 1962).

The waxes were among the first natural products to be studied in modern times but they have received little detailed chemical attention since the outstanding contributions of Chibnall and his collaborators in the 1930's. This is due in large part to the formidable problems of separation involved in the study of such a complex mixture of closely related long-chain aliphatic components. Recent studies employing the combined techniques of gas–liquid chromatography and mass spectrometry have shown the wax composition to be more complex than was earlier suspected, and the present situation is summarized in Table I. The common feature of all major wax constituents is the straight saturated chain of greater length (20–37 carbons) than is found in the acids (12–20 carbons) derived from the fats.

Detailed chemical investigation of the plant "skin", including the

epidermal cells, the cuticular layer, the embedded waxes and the surface waxes, is now feasible. Such work is certain to result in considerable modification and supplementation of the results in Table I and it will be of particular interest to attempt the placing of each new type of compound within the overall scheme of plant wax biogenesis (Section VI). For example, the C.S.I.R.O. group in Australia have just reported the occurrence of a new class of wax constituent, long-chain C_{29}–C_{33} β-diketones, in *Eucalyptus* leaf waxes (Horn and Lamberton, 1962).

TABLE I

Major constituents of leaf waxes

Type	Range	Frequency
Alkanes	Normal: odd C_{21}–C_{37}	Common (especially C_{29} and C_{31})
	Normal: even C_{20}–C_{34}	Common minor constituents
	Branched: C_{27}–C_{33}	Infrequent
Alcohols (usually as esters)	Primary: even C_{22}–C_{32}	Common
	Primary: odd C_{25}–C_{31}	Infrequent
	Secondary: odd C_{21}–C_{33}	Common
	Diols and ketols	Rare
	Terpene alcohols	Infrequent
Aldehydes (as polymers)	Normal: C_{24}–C_{34}	Rare
Ketones	Di-n-alkyl ketones	Rare
Acids (usually as esters)	Normal: even C_{14}–C_{34}	Common
	Normal: odd C_{15}–C_{33}	(?)
	Ketoacids	Rare
	Dibasic acids	Rare
Esters	Between n-acids and primary and secondary alcohols	Common
	Estolides of hydroxyacids	Infrequent (?)

Amongst minor or infrequent constituents one might cite triterpenoids (e.g. ursolic acid), diterpenes, glycerides (Lamberton, 1961) and phenolic substances. Analytical procedures are discussed under Section IV.

C. Taxonomic Applications

The employment of leaf waxes in chemical plant taxonomy would seem advantageous in view of the universal occurrence of these coatings, the now-established species variation in wax composition, the fact that the wax is extracellular and almost certainly an end-product insulated from the regular essential metabolic functions of the plant, the simplicity

in sampling, and the present day availability of precise and rapid micro-analytical tools. Several workers have used leaf waxes taxonomically, thus Barber (1955) and Barber and Jackson (1957) have found that the waxes of two sub-genera of *Eucalyptus* differ in their melting point ranges, while Hopkins and Riley (1961) and Purdy and Truter (1961) have used paper and thin-layer chromatographic procedures, respectively, to show that the surface lipids give patterns ("biochemical profiles") characteristic of the particular species of plant. These rapid qualitative procedures provide useful comparison data without necessitating identification of the compounds involved. Direct gas–liquid chromatography of an unfractionated plant wax is similarly effective in providing a complex profile or "fingerprint" (Eglinton *et al.*, unpublished).

Kreger (1958) has attempted a taxonomic approach based on the nature of the wax constituents as deduced from the X-ray powder photographs of unfractionated waxes. This analytical method is relatively insensitive (Section IV). However, he summarizes his results as follows.

"(i) Closely allied species generally produce waxes of corresponding or identical composition, slight differences being mainly confined to the proportions in which they occur.

"(ii) There are plants of widely divergent families which have cuticle waxes of almost the same composition.

"(iii) Cuticle waxes which consist predominantly of primary alcohols seem to be confined to monocotyledons.

"(iv) There are certain indications that morphological features common to plants of different families are often accompanied by a similarity in the constitution of the cuticle wax."

However, it may reasonably be concluded that a full understanding of the biogenetic pathways involved will be essential for the satisfactory use of leaf wax constituents in plant taxonomy. The biogenesis of the plant alkanes is discussed later (Section VI).

IV. The Isolation and Characterization of Alkanes

Nearly all plant waxes examined so far contain alkanes, though the percentage of alkane varies greatly from one species to another: the wax of the tobacco plant is said to consist almost entirely of alkanes (Chibnall and Piper, 1934a,b) as is the leaf wax of *Cotyledon orbicularis* (Juniper, unpublished). Flower petal waxes and the commercial "candelilla" wax (derived from *Pedilanthus paronis* (Euph.) have alkane fractions in excess of 50% by weight, while two other commercially important products, sugar-cane cuticle wax and the wax of carnauba palm, have less than 10% of alkane. The various procedures now available for the collec-

tion, separation and analysis of the alkane fractions are discussed below. These procedures owe much to prior developments in the petroleum industry (Brooks *et al.*, 1954).

A. Isolation of the Alkane Fraction

The plant waxes can sometimes be removed by scraping the surface of the plant (Kreger, 1948) or by placing it in hot water (Chibnall *et al.*, 1934a, b). However, it is more usual to extract the wax by dipping the unbroken leaves or stems into a solvent such as ether or chloroform. Although there are reports to the contrary (Hall and Donaldson, 1962), this procedure is held to remove all the surface wax without removing any of the cytoplasmic constituents (Martin and Batt, 1958; Martin, 1960; Dewey *et al.*, 1962). Solvent extraction of the macerated plants on the other hand results in the isolation of the total plant lipids, including the galactoglyceride fraction (Garton, 1960; Shorland, 1961).

Physical techniques, alone or in conjunction with chemical procedures, are currently employed in the isolation of the alkane fraction. Alumina column chromatography of the crude wax with light petroleum as solvent is quite often effective, the first fraction containing only the alkanes (Savidan, 1956; Mazliak, 1960b, 1961b, c; Eglinton *et al.*, 1962a, b). Partition between heptane and methanol has been used (Martin, 1960; Dewey *et al.*, 1962) but relatively non-polar substances such as long-chain esters and ketones accompany the alkanes into the heptane layer. Chromatography over alumina or silica is not always effective in removing contaminants and a more thorough approach involves saponification of the crude wax and subsequent treatment with 2,4-dinitrophenylhydrazine to remove ketonic material. Even then a few cases have been encountered where the "alkane" fraction still contained oxygenated compounds after such treatment: the infra-red absorption near 1120 cm^{-1} in the "alkane" fraction of *Arundo conspicua* has been shown to be due to the presence of triterpenoid ethers (Eglinton, Hamilton and Martin-Smith, 1962b). These ethers were readily removed by treatment of the fraction with hot concentrated sulphuric acid (Fig. 2).

A complete analysis of a plant wax requires separation of each class of compound present and here adsorption chromatography must be combined with acid/base or ion-exchange treatment. Neither the formation of inclusion compounds with urea, nor the use of molecular sieves is advised for the purification of alkane fractions as some fractionation may occur. However, these methods have been used by several workers (e.g. Downing *et al.*, 1960; Louloudes *et al.*, 1961, 1962; Carruthers and Johnstone, 1959).

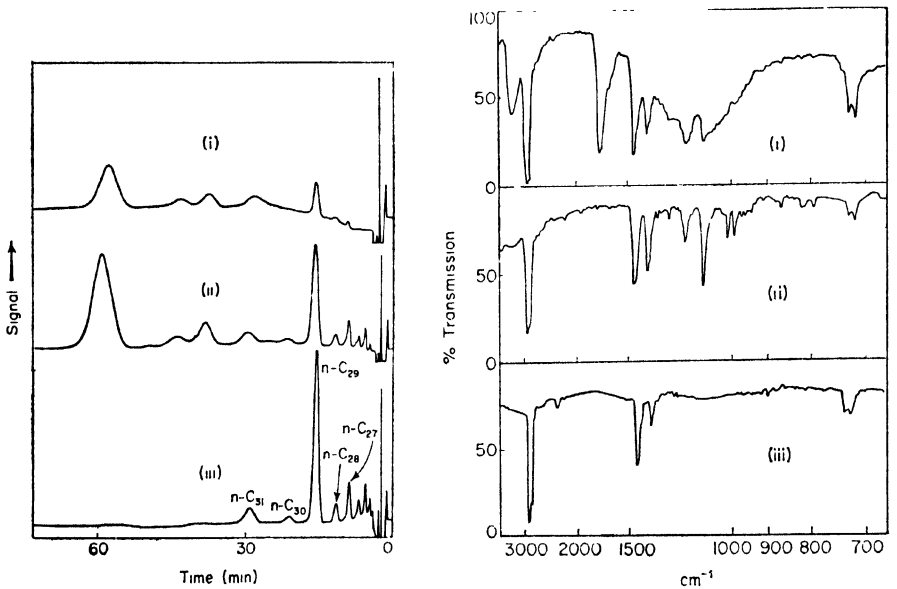


FIG. 2. Isolation of the hydrocarbon fraction from the plant wax of *Arundo conspicua*. (i) The crude plant wax as extracted from the dried plant. (ii) The wax remaining after successive treatments with alcoholic sodium hydroxide and 2,4-dinitrophenylhydrazine reagent, followed by chromatography over alumina. (iii) The wax remaining after treatment of fraction (ii) with hot concentrated sulphuric acid.

Left. Gas–liquid chromatograms. Load approximately 5 μg solid wax; column 130 cm × 0·4 cm, 0·5% Apiezon "L" on Embacel, 80–100 mesh at 225°; gas flow, 45 ml argon per min; detector voltage 1750 V, attenuation × 10.

Right. Infra-red spectra. Solid films. (Reprinted from Eglinton, Hamilton and Martin-Smith, 1962b.)

B. Analysis of the Alkane Fraction

Only two methods are really effective—gas–liquid chromatography and mass spectrometry—and they may be used either alone or in conjunction. They are both micro methods (< 1 mg) but gas–liquid chromatography is probably the most convenient and the most readily available. The technique has been fully described by several authors (see *inter alia* Downing *et al.*, 1960; Eglinton *et al.*, 1962a, b; Louloudes *et al.*, 1962; Mazliak, 1961c; Adlard and Whitham, 1958; Levy *et al.*, 1961) and selective stationary phases are now commercially available.* All members of the n- and iso-homologous series are readily separated from one another but iso- and anteiso- and, possibly, mid-chain branched, alkanes are not. Pre-columns of molecular sieve have been used (Downing *et al.*, 1960), while class reactions carried out in a closed gas chromatographic system

* Inda Chemical, 33rd Street and Cleveland Avenue, Camden 5, N.J., U.S.A.

enabled Rowan (1961) to deal with very complex hydrocarbon mixtures. Temperature-programmed equipment greatly facilitates analysis of alkane mixtures covering a wide range of carbon numbers (Downing *et al.*, 1960; Haahti and Horning, 1961). Only occasional calibration with authentic alkanes is required when a series of alkane fractions is under examination. Automatic signal integration and digital output of the results is now available and should be of considerable assistance in taxonomic surveys of the type described in Section V.

Good examples of the mass spectrometric approach are to be found in the work of Waldron (1961), Brown (1954), Levy (1961), Wanless (1955), Louloudes (1962) and their co-workers. Careful calibration of the instrument and mathematical treatment of the results are required.

The X-ray powder diffraction method (Piper *et al.*, 1931) has been used extensively by Kreger and his co-workers, and by Wiedenhof (1959), but only yields definite results with single alkanes or very simple mixtures (Kranz *et al.*, 1960).

C. Isolation and Identification of Individual Alkanes

It would seem that most plant waxes contain only n- and iso-alkanes and there is ordinarily no need to isolate individual alkanes since the analytical methods provide reasonable proof of homology. If rigid structural proof is required it is recommended that the alkane be collected by preparative gas–liquid chromatography and then identified with the help of the numerous physical aids now available. Mass spectrometry (Ryhage and Stenhagen, 1960) would be the first choice, but infra-red and nuclear magnetic resonance spectra, and X-ray crystallography are also suitable for micro samples (1 mg). Some preliminary fractionation of the plant alkanes prior to preparative gas–liquid chromatography can be of considerable assistance and may be achieved by column chromatography (Wanless *et al.*, 1955), fractional crystallization (Chibnall *et al.*, 1954), normal and amplified fractional distillation (Downing *et al.*, 1960, cite such work), and, as mentioned earlier, by the formation of inclusion compounds or adsorption using molecular sieves.

V. Taxonomic Applications of Alkanes

The use of chemical constituents of plants as an aid to their classification is now a familiar concept, the oustanding example being the extensive work of the Erdtman school on conifers (Erdtman, 1956). Erdtman has pointed out that the most valuable substances taxonomically are not those which are involved in primary metabolic processes but rather those

which are relatively stable by-products in their biological environment. The plant wax hydrocarbons meet this requirement quite well and, further, as already described, the alkane fraction is amenable to rapid isolation and quantitative analysis, while its very complexity serves as a positive advantage in providing a taxonomic fingerprint. However, there are only isolated reports of the alkanes being used in this way. Mirov (1961) discussed the distribution of the lower n-alkanes (C_7, C_9 and C_{11}, especially) in *Pinus* species and proposed an ancestral relationship between the relic pine, *P. jeffreyi*, and a distant group of *Macrocarpae*. Both contain the lower n-alkanes in considerable amounts whereas *P. ponderosa*, which belongs to the *Australes* group and is morphologically close to *P. jeffreyi*, does not. Mirov (1952) had earlier related *P. reflexa* and *P. monticola* by virtue of their mutual content of undecane, but the use of single constituents in this way is undoubtedly dangerous, and it is preferable to employ the full range of alkanes present in the plant. Alkaloid (Eddy *et al.*, 1961), fatty acid (Wheaton, Lyons and their co-workers, 1962) and other types of distribution patterns have been discussed (see Chapters 10 and 14) but the only similar study of plant alkanes has been that of Eglinton and his colleagues (1962a, b). This work is discussed in detail below.

A. The Alkane Distribution Patterns as a Species Characteristic

The principal requirement for a taxonomic criterion is that it be species specific. The results obtained by Eglinton and his colleagues (1962a) indicate that the alkane distribution pattern, as established by gas–liquid chromatography of the hydrocarbon fraction of the wax of a species, is a property characteristic of that species. Table II summarizes some of the data obtained with a single species *Aeonium urbicum* (Crassulaceae), covering variation in place of growth, season, age and part of plant. Such small differences as there are, would not seem to invalidate the general conclusions, though further studies employing a thorough statistical treatment are desirable. Incidentally, Downing *et al.* (1961) have remarked on the constancy in the alkane pattern of bees' wax and Lamberton and Redcliffe (1960) found a similar constancy in the composition of sugar cane wax. One point requiring early attention is to determine the extent of any variation in the alkane pattern for wax derived from different parts of the plant—leaves, petals, roots, stem, etc. Further studies are also required on the effects of different extraction procedures (see Section V, C). Even pollen waxes have been examined (Nilsson *et al.*, 1957); the species investigated contained n-C_{25}, C_{27} and C_{29} alkanes.

TABLE II

Distribution in mole-% of the alkane constituents* of the leaf wax of *Aeonium urbicum* (Crass). Variation with repeat sampling, station and age of leaf

Station	Season	Comment	Percentage alkanes in crude wax	n-C_{25}	n-C_{26}	iso-C_{27}	n-C_{27}	n-C_{28}	iso-C_{29}	n-C_{29}	n-C_{30}	iso-C_{31}	n-C_{31}	n-C_{32}	iso-C_{33}	n-C_{33}	n-C_{34}	iso-C_{35}	n-C_{35}
La Laguna	Sept. 1960	Repeat sampling	25	†	†		†	†		1	†	1	20	3	3	68	2		3
			55				†	†		1	1	1	21	4	2	63	†	1	6
			60				†	†		†	†	†	15	2	4	69	3		6
			50				†	†		†	†	†	16	3	6	70	1		3
Las Mercedes	Nov. 1960	Immature leaves	35				1	†		1	†	1	22	2	1	68	1		2
			65				†	†		†	†	1	15	4	4	72	1		2
Santa Ursula	Feb. 1961	Dead leaves	55				†	†		†		†	12	3	1	69	2		13
Buenavista	Dec. 1960	Different altitudes	—				1	†	†	1	†	†	12	3	2	74	1	†	5
			25				†	†	†	1	†	1	21	3	1	64	2	†	5

* The iso-alkanes have been so termed by analogy with results obtained for other species. Anteiso- or other types of branched alkane may be present (see text).

† Indicates alkanes present in trace quantities only (< 1%). Alkanes absent for all examples have been omitted from the table.

B. The Alkane Distribution Patterns of Closely-related Species and Genera

The only relevant study would appear to be that of Eglinton and his colleagues (1962a). These workers examined the leaf waxes of a compact grouping of closely related genera of the sub-family Sempervivoideae (Crassulaceae: Fig. 3) endemic to the Canary Islands, which had already

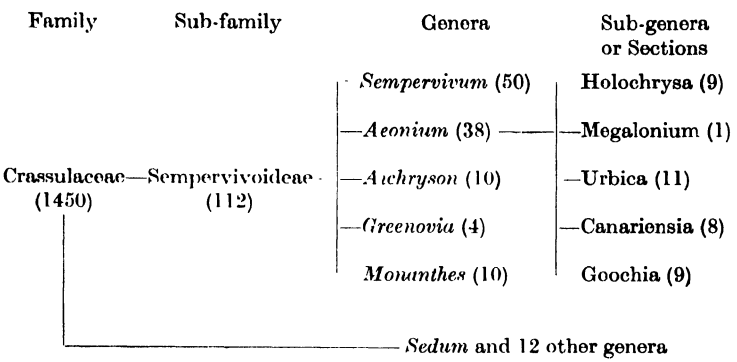


Fig. 3. The Crassulaceae family showing the sub-division (Lems, 1960) of the sub-family Sempervivoideae. Number of species in parentheses

been extensively studied botanically. They are believed to be descended from a common ancestor which initially colonized the islands and to have been developed in isolation from the mainland. Because of the wide climatic variation on these islands of diverse terrain, the variety of forms encountered presents an excellent example of "adaptive radiation". The members of this family are all xeromorphic and generally possess quite substantial waxy coatings. Lems (1960) devotes a recent lengthy paper to the evolutionary aspects of the group in which he states his belief that the *Aeoniums* present "a situation comparable in many ways to the finches of the Galapagos". His study concerns the plant forms of the Sempervivoideae and his conclusion is that "this group is composed of species of many forms, from shrub to biennial and annual herb; it is possible to derive all of them from a shrubby ancestor".

The plants studied included examples from all the constituent genera of the Sempervivoideae (Fig. 3), with the exception of *Sempervivum*. The distribution of the hydrocarbon constituents is shown in histogram form in Fig. 4, a single diagram for each authenticated species. The botanical classification is delineated, and within this the species are arranged such that in general, the branched-chain isomer content increases from left to

right and from top to bottom; the proportion of C_{31} to C_{33} seems to increase in a similar progression.

The most extensive survey was centred on the genus *Aeonium*. Within the section Holochrysa the three species examined show closely similar hydrocarbon patterns and the same is true for the different hydrocarbon pattern obtained for the species in the section Urbica. The botanical subdivision of Urbica proposed by Lems is not paralleled by these results. The species of the section Goochia give mutually rather similar hydrocarbon patterns but two seem out of step in this respect, *A. spathulatum* (34)* and *A. cruentum* (35). The species of the section Canariensia fall into two groupings. The first three members (21, 22, 23) show a close hydrocarbon pattern relationship allied to the Holochrysa and the Goochia species 27 and 28, while the patterns of the next two (24, 25) are much more akin to those of the section Urbica; one member of the Canariensia, the distinctive plate-like *A. tabulaeforme* (26), is quite anomalous in the reversal of its C_{31} to C_{33} n-alkane ratio.

In the less extensively examined genera *Greenovia*, *Aichryson* and *Monanthes* the hydrocarbon patterns are internally consistent with the exception of one of the last, *M. amydros* (7). Lems has suggested that the genus *Greenovia* is evolutionarily related to the Canariensia section of the genus *Aeonium* but the hydrocarbon patterns of the two are quite different and on this latter basis Eglinton *et al.* (1962a) suggested a relationship between the genera *Greenovia* and *Monanthes* and the sections Urbica and Megalonium of the genus *Aeonium*. On the other hand, the branched hydrocarbon content of the *Aichryson* species examined does give some support to Lems' contention that this genus is related to the Goochia section of the genus *Aeonium*.

The conclusions drawn from the above results by Eglinton *et al.* (1962a) were that although such comparisons might serve to confirm relationships between closely related species, the differences between related genera might be insufficiently discriminating. Even so, similar species sometimes had widely different patterns and there was only a rough parallelism of hydrocarbon pattern and botanical classification.

One interesting point, which will be discussed under Section V, D, is the high content of iso-alkanes in some of the plants.

C. The Alkane Distribution Patterns of Species Belonging to a Variety of Genera and Families

The alkane distribution patterns represented diagrammatically in Fig. 5 have been constructed from literature data obtained either by

* The numbers refer to Fig. 4.

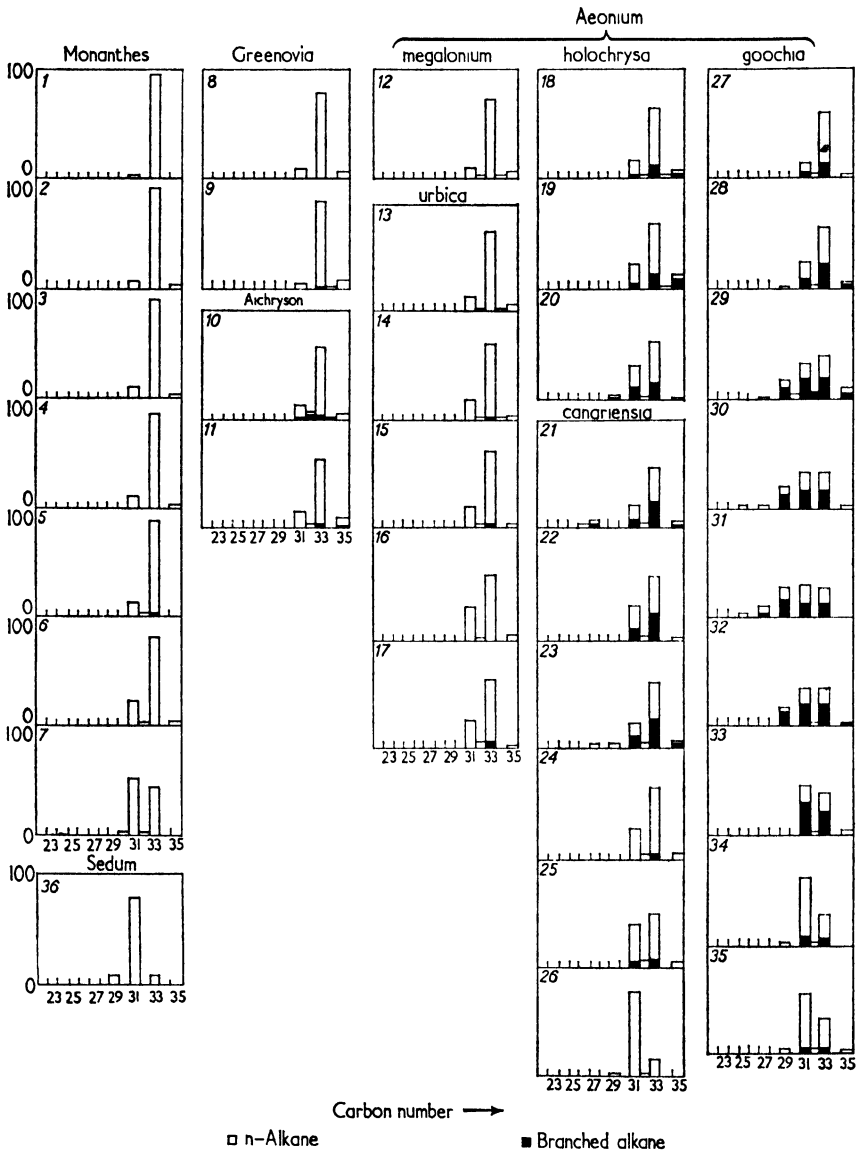


FIG. 4. Distribution (mole-%) of n- and branched alkanes C_{23}–C_{35} in the hydrocarbon fraction of the surface waxes from the leaves of individual species of the constituent genera *Monanthes*, *Greenovia*, *Aichryson* and *Aeonium* of the sub-family Sempervivoideae (Fig. 3).

mass spectrometric or gas–liquid chromatographic analysis of unfractionated plant alkanes (Eglinton, Hamilton and Martin-Smith, 1962b and refs. cited therein). As mentioned earlier, little reliance can be placed upon claims for the separation and identification of alkanes by other methods.

The patterns may not be strictly comparable with those obtained with the Sempervivoideae (Section V, B), since some of them refer to wax derived from the whole plant or to parts other than the leaves. Although the alkane components are probably restricted to the surface coatings there is no reason to suppose that the alkane distribution patterns are the same for different anatomical portions of the plant. In fact the marked difference between the alkane distribution in the rhizomes of *Cordyline australis* (16, Fig. 5) and that in the leaves of *Draceana draco* (17), plants which are considered to be closely related botanically, shows the need for studies on the relative alkane distribution within the same plant.

The variety of the hydrocarbon patterns is striking when Fig. 5, in which thirteen families and twenty-one genera are represented, is considered as a whole, and it may be that there is indeed some future for taxonomic correlations based on this approach. However, this diversity is itself disconcerting as no general patterns are as yet apparent which might permit monocotyledons (e.g. the Gramineae and Liliaceae species shown) to be distinguished from dicotyledons (the remaining families illustrated), or which would allow the assignment of a species to a given family, or even to a particular genus. With regard to the last point there is little constancy within the seven species of the genus *Euphorbia*, but this genus (750 species) is in any case not closely knit botanically. On the other hand the two *Lolium* species (29, 30) have very similar patterns and the interesting correlations within the sub-group Sempervivoideae have been discussed in the preceding section (V, B).

Alkanes present as less than 2% have been omitted. The genus *Aeonium* has been subdivided into the sections given by Lems (1960). (Redrawn from Eglinton *et al.*, 1962a.)

SPECIES:

MONANTHES:

1. *M. laxiflora*
2. *M. anagensis*
3. *M. muralis*
4. *M. brauchycaula*
5. *M. polyphylla*
6. *M. pallens*
7. *M. amydros*

SEDUM:

36. *S. anglicum*

GREENOVIA:

8. *G. aurea*
9. *G. diplocycla*

AICHRYSON:

10. *Ai. dichotomum*
11. *Ai. punctatum*

AEONIUM:

12. *A. nobile*
13. *A. percarneum*
14. *A. urbicum*
15. *A. haworthii*
16. *A. castello-paive*
17. *A. decorum*
18. *A. rubrolineatum*
19. *A. holochrysum*
20. *A. manriqueorum*
21. *A. virgineum*
22. *A. canariense*
23. *A. palmense*
24. *A. cuneatum*
25. *A. subplanum*
26. *A. tabulaeforme*
27. *A. caespitosum*
28. *A. sedifolium*
29. *A. goochiae*
30. *A. lindleyi*
31. *A. viscatum*
32. *A. saundersii-Bolle*
33. *A. smithii*
34. *A. spathulatum*
35. *A. cruentum*

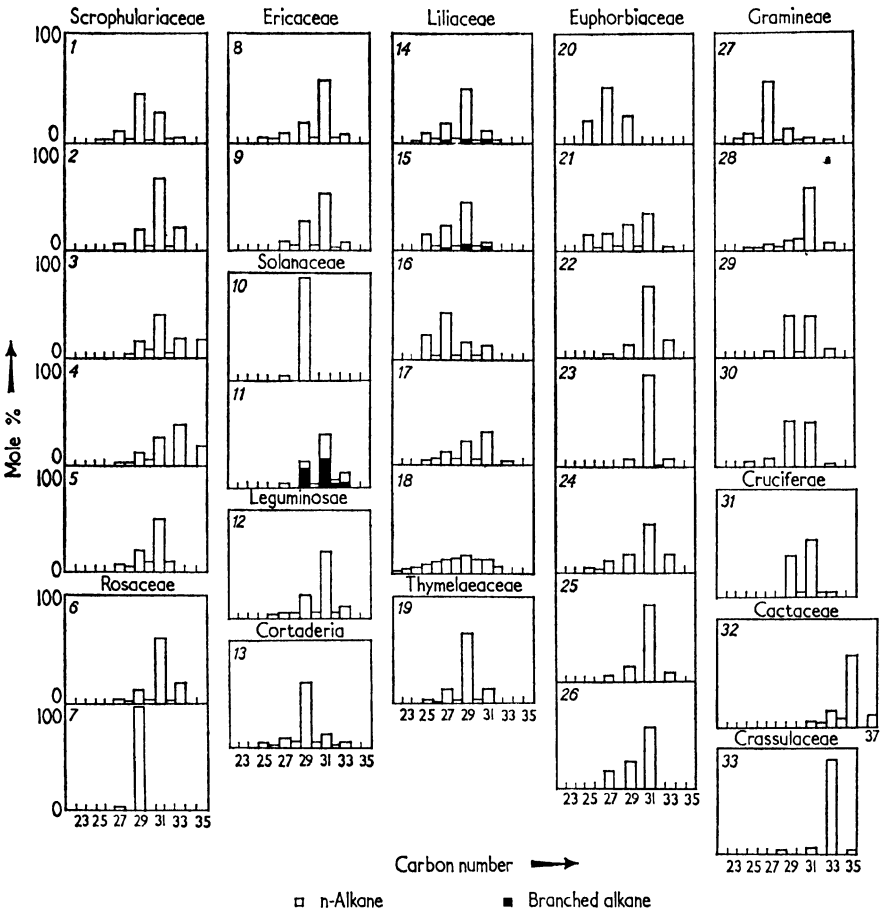


FIG. 5. Distribution (mole-%) of n- and branched alkanes C_{22}–C_{35} in the hydrocarbon fraction of the waxes derived from plants representing several different genera and families. Alkanes present as less than 2% have been omitted. (Redrawn from Eglinton *et al.*, 1962b.)

SPECIES

1. *Hebe odora*
2. *H. parviflora*
3. *H. diosmaefolia*
4. *H. stricta*
5. *Bacopa monnieri*
6. *Acaena anserinifolia*
7. *Pyrus malus*
8. *Gaultheria subcorymbosa*
9. *G. antipoda*
10. *Solanum tuberosum*
11. *Nicotiana tabacum*
12. *Phaseolus aureus*
13. *Arundo conspicua*
14. *Phormium tenax* (var. SS)
15. *P. tenax* (var Ngaro)
16. *Cordyline australis*
17. *Dracaena draco*
18. *Copernicia cerifera*
19. *Pimelea prostrata*
20. *Euphorbia balsamifera*
21. *E. aphylla*
22. *E. regis-jubae*
23. *E. cerifera*
24. *E. peplus*
25. *E. atropurpurea*
26. *E. bourgianea*
27. *Saccharum officinarum*
28. *Leptochloa dyntata*
29. *Lolium perenne*
30. *L. multiflora*
31. *Brassica nigra*
32. *Cactus cactus*
33. *Cotyledon orbicularis*

The diversity within the genus *Hebe* is of interest since the four *Hebe* spp. (1–4) were included by Eglinton, Hamilton and Martin-Smith (1962b) in order to ascertain whether it would be possible to differentiate them chemically. This genus is well known for the ease with which hybridization occurs, making botanical classification extremely difficult, and the species chosen had been carefully selected as representative of definite groups. In the case of the two *Gaultheria* spp. (8, 9) the differences in the alkane distribution patterns are not nearly so clear cut but they are still easily distinguishable. The fact that the two varieties (14, 15) of *Phormium tenax* give virtually superimposable patterns may strengthen the utility of plant hydrocarbon analysis rather than weaken it, as distinction between species without differentiation between varieties would be useful in taxonomy.

An interesting point which may have taxonomic significance emerges from a comparison of Figs. 4 and 5: thirty-three of the thirty-seven species shown of the family Crassulaceae possess hydrocarbon fractions consisting almost entirely of C_{31} and/or C_{33} alkanes, whereas the thirty-one species drawn from twelve other families show no such regularity. More extensive study of the Crassulaceae would be welcome.

D. The Range of Alkanes Present in Plant Waxes

Although the alkanes known to be present in plants range in chain length from C_7 to *ca.* C_{62}, it is clear from the data quoted in the preceding two sections that the following generalizations hold reasonably well.

(a) Alkanes of carbon number less than C_{25} and more than C_{35} are rarely present to any appreciable extent.

(b) The content of odd-carbon-number alkanes is usually greater than that of even-carbon-number alkanes by a factor of more than ten, but small amounts of the latter are almost invariably present.

(c) The major constituent is often C_{27}, C_{29}, C_{31}, or C_{33} n-alkane.

(d) A high proportion of iso-alkane is rare, and even trace quantities are uncommon.

(e) For the major odd-carbon-number constituents (e.g. C_{31} and C_{33}) of any given leaf wax there are indications of a parallelism in the iso- to normal-hydrocarbon ratio.

The iso-alkane structures assigned to the relatively plentiful odd-numbered C_{31} and C_{33} branched alkanes have been rigorously proved in a few key cases (Eglinton *et al.*, unpublished) but it is quite possible that the even-numbered branched alkanes which have not been so examined belong to the anteiso- series. Should this be so, the situation would be the

inverse of that reported for the branched hydrocarbons of wool wax (Downing *et al.*, 1960). This point merits further attention, though it is possible that wool wax is largely derived indirectly from the complex mixture of straight and branched acids generated (Section VI A) by the bacterial micro-flora of the rumen (cf. Keeney *et al.*, 1962).

In most cases the shortest and longest n-alkane chains differ by about ten carbon atoms with varying proportions of each homologue between these limits. However, in certain waxes such as that of *Solanum tuberosum* and *Pyrus malus* (Fig. 5, spp. 10 and 7, respectively), there appear to be

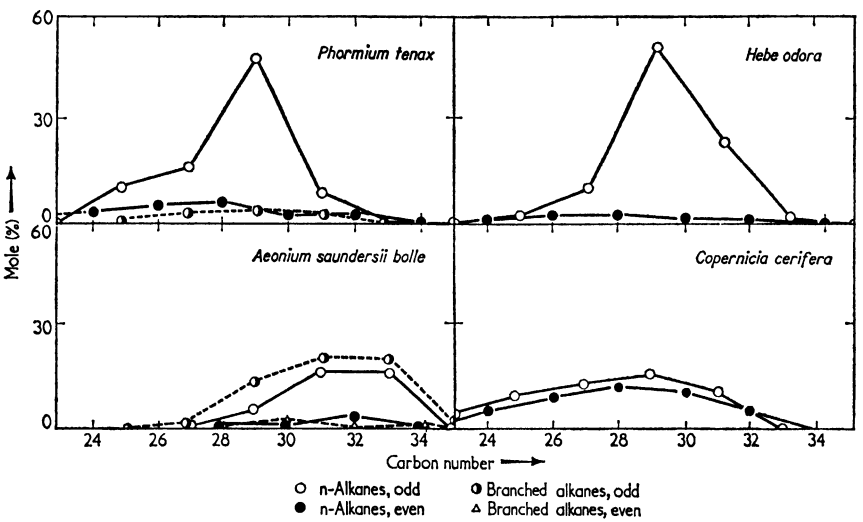


FIG. 6. Content (mole-%) of odd- and even-carbon-number alkanes plotted separately against carbon number. Four types of distribution.

only one or two individual alkanes present in appreciable quantities. An intriguing general point which emerges is that when the odd and even members are considered separately, in both cases the plot of percentage of constituent present against the number of carbon atoms is a simple distribution curve with a single maximum. This generalization also seems to apply with the odd and even branched alkanes, though less data are available (Fig. 6). Since the alkanes can be assumed to be end products of plant metabolism this may be of significance in terms of the specificity of the enzyme systems which are involved in the elaboration of the alkanes from acetate units. These observations again bring to the fore the urgent need for an understanding of the mode, or modes, of biogenesis of the alkanes (Section VI).

VI. The Biogenesis of Alkanes

The range of alkanes encountered as constituents of plant waxes has been summarized under Section V. Any biogenetic proposals must adequately explain these data. Unfortunately plant biochemistry has received relatively little attention apart from the work of Stumpf and his collaborators, and most workers have relied on the availability of suitable analogies in avian or mammalian biochemistry.

It is most probable that one route to the plant hydrocarbons involves decarboxylation of the corresponding long-chain fatty acids or their immediate precursors. Thus the "liquid" (non-crystalline) fraction of apple cuticle wax has been shown to contain acids and alkanes ranging from C_{16} to C_{30} and from C_{15} to C_{29}, respectively (Mazliak, 1960a, 1961a).

The acids of chain length C_{20}–C_{34} which might give rise to the typical n-alkanes (cf. Table I) are common constituents of leaf waxes and are presumably derived in fundamentally the same fashion as the "glyceride" C_{14}–C_{18} series of fatty acids. The current biogenetic proposals for these latter acids are therefore reviewed briefly below.

A. Biogenesis of Fatty Acids

The subject of glyceride fatty acid biogenesis has received intensive study and has been reviewed at frequent intervals (*inter alia*, Cornforth, 1959; Stumpf and Bradbeer, 1959; Bloch, 1960; Wakil, 1961; Lynen and Tada, 1961; Lynen, 1961; Mercer, 1961; Bressler and Wakil, 1962; Birch, 1962; Harlan and Wakil, 1962).

With particulate enzyme systems, two major biosynthetic pathways to the normal fatty acids of even carbon numbers C_{14}–C_{18} have been proposed: viz. the acetate route and the malonate route.

The acetate route was the first to be established and represents the reversal of the now-classical β-oxidation process for chain shortening (see Stumpf and Bradbeer, 1959, for a discussion of plant metabolism). Stepwise addition of acetyl coenzyme A to acetyl and other short-chain acyl-CoA derivatives results in chain lengthening, two carbon atoms at a time. In simplified form the process, which has been demonstrated in liver mitochondria by Wakil *et al.* (1961), is said to be as shown in Fig. 7. Thus palmitolyl-CoA gives stearoyl-CoA. The chain length distribution of the acids found in the glyceride pool has been discussed in terms of the relative rates of transfer of the synthesized fatty acids into the pool (Lynen, 1961).

The malonate route for palmitate synthesis, involves an apparently concerted addition of seven malonyl-CoA units to an acyl-CoA starter

("primer"). This is shown in Fig. 7 for acetyl-CoA as starter (Bressler and Waki, 1962). The enzyme systems which effect this malonate pathway are present in many animal (and insect) and plant tissues, where they are found in the cytoplasm and mitochondria respectively. The malonyl-CoA is derived by biotin dependent carboxylation of acetyl-CoA by bicarbonate.

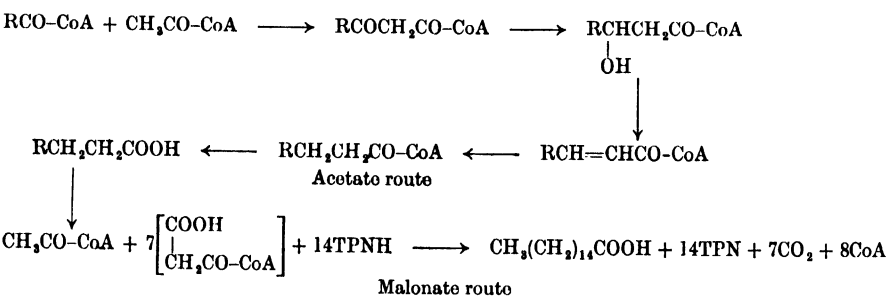


FIG. 7. Biosynthetic pathways to the n-fatty acids.

In the malonate system, the distribution of the fatty acids actually encountered is explained by the relative availability of starter units other than acetyl-CoA. Thus propionyl-CoA would furnish the odd-carbon-number C_{17} acid and n-butyryl-CoA the even-carbon-number C_{18} acid. The lipids of certain bacteria have recently been shown to be made up very largely of branched acids; for example, the odd-carbon-number anteiso acid (I) in *Micrococcus lysodeikticus* (Macfarlane, 1961), and the iso acid (also odd) (II) in *Bacillus subtilis* and *B. natto* (Saito, 1960).

$$CH_3CH_2CH(CH_3)(CH_2)_{10}COOH$$
(I)

$$(CH_3)_2CH(CH_2)_{11}COOH$$
(II)

$$CH_3(CH_2)_7CH{=}CH(CH_2)_nCOOH$$
$n = 7, 9, 11, 13, 15$ and 17.
(III)

Horning and her co-workers (1961) have demonstrated that branched-chain acids of these types can be obtained by providing an enzyme system (derived from rat epididymal adipose tissue) with initiator units other than acetyl-CoA (Table III). These elegant studies have clear implications for branched hydrocarbon biogenesis and are discussed further under Section VI, C.

Little is known of the biogenesis of the longer chain fatty acids (C_{20}–C_{34}) which occur mainly in the plant waxes. It is reasonable to suppose that

either the acetate or the malonate routes (or both), might operate. In either case preformed C_{14}–C_{18} starter units derived from the glyceride pool might take the place of the short-chain acyl-CoA starters. Alternatively, different enzyme specificities would be required to release the acids at the right chain length. A good illustration of what would appear to be incorporation of a long-chain starter unit is provided by the constituent fatty acids of the seed fats of certain *Ximenia* species (Lighthelm *et al.*, 1954). The unsaturated acids of chain length 18–30 carbon atoms are of the general formula (III) and it seems likely that they are derived by successive acetate elongation beginning with oleic acid. The recent work of James (1962a, b) on an excised plant leaf demonstrates that the n-C_8, C_{10} and C_{12} acids can be converted into oleic acid without breakdown.

TABLE III

Biogenesis of n- and branched fatty acids (C_{14}-C_{18}) (Horning *et al.*, 1961)

$$RCO\text{-}CoA + n\begin{bmatrix} COOH \\ | \\ CH_2CO\text{-}CoA \end{bmatrix} \longrightarrow R(CH_2CH_2)_nCOOH$$

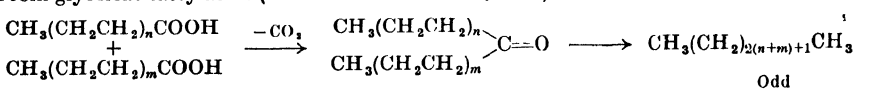
Coenzyme A derivative	R	Acids C_{14}–C_{18} formed
Acetyl	CH_3	n-even
Propionyl	CH_3CH_2-	n-odd
Isobutyryl	$(CH_3)_2CH$-	iso-even
Isovaleryl	$(CH_3)_2CH—CH_2$-	iso-odd
Isocaproyl	$(CH_3)_2CH—CH_2—CH_2$-	iso-even
α-Methylbutyryl	$CH_3CH_2CH(CH_3)$-	anteiso-odd

B. Derivation of the Alkanes

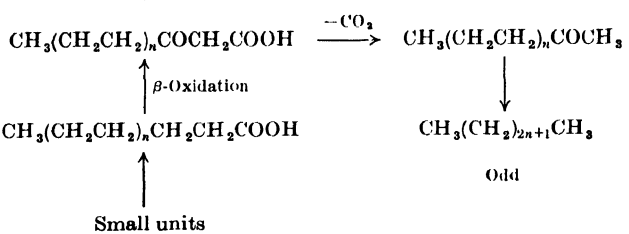
The routes which have been proposed at various times are summarized in Fig. 8. Channon and Chibnall (1929) found it difficult to explain the common occurrence of the C_{29} n-alkane; this compound might result from the coupling of two C_{15} acid units together, but the latter, being

odd-numbered, is present in fats in trace quantities only. Another possible derivation requires the mixed coupling of n-C_{16} and C_{14} acids. However, Gastambide-Odier and Lederer (1959) have shown that *Corynebacterium diphtheriae* will incorporate two complete molecules of

From glyceride fatty acids (Channon and Chibnall, 1929)



β-Oxidation and decarboxylation (Chibnall and Piper, 1934)



Routes involving acyl-CoA units. Route (1) leads to odd carbon number alkanes, and routes (2), (3) and (4) to even number alkanes. (Wanless, King and Ritter, 1955†)

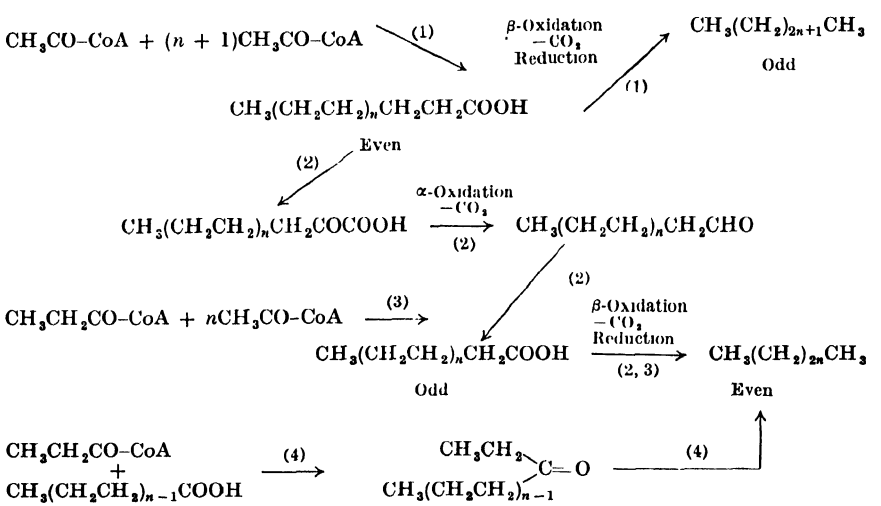


FIG. 8. Biogenesis of long-chain alkanes.

† The acetate pathway is as given by Wanless *et al.*; the chain extension in routes (1) and (3) might conceivably proceed by either the acetate or the malonate pathway (Section VI, B).

palmitic acid into corynomycolic acid. Palmityl coenzyme A is suggested as the intermediate (Fig. 9.)

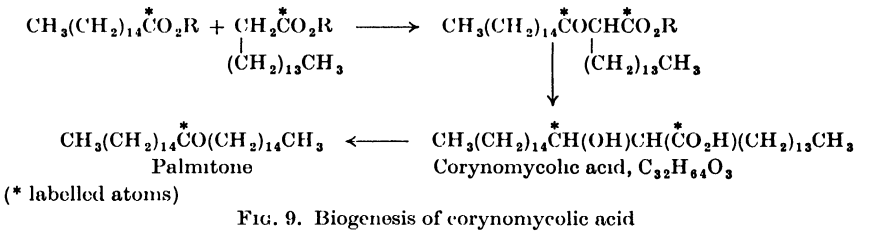


FIG. 9. Biogenesis of corynomycolic acid

The labelling pattern was established by subsequent *in vitro* conversion to palmitone. Palmitone occurs naturally in *C. diphtheriae* lipids and is almost certainly formed from palmitic acid. Thus, the original Channon and Chibnall route is valid for at least one long-chain compound, and, although the alkane fraction was not examined in this case, it may well be that the n-C_{31} hydrocarbon is present.

The β-oxidation step of Chibnall and Piper (1934; Fig. 8) is probably of minor importance in the biogenesis of alkanes, since a suitable β-keto derivative may be formed by the addition of the final malonate unit during normal fatty acid synthesis. Wanless and his colleagues (1955; Fig. 8) suggested three possible routes to the even numbered plant hydrocarbons, based respectively on loss of the carboxyl grouping as a result of α-oxidation after acetate starting (Fig. 8, route 2), β-oxidation after propionate starting (route 3) and formation of a β-keto system after condensation between propionate and long-chain acid units (route 4). These authors discussed several ways in which the observed high proportion of odd-numbered hydrocarbons could be explained.

The work of Horning *et al.* (1961) on the biogenesis of the n- and branched glyceride fatty acids (Section VI, A) would certainly lead one to infer that the corresponding longer chain plant-wax acids are similarly derived. The odd-carbon number iso-alkanes of chain length C_{29}, C_{31}, etc. encountered by Carruthers and Johnstone (1959) and by Eglinton *et al.* (1962a, b), would then be explicable as shown in Fig. 10.

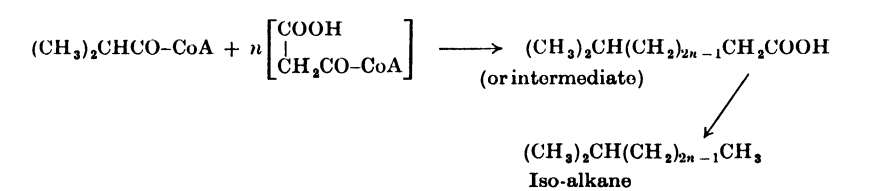


FIG. 10. Biogenesis of iso-alkanes

Similarly, the small proportion of even-numbered n- and branched-alkanes would reflect the low concentrations of the appropriate acyl coenzyme A esters available as initiators for fatty acid synthesis as compared with the concentration of acetyl coenzyme A. Since the acyl portions of these coenzyme A esters are ultimately derived from amino acids some correlation might be sought with the species distribution of the latter. However, it should be pointed out that Birch and his colleagues (1962) have shown that methyl groups can be introduced by formate, probably while the malonate-derived β-polyketone chain still exists.

It might be thought that the chain-length distribution of the constituent acids of plant waxes would parallel that of the alkanes. Few comparative data of this sort are available, but that depicted in Fig. 11 does

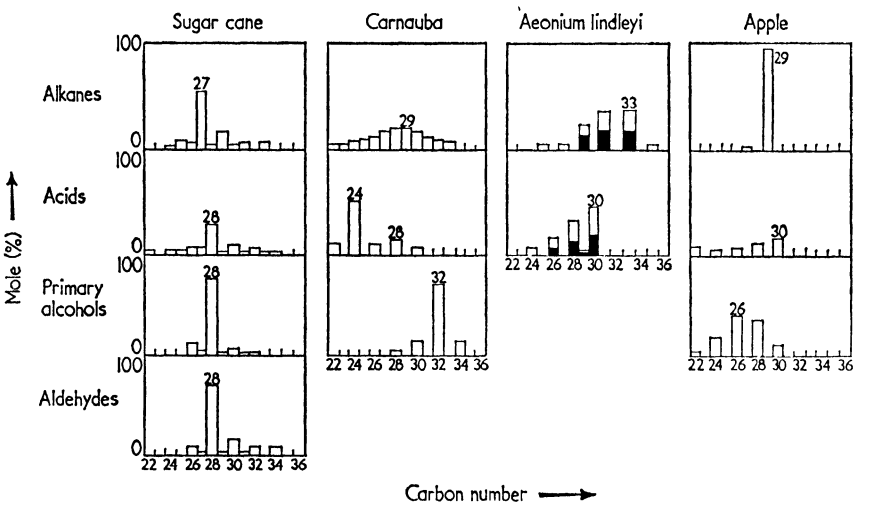
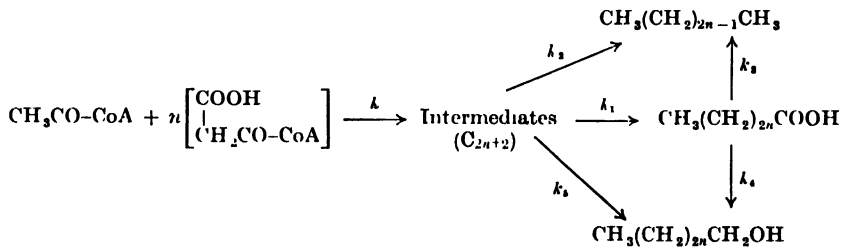


FIG. 11. Carbon-number distribution diagrams for the constituent alkanes, acids, primary alcohols and aldehydes of certain waxes. The waxes are the stem wax of the sugar cane (*Saccharum officinarum*; Kranz *et al.*, 1960), the cuticle wax of the carnauba palm (*Copernicia cerifera*; Mazliak, 1961b), the leaf wax of *Aeonium lindleyi* (Eglinton *et al.*, unpublished; the acids indicated by the black blocks are believed to be iso-acids, though this has not yet been proved), and the cuticle wax of the apple fruit (*Pyrus malus*; Mazliak, 1960b).

not show any consistent relationship other than the expected pattern of alkanes predominantly odd, acids and primary alcohols predominantly even. However, it is significant that *both* the alkane and the acid fractions of the wax of *Aeonium lindleyi* contain normal and branched isomers (though it should be mentioned that the constitution of the branched acids is not yet established). The explanation of the apparently random fluctuation in the chain length distribution when the hydrocarbons, acids

and primary alcohols are compared, must lie in the differing specificity and kinetic control of the individual enzyme systems (cf. Section V, D). The situation might be rather as shown in Fig. 12.



(k, k_1, k_2 . . . rates of the reactions)

FIG. 12. Possible kinetic explanation of the differing distribution patterns (cf. Fig. 11).

Of course, reverse reactions would complicate this too simple picture; even the alkanes can be attacked, as was shown some years ago by Hopkins and Chibnall (1932) who found that fungi could metabolize pure n-alkanes up to C_{35}.

Labelling experiments are clearly necessary at this juncture. However, Birch *et al.* (1962) in remarking on the biogenetic relationship of polyketides and straight-chain fatty acids, state their belief that a fairly closely concerted assembly of the major units must occur, without pools of intermediates. The recently announced (Horn and Lamberton, 1962) β-diketones (e.g. $CH_3(CH_2)_{14}COCH_2CO(CH_2)_{14}CH_3$) would make an interesting start for labelling experiments, provided the same leaf waxes contain alkanes.

C. The Present Situation

It would seem from the foregoing discussion that the biogenesis of the known plant wax long-chain alkanes should fit in with the established acetate–malonate pathway, though the definitive labelling experiments are yet to be made. Indeed, Sanderman and Schweers (1960) have already shown that the n-heptane produced by *Pinus jeffreyi* is formed from four acetate units, the labelling from carboxy-labelled acetic acid being 2, 4, 6 (IV).

Labelling experiments are also in progress with isolated apple cuticle (Mazliak, 1961c) and with insects (Clark and Bloch, 1959; Louloudes *et al.*, 1962). If indeed the long-chain alkanes are derived from, or in parallel with, the long-chain acids, we can confidently expect the alkane fractions from mycobacteria and other micro-organisms to contain branched alkanes analogous to the acids (V–VII) (Lederer, 1960; Miller, 1961;

Kates *et al.*, 1962). Some are clearly derived by methylmalonyl extension. A further point is that terpenoid biogenesis and fatty acid biogenesis are likely to be interdependent now that malonyl-CoA is believed to be an intermediate in the formation of mevalonate (Brodie *et al.*, 1962). At least one saturated alkane with an isoprenoid skeleton is known to occur in

$$CH_3\overset{*}{C}H_2CH_2\overset{*}{C}H_2\overset{*}{C}H_2CH_2CH_3$$
(IV)

$$CH_3(CH_2)_5-\underset{\diagdown\,\diagup}{\overset{CH_2}{}}CH-CH-(CH_2)_9COOH$$
(V)
Lactobacillic acid
(Chalk and Kodicek, 1961)

$$CH_3(CH_2)_{16}[CH_2CH(CH_3)]_2CH=C(CH_3)COOH$$
(VI)
Mycolipenic acid

$$CH_3(CH_2)_{18}[CH_2CH(CH_3)]_4COOH$$
(VII)
Mycocerosic acid
(Asselineau *et al.*, 1959, 1961)

$$H[CH_2CH(CH_3)CH_2CH_2]_3CH_2CH(CH_3)_2$$
(VIII)
Pristane

nature; pristane (VIII) which is found in *Elasmobranch* liver oils (together with the unsaturated hydrocarbons squalene and zamene) has been shown by Sørensen and his co-workers (1949, 1950) to be a C_{19} norditerpane, presumably derived from, or in parallel with, phytol. It is entirely possible that alkane fractions will contain unusual branched hydrocarbons whenever terpenoid and fatty acid biogeneses proceed together. The great variety of straight, branched, unsaturated and cyclic hydrocarbons encountered in the wax of the house fly (Louloudes *et al.*, 1962) will require a major analytical and biochemical effort, but the results are likely to be of considerable interest.

VII. Fossil Alkanes

Alkanes are prominent among the constituents of petroleums, shale oils, bitumens, asphalts, earthwaxes and other fossil fuels. It has even been suggested that the lunar "seas" are composed of asphalt of abiological origin (Wilson, 1962)! In his recent survey, Sir Robert Robinson (1961) has proposed a duplex origin for petroleum—partly abiological

from methane and carbon dioxide, and partly biological. There is more or less universal agreement as to the biological origin of at least part of the oil, in view of the presence of optical activity, porphyrins, naphthenes derived from steroids, triterpenoids, etc. However, much discussion has

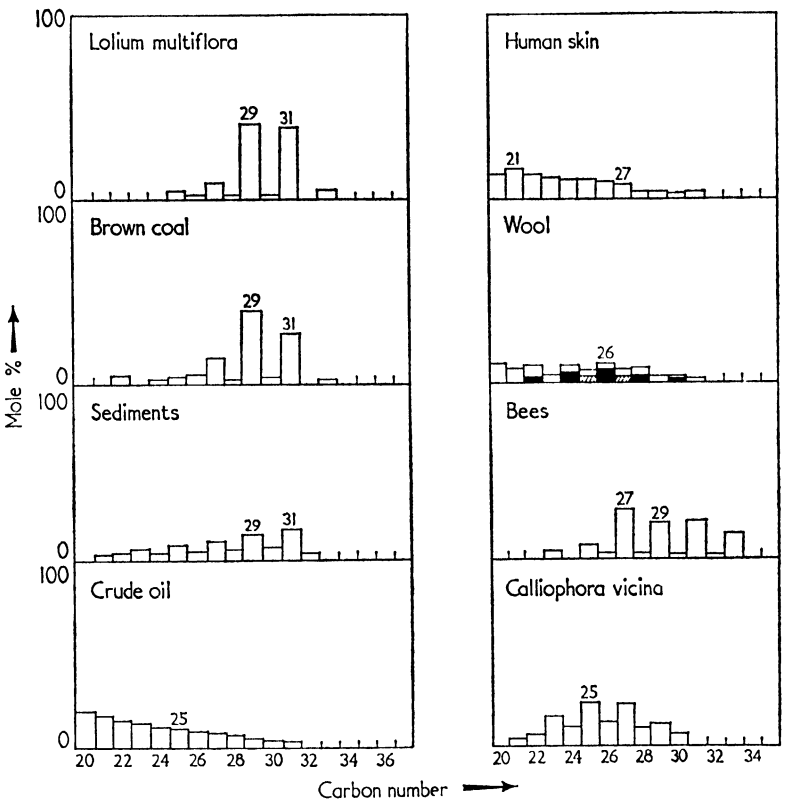


FIG. 13. Carbon-number distribution diagrams (moles-%) for the alkane fractions derived from a variety of natural sources. The sources are the leaf wax of *Lolium multiflora* (Italian rye grass, Eglinton *et al.*, 1962b), the wax extracted from brown coal (Montan wax, Wollrab *et al.*, 1962), recent marine sediments and crude oil (Baker, 1960), human skin wax (Haahti, 1961), wool wax (Downing *et al.*, 1960), bees' wax (*Apis mellifica*, Downing *et al.*, 1961) and the n-alkane content of the lipids extracted from the fly, *Calliophora vicina* (Louloudes *et al.*, 1962).

centred round the smooth distribution of n-alkanes typical of nearly all crude oils which is in sharp contrast to the very high odd-even ratio found for most alkane mixtures from biological sources (Fig. 13). Marine organisms such as phytoplankton—would be the likely biological source material though little is known of their chemical constitution (Whitmore,

1943; Oakwood, 1944; cf. Robinson, 1961) or of their breakdown. But it is unlikely that the hydrocarbons released would have the crude oil pattern and current work (e.g. Bray and Evans, 1961; Baker, 1960; Meinschein, 1961) has revealed that Recent marine sediments (Ages, $2–20 \times 10^3$ years) contain alkane fractions with a marked odd-even alternation strongly peaked at C_{29} and C_{31}, as in most plants (Fig. 13). This alternation becomes less and less pronounced with increasing age of the sediments and the sedimentary oil-bearing rocks (Hunt, 1961). Preferential biological attack (Cooper, 1962), and preferential micelle formation followed by migration (Baker, 1960) have been invoked to explain this gradual transition from the "biological" alkane pattern of Recent sediments to the crude oil pattern.

In conclusion one might mention firstly the recent work (Wollrab *et al.*, 1962) on the constituents of montan wax from brown coal, where, as one might expect, the distribution patterns (Fig. 13) are quite typically those of plants, and secondly, the work on the alkane fraction derived from a fossil tree cuticle (about $1{\cdot}5 \times 10^8$ years) which contains an unusually wide spread of chain lengths (Eglinton *et al.*, unpublished). Most interestingly, Nagy, Meinschein and Hennessy (1961) have claimed "that the hydrocarbons in the Orgueil meteroite resemble . . . the hydrocarbons in the products of living things and sediments on earth". They go on to suggest that "Based on these preliminary studies it appears that biogenic processes occur and that living forms exist in regions of the Universe beyond the earth".

VIII. Conclusions

Our knowledge of the distribution of alkanes in plants is likely to increase rapidly in the near future as a result of the application of physical analytical methods. Branched and cyclic alkanes related to already known acids and alcohols will almost certainly be encountered. Cuticular and surface leaf waxes as a whole merit particular attention since it is probable that the various long-chain alkanes, acids, alcohols and other oxygenated constituents are inter-related biogenetically. Taxonomic applications of the quantitative distribution patterns of the leaf wax constituents and, in particular, of the very readily examined alkane fractions, will require an approach firmly based on statistical and biogenetical grounds.

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CHAPTER 9

Chemical Taxonomy of Acetylenic Compounds

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I. Introduction

Acetylenic derivatives belong to the so-called secondary plant products. Very little is known about their function. In some perennials qualitative and quantitative changes have been observed during the season, and so obviously these substances are not irreversible end products of metabolism. However, when assessing the taxonomical usefulness of the occurrence of secondary components it is not important (a) whether they are useful to the plant or not; (b) whether they are intermediates in the biosynthesis of primary metabolites and are thus usually not detectable in plants; or (c) whether they are the result of unavoidable side reactions of normal metabolism. What is of importance is (i) that their distribution is not too widespread; (ii) that there is some correlation between their distribution and the botanical classification; and (iii) that their occurrence is as independent as possible of the two main disturbing factors in classical morphology: convergence and divergence.

In this chapter acetylenic compounds are considered from these points of view. To some extent they appear to fulfil our definition of useful compounds for chemotaxonomical purposes, but it will be obvious that the chemical investigations so far carried out are very unsatisfactory for this

purpose, and that the number of new acetylenic compounds discovered each year is growing so rapidly that continual revision may have to be made. Our present knowledge of acetylenic compounds is probably sufficient to examine taxonomic problems at the level of genus, section or species; but is far too incomplete for problems concerning higher groups in the classification system.

II. Acetylenic Fatty Acids

In 1892 Arnaud isolated tariric acid from seeds of the Genus *Picramnia* Swartz (synonymous with Genus *Tariri* Aublet) (Arnaud, 1892). The constitution of this acid as 6-octadecynoic acid (Table 1) was proved ten years later (Arnaud, 1902). Tariric acid was subsequently isolated from *P. carpinterae* Polak., *P. lindeniana* Tul. and *P. camboita* Engl. (Grimme, 1910, 1912; Steger and van Loon, 1933). *Picramnia* is placed in Simarubaceae, and although no extensive investigation has been carried out of the glycerides of this family, about half a dozen other fats from trees which have been examined have been shown to contain only normal fatty acids (Hilditch, 1956; Eckey, 1954) and not acetylenic derivatives. So far tariric acid has not been found in any other plant.

The occurrence of fats peculiar to a genus rather than to the family is met again in Olacaceae. Here again the glycerides of some seed fats investigated (*Coula edulis* Baillon, *Mappia foetida*, *Agonandra brasiliensis*) are composed of normal saturated and ethenoid fatty acids, whereas the genera *Ximenia* and *Ongokea* contain very unusual seed fats. (Present-day botanists place *Mappia* in the Icacinaceae and *Agonandra* in the Opiliaceae, and so the conclusions drawn by Hilditch (1956) and Eckey (1954) may be premature.)

No less than four of the acids in Table I have been isolated from the seed fat (Boleka oil) of *Ongokea klaineana* Pierre. The constitution of one or two of them have not been finally established, the formulas given being only proposals, but it is quite certain that Boleka oil contains at least this number. Glycerides from other parts of the tree do not seem to have been investigated.

Ripe Ongokea nuts are yellow, but if not properly stored they turn dark. The unsaponifiable part of the oil then increases from the normal value of 1·1–1·5% to above 10%. Castille (1939) showed that this increase is due to the formation of deca-1,3-diynes and decadiynenes. These compounds most probably originate from the hydroxy-acids, but so far it is not known if the process is autolytic, or if it is due to the action of micro-organisms. These changes need to be further studied in connection with the biogenesis of the acetylenes in fungi and the Compositae.

TABLE I

C_{18} Acetylenic fatty acids

Structure	Genus first isolated	Family	References
$H_3C-(CH_2)_{10}-C\equiv C-(CH_2)_4-COOH$	*Picramnia*	Simarubaceae	Arnaud (1892; 1902)
$H_2C=CH-(CH_2)_4-C\equiv C-C\equiv C-(CH_2)_7-COOH$	*Ongokea*	Olacaceae	Castille (1939); Meade (1951)
$H_2C=CH-(CH_2)_2-CH=CH-C\equiv C-C\equiv C-(CH_2)_7-COOH$	*Ongokea*	Olacaceae	Castille (1939); Meade (1951)
$H_2C=CH-(CH_2)_4-C\equiv C-C\equiv C-CHOH-(CH_2)_6-COOH$ (?)	*Ongokea*	Olacaceae	Kaufmann *et al.* (1937); Riley (1951)
$H_2C=CH-(CH_2)_2-CH=CH-C\equiv C-C\equiv C-CHOH-(CH_2)_6-COOH$ (?)	*Ongokea*	Olacaceae	Kaufmann *et al.* (1937): Riley (1951)
$H_3C-(CH_2)_5-CH=CH-C\equiv C-CHOH-(CH_2)_6-COOH$	*Ximenia*	Olacaceae	Lighthelm (1954)
$H_3C-(CH_2)_5-CH=CH-C\equiv C-(CH_2)_7-COOH$	*Ximenia* *Santalum*	Olacaceae Santalaceae	Lighthelm *et al.* (1952); Hatt and Szumer (1954); Gunstone and McGee (1954)
$H_3C-(CH_2)_3-CH=CH-C\equiv C-C\equiv C-(CH_2)_7-COOH$	*Exocarpus*	Santalaceae	Hatt *et al.* (1959)
$H_3C-(CH_2)_3-CH=CH-CH=CH-C\equiv C-(CH_2)_7-COOH$	*Eucarya*	Santalaceae	Hatt and Szumer (1954); Hatt and Schoenfeld (1954)
$H_3C-CH_2-CH=CH-CH=CH-C\equiv C-C\equiv C-(CH_2)_7-COOH$ (?)	*Leptomeria*	Santalaceae	Hatt *et al.* (1960)
$H_3C-CH_2-CH=CH-C\equiv C-C\equiv C-C\equiv C-(CH_2)_7-COOH$ (?)	*Leptomeria*	Santalaceae	Wailes *et al.* (unpublished)

The genus *Ximenia* has given its name to ximenynic acid, octadec-11-en-9-ynoic acid. It was first isolated by Lighthelm *et al.* (1952) from the seed fat of *X. americana* L., and has subsequently been shown to be present in *X. caffra* Sond., *X. caffra natalensis* and *X. americana microphylla* (Lighthelm *et al.*, 1954). The glycerides present in the seeds of these plants also contain unusual ethenoic acids, viz. the C_{26} acid *cis*-hexacos-17-enoic acid, and the C_{30} acid *cis*-triacont-21-enoic acid, which have not been isolated from other vegetable oils.

Hatt *et al.* (1960) recently investigated the glycerides from the bark and xylem of the roots of *X. americana* L. and found they contained octadeca-11,13-dien-9-ynoic and octadeca-13-ene-9,11-diynoic acid. Hatt and his collaborators (1959) had earlier demonstrated that in plants belonging to the family Santalaceae the glycerides regularly increased in unsaturation from the seed fat through those of the other aerial parts to the lipids of root and root bark.

The main fatty acid of *Santalum album* was originally described (Madhuranath and Manjunath, 1938) as a trienic acid; in 1954 it was shown to be identical with ximenynic acid (Gunstone and McGee, 1954; Hatt and Szumer, 1954; Hatt and Schoenfeld, 1954).

From a chemotaxonomic point of view the contributions from the Australian group has yielded a number of interesting facts. Acetylenic acids have been found in all members of the Santalaceae so far investigated (about seventeen species). The genera *Exocarpus* Labill., *Omphacomeria* D. C. and *Anthobolus* R. Br., which botanists since the days of R. Brown have united in the section Anthobolearum (Brown, 1810; Stauffer, 1959) are nearly identical as regards the constitution of their glycerides. In ripe seeds ximenynic acid is the only acetylenic acid present; in the stem and stem bark the octadeca-11,13-dien-9-ynoic acid is readily recognizable; and the root glycerides are characterized by the presence of octadeca-13-ene-9,11-diynoic acid. In the genus *Exocarpus* no less than six species have been investigated (*E. cupressiformis* Bill., *E. strictus* R. Br., *E. sparteus* R. Br., *E. aphyllus* R. Br., *E. humifusus* R. Br., and *E. nanus* Hook.), and the picture obtained is also very uniform (Wailes *et al.*, 1960; Wailes, private communication).

In other members of the Santalaceae such as *Comandra richardsiana* Fernald, *Leptomeria aphylla* R. Br., *L. billardieri* R. Br., and *Thesium australe* R. Br., and in the members of Santalum in a wider sense which have been investigated (*Eucarya acuminata* (D.C.) Spr. et Summ., *E. murrayana* (F. v. M.) Spr. et Summ., *E. spicata* (R. Br.) Spr. et Summ., *Santalum lanceolatum* R. Br., *S. freycinetianum* Gaudich.) the picture varies a little more and diene-diynic and ene-triynic acids have been added to the series. It is important to note that whereas most natural ethenoid

fatty acids appear to have a *cis*-configuration, all the enynic fatty acids isolated so far have double bonds with a *trans*-configuration. Another interesting observation by the Australian group is that the glycerides of unripe seeds are more unsaturated than those of the ripe ones.

The Australian group has further demonstrated the presence of acetylenes in plants belonging to other families of the order Santalales such as *Opilia* spp. (Opiliaceae), *Nuytsia floribunda* (Labill.) R. Br., and *Viscum album* (Loranthaceae). But as in the Olacaceae, the occurrence of acetylenic acids does not seem to be a regular characteristic of all these families as it is in the Santalaceae. Plants belonging to the Santalales have been shown to exist from late cretaceous times, so this order is old and morphologically rather conservative. The occurrence of acetylenic fatty acids, therefore, should be a good chemotaxonomic character.

The Australian research on the Santalales very clearly demonstrates that investigations of the families Simarubaceae and Olacaceae are clearly required before any general conclusions can be drawn.

III. Acetylenic Compounds from Micro-organisms

About 1950 a group at New York Botanical Garden (Kavanagh *et al.*, 1950; Anchel *et al.*, 1950) recognized that certain antibiotic substances produced by particular moulds were characterized by having a u.v. spectra showing fine structure. These antibiotics were first supposed to be polyenes, but comparison with polyacetylenes from Compositae tentatively established their acetylenic structure.

The isolation of the antibiotic mycomycin from the Actinomycete *Nocardia acidophilus* and rapid elucidation of its structure (C_{13}, Table II) focused attention on this field, and development has been extremely rapid during the last ten years especially due to the work of Professor E. R. H. Jones and his groups in Manchester and Oxford (cf. Jones, 1959) and by Dr. Marjori Anchel in New York (Anchel, 1953, 1955, 1959).

The acetylenic compounds which have been isolated are shown in Table II. Before briefly discussing some of them, I think a few peculiarities should be remembered. As mentioned above, the first members of this series were found by screening moulds for antibiotics. Moulds which did not show antibiotic action were, as usual, not investigated at all. Further, most of these antibiotics are excreted into the culture medium; the mycelium itself apparently often contains very little acetylenes. Only in one single case in higher plants, Tagetes, has an investigation been carried out concerning excretory products. However, although the constitutional formulae demonstrates a very close similarity (in one case identity) of the acetylenes from Basidiomycetes and Compositae, we should not

TABLE II

Acetylenic compounds isolated from micro-organisms

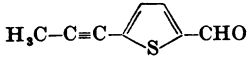
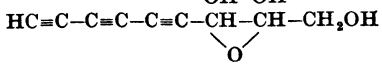
Number of C atoms	Structure	Organism	Reference
8	$HOOC-CH=CH-C\equiv C-CH=CH-COOH$	*Polyporus anthracophilus*	Bu'Lock *et al.* (1957); Gardner *et al.* (1960); Jones *et al.* (1960)
	$NH_2CO-C\equiv C-C\equiv C-CH=CH-CH_2OH$	*Agrocybe dura*	Ashworth *et al.* (1958)
	$NH_2CO-C\equiv C-C\equiv C-C\equiv C-CH_2OH$	*A. dura*	Ashworth *et al.* (1958)
	$NH_2CO-C\equiv C-C\equiv C-CH=CH-COOH$	*Clitocybe diatreta*	Anchel (1953)
	$CN-C\equiv C-C\equiv C-CH=CH-COOH$	*C. diatreta*	Anchel (1955); Ashworth *et al.* (1958)
		*Daedalea juniperina*	Birkinshaw and Chaplen (1955)
9	$HC\equiv C-C\equiv C-CH=C=CH-CH_2CH_2OH$	*Marasmius* spp.	Bendz, G. (1959)
	$HC\equiv C-C\equiv C-C\equiv C-CH=CH-CH_2OH$	*Coprinus quadrifidus*	Jones and Stephenson (1959)
	$HC\equiv C-C\equiv C-C\equiv C-CH(OH)-CH(OH)-CH_2OH$	*C. quadrifidus*	Jones and Stephenson (1959)
		*C. quadrifidus*	Jones and Stephenson (1959)
	$HC\equiv C-C\equiv C-C\equiv C-CH=CH-CHO$	*C. quadrifidus*	Jones and Stephenson (1959)
	$HC\equiv C-C\equiv C-CH=CH-CH_2-CH_2-COOH$ *cis*	*Drosophila subatrata*	Gardner *et al.* (1960); Jones *et al.* (1960)
	$HC\equiv C-C\equiv C-C\equiv C-CH=CH-COOH$	*Psilocybe sarcocephala*	Jones (1959)
10	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH_2OH$ *cis, trans*	*Leptoporus kymantodes* *Polyporus guttulatus*	Gardner *et al.* (1960); Jones *et al.* (1960)
	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH_2OH$ all *trans*	*P. anthracophilus*	Bu'Lock *et al.* (1957)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH_2OH$	*Pleurotus ulmarius*	Gardner *et al.* (1960); Jones *et al.* (1960)
	$HOH_2C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH_2OH$	*Coprinus quadrifidus*	Jones and Stephenson (1959)

TABLE II—*cont.*

Number of C atoms	Structure	Organism	Reference
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CHO$	*Pleurotus ulmarius*	Gardner *et al.* (1960); Jones *et al.* (1960)
	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-COOH$ (Me)	*Polyporus anthracophilus*	Bu'Lock (1957)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-COOH$	*Pleurotus ulmarius*	Gardner *et al.* (1960)
	(Me)HOOC$-CH=CH-C\equiv C-C\equiv C-CH_2-CH_2-COOH$ (Me) *trans*	*Merulius lachrymans* *Polyporus anthracophilus*	Gardner *et al.* (1960); Bu'Lock (1957)
	$HOOC-C\equiv C-C\equiv C-C\equiv C-CH_2-CH_2-COOH$	*Merulius lachrymans* *Polyporus anthracophilus*	Gardner *et* al. (1960); Bu'Lock (1957)
	(Me)HOOC$-CH=CH-C\equiv C-C\equiv C-CH=CH-COOH$ 8 *trans*	*P. anthracophilus*	Bu'Lock *et al.* (1957)
	(Me)HOOC$-CH=CH-C\equiv C-C\equiv C-CH_2CH_2-CH_2OH$ 8 *trans*	*P. anthracophilus*	Bu'Lock *et al.* (1957)
	$HOOC-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH_2OH$ 8 *trans*	*Pleurotus ulmarius* *Tricholoma paneolum*	Gardner *et al.* (1960); Jones *et al.* (1960)
11	$H_2C=C=CH-C\equiv C-C\equiv C-CH=CH-CH_2-COOH$	*Drosophila subatrata*	Gardner *et al.* (1960); Jones *et al.* (1960)
	$HC\equiv C-CH_2-C\equiv C-C\equiv C-CH=CH-CH_2-COOH$	*D. subatrata*	Gardner *et al.* (1960); Jones *et al.* (1960)
	$HC\equiv C-C\equiv C-CH=C=CH-CH(OH)-CH_2-CH_2-COOH$	*Poria tenuis* *P. corticola*	Bu'Lock *et al.* (1955, 1957)
	$HC\equiv C-C\equiv C-CH=C=CH-CH-CH_2-CH_2-CO$ (O)	*P. tenuis* *P. corticola*	Bu'Lock *et al.* (1955, 1957)
12	$H_3C-C\equiv C-C\equiv C-CH=C=CH-CH(OH)-CH_2-CH_2-COOH$	*P. tenuis* *P. corticola*	Bu'Lock *et al.* (1955, 1957)
	$H_3C-C\equiv C-C\equiv C-CH=C=CH-CH-CH_2-CH_2CO$ (O)	*P. tenuis* *P. corticola*	Bu'Lock *et al.* (1955, 1957)
13	$HC\equiv C-C\equiv C-CH=C=CH-CH\equiv CH-CH=CH-CH_2COOH$ 3 *trans* 5, *cis*	*Nocardia acidophilus*	Celmer and Solomons (1952, 1953)
14	$HOOC-C\equiv C-C\equiv C-C\equiv C-CH_2-CH=CH-CH_2-CH_2-CH_2-COOH$ *cis*	*Poria sinuosa*	Jones (1960)

forget that they are really completely different types of metabolic product in the two taxa.

When Jones and his co-workers started their broad investigations into acetylenes from fungi they changed the screening technique from an antibiotic test to one of determining the u.v. spectra of culture fluids. As Jones has pointed out, this mainly limits the recognition of the presence of acetylenes to ene-diynes and longer chromophores. Since in the acetylenes of the Basidiomycetes, interruption of the chromophores is rather common there is a great danger that a number of acetylenic metabolites may have been overlooked.

It is well known that the production of metabolites by fungi depends widely on the composition of the culture medium. Very little is known as to what extent fungi which have been shown to produce acetylenes, will do so on all media which promote their development, nor if some of the numerous cases where no acetylenes have been recognized could these observations have been reversed by suitable changes in culture medium.

The isolation of two thiophene derivatives from *Daedelia juniperina* (Birkinshaw and Chaplen, 1955) is the only case where fungal acetylenes have been shown to contain a ring structure. Only the main product, junipal, has had its constitution completely determined (C_8, Table II). Jones has stated that the formation of junipal ceases on a growth medium low in sulphur. Instead some new aliphatic polyacetylenes are excreted.

Due to restrictions placed on editors of chemical journals almost all knowledge is lacking about the numerous species of fungi which do not contain acetylenes. What we are able to do from a chemotaxonomic point of view is only to see if the known acetylene-producers fall into certain sections, families (or sub-families) of the Basidiomycetes or not.

The Actinomycetes are today classed together with Streptomyces and Mycobacteria. Acetylene-dicarboxylic acid diamide has been isolated from *Streptomyces chibaensis* and *S. reticuli aquamyceticus* by Suzuki *et al.* (1958); 10-undecynoic acid from the yeast *Rhodotorula glutinis lusitanica* by Prista (1954). With these exceptions acetylenes do not seem to be known from micro-organisms outside the Basidiomycetes.

In the case of the Basidiomycetes we can briefly conclude:

1. All the acetylenes present are either straight-chain aliphatic compounds, or lactones or thiophenes obviously formed from straight chain precursors.

2. Carbon chain are odd or even and vary from C_8 to C_{14}.

3. Comparison of the formulae strongly indicates that ω-oxidation of methyl groups takes place frequently, and that shortening of chains follows by decarboxylation. Gardner *et al.* (1961) have demonstrated the

presence of the decarboxylation stage by adding a synthetic acetylenic carboxylic acid to cell free extracts of *Coprinus quadrifidus*.

4. The chromophores mostly contain one or more double bonds. The configuration at the double bond is *trans* with only four exceptions.

5. Hydrocarbons have so far not been isolated, but alcohols, glycols, epoxides, aldehydes, acids (with lactones and esters), amides and nitriles are all represented. Where bifunctional acetylenes appear (as in *Polyporus anthracophilus*) the ester mixture is very complicated. Only the acetylenic alcohols and acids are given in Table II, not the mixed esters.

In Fig. 1 the species of the Basidiomycetes which synthesize acetylenes are shown in one of the numerous systems used in sub-division of this group of fungi. It does not matter very much if other systems had been

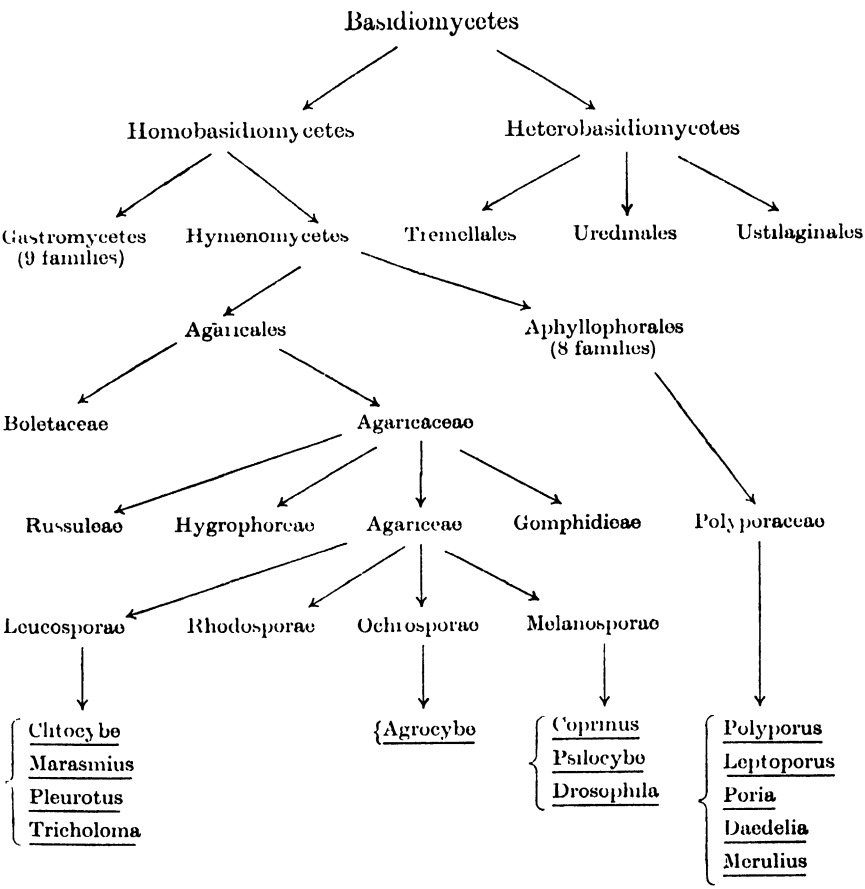


FIG. 1.

chosen. The acetylene-synthesizing species are clearly seen to be restricted to two families Agaricaceae and Polyporaceae belonging to two different orders of this very large group of fungi. It is obvious that a number of species where no acetylenes have been found belong to these same two families. Nothing is known, however, as to what extent all the other orders and families in this group have been covered by the thousand or so species investigated. It seems very desirable to obtain this information before any firm conclusions are made.

IV. Acetylenic Compounds from Dicotyledons

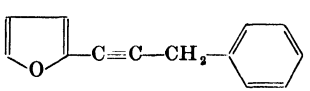
A. Compositae

The identification of polyacetylenes from Compositae had a very slow start. More than 130 years ago the crystals of dehydromatricaria ester (I) were observed in the essential oil from the root of *Artemisia vulgaris* L. (Bretz and Elieson, 1826), but its constitution remained unknown until 1950 (Stavholt and Sørensen, 1950).

$CH_3—C\equiv C—C\equiv C—C\equiv C—CH=CH—COOCH_3$ 2-*cis*

(I)

Dehydromatricaria ester



(II)

Carlina oxide

$R—C\equiv C—C\equiv C—CH=CH—COOCH_3$ 2-*cis*

(IIIa) R = $CH_3—CH_2—CH_2—$

(IIIb) R = $CH_3—CH=CH—$ (*cis*)

(IIIc) R = $CH_3—C\equiv C—$

Semmler (1889 and 1909) obtained carlina oxide (II) from the root of the carline thistle (*Carlina acaulis* L.) in a pure state in 1889, and in 1909 restricted the possible structural formulae to three, two containing acetylenic linkages and the other allenic links. Due to prejudice against the possibility of the occurrence of acetylenic compounds in Nature, Semmler selected the allenic structure and we had to wait to the publications of Gilman *et al.* (1933) and Pfau *et al.* (1935) before the correct formula (II) was presented.

TABLE III

Acetylenic hydrocarbons from Compositae

Number of C atoms	Constitution	Genus	Tribe	References
13	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH-CH=CH_2$	*Coreopsis*	V	Sørensen and Sørensen (1954a)
	$H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH_2$	*Coreopsis*	V	Sørensen and Sørensen (1958a)
		Centaurea	XI	Bohlmann *et al.* (1958)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-CH=CH_2$ *all trans*	*Achillea, Anthemis, Artemisia, Chrysanthemum*	VII	Bohlmann *et al.* (1962b)
		Bidens, Coreopsis	V	Sørensen and Sørensen (1954b)
	$H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-C\equiv C-CH=CH_2$ *cis trans*	*Arnica*	VIII	
		Carthamus, Centaurea, Cnicus Crupina, Serratula, Silybium	XI	
		Blumea, Cassinia, Gnaphalium Helipterum, Pulicaria	IV	Sørensen (1954d)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-C\equiv C-C\equiv C-CH=CH_2$	*Sanvitalia, Synedrella, Xanthium*	V	Sørensen (1954d)
		Arnica	VIII	
		Calendula, Cynara	IX	
16	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-(CH_2)_3-CH=CH_2$ *all trans*	*Chrysanthemum*	VII	Ve (unpublished)
17	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH-(CH_2)_4-CH=CH_2$	*Artemisia*	VII	Bohlmann *et al.* (1958)
		Centaurea	XI	
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-(CH_2)_6-CH=CH_2$	*Artemisia*	VII	Bohlmann *et al.* (1960b)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-(CH_2)_4-CH=CH_2$ *all trans, 7-cis*	*Artemisia*	VII	Bohlmann *et al.* (1957a, b)
		Centaurea	XI	

TABLE IV

Acetylenic alcohols from Compositae

Number of C atoms	Constitution	Genus	Tribe	References
10	$H_3C-CH=CH-C\equiv C-CH=CH-CH=CH-CH_2-O-COCH_3$ 4-*cis*	*Brachycome*	III	Sørensen (1961)
	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH_2-O-COCH_3$ 2-*trans*, 8-*trans* 2-*trans*, 8-*cis*	*Aster, Calotis, Grindelia, Xanthisma*	III	Holme and Sørensen (1954); Sunde (unpublished)
13	$H_3C-C\equiv C-C\equiv C-CH=CH-CH=CH-CH=CH-CH_2-CH_2-O-COCH_3$ 5-*cis*	*Matricaria*	VII	Christensen (1959)
	$H_2C=CH-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH_2-O-COCH_3$ 10-*cis*	*Carlina*	XI	Sørensen and Sørensen (1954c)
	$H_2C=CH-C\equiv C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH_2-O-COCH_3$ *trans*	*Bidens, Coreopsis, Leptosyne*	V	Sørensen (1960); Bohlmann *et al.* (1962a)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-CH_2-CH_2-O-COCH_3$ *trans*	*Chrysanthemum*	VII	Bohlmann *et al.* (1961d); Ve (unpublished)
14	$H_3C-CH_2-CH=CH-CH=CH-CH=CH-CH=CH-(CH_2)_3-CH_2-O-COCH_3$	*Centaurea*	XI	Bohlmann *et al.* (1961c)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-(CH_2)_2-CH_2-O-COCH_3$	*Chrysanthemum, Artemisia*	VII	Bohlmann *et al.* (1960b) Bohlmann *et al.* (1962c)

TABLE V

Acetylenic glycols and chlorhydrins from Compositae

Number of C atoms	Constitution	Genus	Tribe	References
13	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH-\underset{(AcO)HO}{CH}-\underset{OH(OAc)}{CH_2}$	*Centaurea*	XI	Bohlmann *et al.* (1961c)
	$H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-\underset{(OAc)OH}{CH}-\underset{OH(OAc)}{CH_2}$	*Centaurea*	XI	Bohlmann *et al.* (1961c)
15	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-CH_2-\underset{AcO}{CH}-CH_2-\underset{OAc}{CH_2}$	*Centaurea*	XI	Bohlmann *et al.* (1958)
	$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH-CH_2-\underset{AcO}{CH}-CH_2-\underset{OAc}{CH_2}$	*Centaurea*	XI	Bohlmann *et al.* (1958)
13	$H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-\underset{Cl}{CH}-\underset{OH(OAc)}{CH_2}$	*Centaurea*	XI	Bohlmann *et al.* (1958)
	$H_3C-C\equiv C-C\equiv C-C\equiv C-C\equiv C-CH=CH-\underset{Cl}{CH}-\underset{OH(OAc)}{CH_2}$	*Centaurea*	XI	Bohlmann *et al.* (1961c)

TABLE VI

Acetylenic aldehydes, ketones and epoxides from Compositae

Number of C atoms		Genus	Tribe	References
13	$H_2C{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CHO$	*Bidens, Leptosyne*	V	Bohlmann *et al.* (1962a)
14	$H_3C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH_2{-}CH_2{-}CO{-}CH_2CH_3$ *trans*	*Artemisia, Calotis, Chrysanthemum, Matricaria*	VII	Stavholt and Sørensen (1950); Bohlmann *et al.* (1955)
17	$H_2C{=}CH{-}(CH_2)_5{-}CH{=}CH{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}CO{-}CH{=}CH_2$ *cis*	*Galinsoga, Helianthus, Iva*	V	Bohlmann *et al.* (1962b)
		Lagascea, Tithonia, Tridax	V	
		Artemisia, Cotula	VII	
		Eriocephalus	VII	
	$H_3C{-}(CH_2)_6{-}CH{=}CH{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}CO{-}CH{=}CH_2$ *cis*	*Galinsoga*	V	Bohlmann *et al.*(1961b); Bohlmann and Bernowski (1961)
13	$H_3C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{-}CH{-}CH{=}CH_2$ (O)	*Achillea, Artemisia*	VII	Bohlmann *et al.* (1960b)
		Chrysanthemum, Cladanthus	VII	Sørensen *et al.* (1961)
	$H_3C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{-}CH{-}CH{=}CH{-}CH{=}CH_2$ (O)	*Anthemis*	VII	Bohlmann *et al.* (1962b)
	$H_3C{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{-}CH_2$ (O)	*Centaurea*	XI	Bohlmann *et al.* (1961c)
	$H_3C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{-}CH_2$ (O)	*Centaurea*	XI	Bohlmann *et al.* (1961c)

It is quite obvious from the experimental data given in his paper that Carthaus (1907) had rather large amounts of a pure diacetylenic compound. The source of this compound named "*Artemisia lavandulaefolia*" is, however, a *nomen absurdum*, and all efforts to trace the actual plant Carthaus used has been in vain; the probability that the plant was a member of the Compositae, however, seems overwhelming.

The three Russian scientists Viljams, Smirnov and Goljmov (1935) described the isolation of methyl-dec-*cis*-2-ene-4,6-diynoate (IIIa) from the Aserbeidschan plant *Lachnophyllum gossypinum* Bge. and determined both its constitution and configuration. This remarkable contribution, however, did not attract any interest at the time.

Due to the war our researches in Trondheim were directed to the closely related acetylenic esters (IIIb) and (IIIc), and since then this field has undergone an explosive development especially due to the very extensive investigations of Bohlmann and his collaborators. A compilation of our knowledge of the polyacetylenes of the Compositae (and of derivatives obviously derived from polyacetylenes) is given in Tables III to XII. The number of established compounds is about 85.

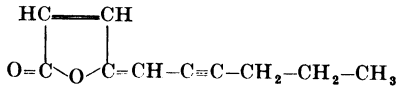
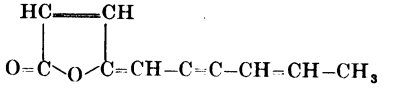
Before discussing the Compositae acetylenes from a taxonomical point of view I would like to make a few general remarks.

As very often with natural products the first compounds of this class isolated (carlina oxide, lachnophyllum- and matricaria ester) were found accidentally purely because they happened to be present in reasonable amounts and were easily purified. Since the aliphatic polyacetylenes showed very characteristic u.v. spectra, the hunt for further members, as with the Basidiomycetes, was led by application of this technique. Although carlina oxide, which has an indistinct u.v. maxima at short wavelength, should have given a warning, the number of new acetylenes which were detected by the aid of u.v. spectroscopy was great enough for this to be ignored. This one-sided development was only corrected when another plant happened to yield substantial amounts of a compound with a broad-banded indistinct u.v. spectrum, which could only be proved to be acetylene after infra-red spectroscopy and a full study of its constitution.

Most of the aromatic compounds in Tables VIII and IX and the heterocycles in Tables X–XII have broad and indistinct u.v. spectra, but at least they have strong chromophores. The greatest danger is (as in the research on fungi)—that compounds containing isolated acetylenic bonds or short chromophores such as ene-ynes may very easily have been overlooked. The task to prove that such acetylenes are absent, is obviously much more difficult than the isolation and structural elucidation of a compound possessing a strong chromophore.

TABLE VII

Acetylenic carboxylic acid derivatives from Compositae

Number of C atoms	Constitution	Genus	Tribe	References
	Methylesters			
10	$H_3C-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-COOCH_3$ 2-*cis*, 2-*trans*	*Lachnophyllum, Erigeron, Bellis, Calotis*	III	Viljams *et al.* (1935); Holme and Sørensen (1954)
	$H_3C-CH=CH-C\equiv C-C\equiv C-CH_2-CH_2-COOCH_3$ 8-*cis*	*Amellus*	III	Stavholt-Baalsrud *et al.* (1952)
		Tripleurospermum	VII	
	2-*cis*, 8-*cis* $H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-COOCH_3$	*Calotis, Erigeron*	III	Sørensen and Stene (1941)
		Tripleurospermum	VII	
	2-*trans*, 8-*cis*	*Amellus*	III	Stavholt-Baalsrud *et al.* (1952)
		Tripleurospermum	VII	
	2-*cis* $H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-COOCH_3$ 2-*trans*	*Achillea, Anthemis, Artemisia, Chrysanthemum*	VII	Stavholt and Sørensen (1950)
		Calotis	III	
		Achillea, Cotula, Tripleurospermum	VII	Sørensen *et al.* (1954a)
	Lactones			
10		*Boltonia, Erigeron*	III	Christensen (1959)
		*Chrysothamus, Conyza, Erigeron*	III	Christensen *et al.* (1957)
		Tripleurospermum	VII	
	Isobutylamides			
10	$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-CO-NH-CH_2-CH(CH_3)_2$	*Achillea*	VII	Bohlmann and Jartrow (1962)
14	$H_3C-CH_2-CH_2-C\equiv C-C\equiv C-CH_2-CH_2-CH=CH-CH=CH-CO-NH-CH_2-CH(CH_3)_2$	*Anacyclus*	VII	Crombie (1955)
	$H_3C-CH=CH-C\equiv C-C\equiv C-CH_2-CH_2-CH=CH-CH=CH-CO-NH-CH_2-CH(CH_3)_2$	*Anacyclus*	VII	

Acetylenes are present in most organs of the compositae, the root usually being the richest source, and very often, acetylenes can only be isolated from this part. Remembering the distribution of the acetylenic fatty acids in plants (cf. Chapter 9) it ought to be noted that most seed fats of the Compositae so far investigated have turned out to be normal glycerides. Clearly, the existence of a seed fat of normal composition ought not to deter any chemist from investigating other parts of the plant. How much the currently observed restriction of the acetylenes to some few families is due to this fact is completely unknown.

Since the days of Hooker the Compositae have been usually divided into thirteen tribes. The tribe Cichorieae, generally placed at the end, is botanically rather distinct from the rest; all the flowers of this tribe are ligulate and all members contain a milky sap. This latter property is partly anatomical, anastomosed sap channels, and partly biochemical, the enzyme system producing the sap. So the botanists themselves have partly used a "chemical character" for distinguishing this tribe from the rest of the family. Very often the Cichorieae is treated as a separate family (the Liguliflorae) whereas the twelve other tribes are united in another,

VII

derivatives from Compositae

Genus	Tribe	References
Lachnophyllum, Erigeron, Bellis, Calotis	III	Viljams *et al.* (1935); Holme and Sørensen (1954)
Amellus	III	Stavholt-Baalsrud *et al.* (1952)
Tripleurospermum	VII	
Calotis, Erigeron	III	Sørensen and Stone (1941)
Tripleurospermum	VII	
Amellus	III	Stavholt-Baalsrud *et al.* (1952)
Tripleurospermum	VII	
Achillea, Anthemis, Artemisia, Chrysanthemum	VII	Stavholt and Sørensen (1950)
Calotis	III	
Achillea, Cotula, Tripleurospermum	VII	Sørensen *et al.* (1954a)
Boltonia, Erigeron	III	Christensen (1959)
Chrysothamus, Conyza, Erigeron	III	Christensen *et al.* (1957)
Tripleurospermum	VII	
Achillea	VII	Bohlmann and Jartrow (1962)
Anacyclus	VII	Crombie (1955)
Anacyclus	VII	

Since the days of Hooker the Compositae have been usually divided into thirteen tribes. The tribe Cichorieae, generally placed at the end, is botanically rather distinct from the rest; all the flowers of this tribe are ligulate and all members contain a milky sap. This latter property is partly anatomical, anastomosed sap channels, and partly biochemical, the enzyme system producing the sap. So the botanists themselves have partly used a "chemical character" for distinguishing this tribe from the rest of the family. Very often the Cichorieae is treated as a separate family (the Liguliflorae) whereas the twelve other tribes are united in another,

TABLE VIII

Phenyl-containing acetylene derivatives from Compositae

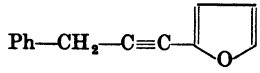
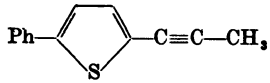
Number of C Atoms	Constitution	Genus	Tribe	References
11	Ph—CH_2—C≡C—C≡CH	*Artemisia*	VII	Bohlmann and Kleine (1962a)
12	Ph—CH_2—CH=CH—C≡C—CH_3	*Artemisia*	VII	Goljmov and Afanesev (1957)
	Ph—CH_2—C≡C—C≡C—CH_3	*Artemisia*	VII	Havada (1954 and 1957)
13	Ph—C≡C—C≡C—CH=CH—CH_3	*Coreopsis*	V	Sørensen and Sørensen (1958a)
	Ph—C≡C—C≡C—C≡C—CH_3	*Coreopsis*	V	Sørensen and Sørensen (1958b)
	Ph—C≡C—C≡C—CH=CH—CH_2OH	*Coreopsis*	V	Sørensen and Sørensen (1954b)
12	Ph—CO—C≡C—C≡C—CH_3	*Artemisia* *Chrysanthemum*	VII	Havada (1954) Bohlmann and Kleine (1962b)
	Ph—CH(OAc)—C≡C—C≡C—CH_3	*Chrysanthemum*	VII	Bohlmann and Kleine (1962b)
13		*Carlina*	XI	Semmler (1889)
		*Coreopsis*	V	Sørensen and Sørensen (1958c)

TABLE IX

Substituted aromatic acetylenic compounds from the Compositae

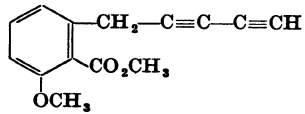
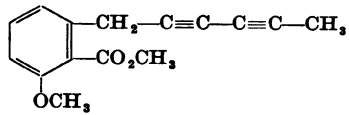
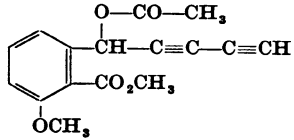
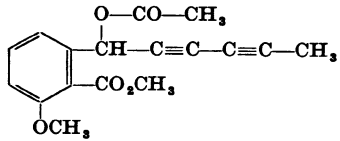
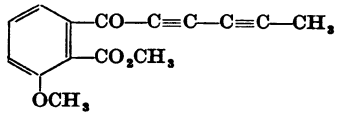
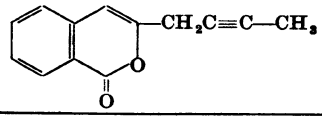
Number of C atoms	Constitution	Genus	Tribe	References
12		*Chrysanthemum*	VII	Bohlmann and Kleine (1962b)
13		*Chrysanthemum*	VII	Bohlmann and Kleine (1962b)
12		*Chrysanthemum*	VII	Bohlmann and Kleine (1962b)
13		*Chrysanthemum*	VII	Bohlmann and Kleine (1962b)
		*Chrysanthemum*	VII	Bohlmann and Kleine (1962b)
		*Artemisia* *Chrysanthemum*	VII	Bohlmann and Kleine (1962b) Bohlmann and Kleine (1962a)

TABLE X

Acetylenic furans from Compositae

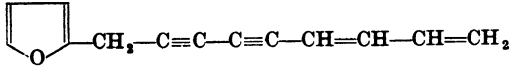
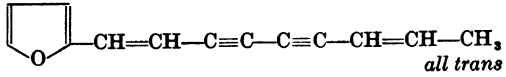
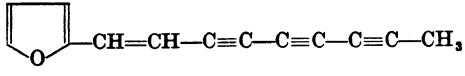
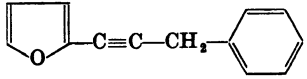
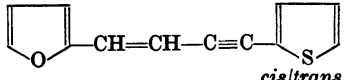
Number of C atoms	Constitution	Family	Tribe	References
13		*Carlina*	XI	Sørensen (1961)
	 *all trans*	*Atractylis spp.*= *Atractylodes spp.*	XI	Yosioka *et al.* (1960)
		*Chrysanthemum*	VII	Bohlmann *et al.* (1961d)
		*Carlina*	XI	Semmler (1889)
12	 *cis/trans*	*Santolina*	VII	Bohlmann *et al.* (1961d)

TABLE XI

Acetylenic thiophenes from Compositae

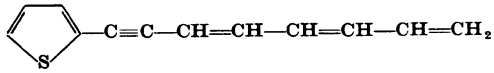
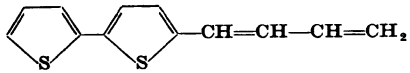
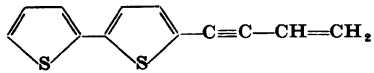
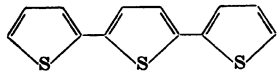
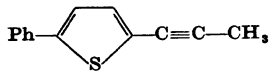
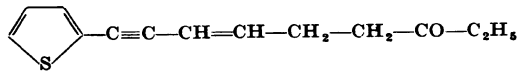
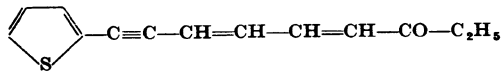
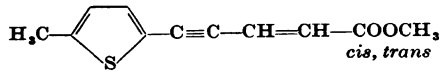
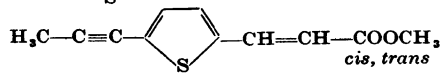
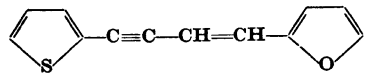
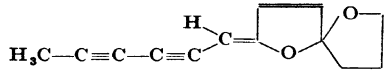
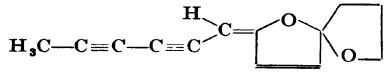
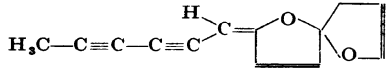
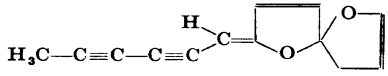
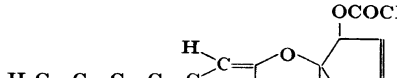
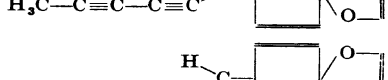
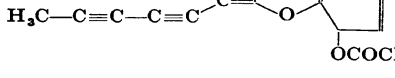
Number of C atoms	Constitution	Genus	Tribe	References
12		*Tripleurospermum*	VII	Sørensen (1960)
		*Bidens*	V	Liaaen-Jensen and Sørensen (1961)
		*Berkheya* *Tagetes* *Echinops*	VII XI	Uehlenbrook *et al.* (1958)
		*Berkheya* *Tagetes* *Echinops*	V VII XI	Zechmeister and Sease (1947) Sørensen (1961)
13		*Coreopsis*	V	Sørensen and Sørensen (1959c)
12		*Tripleurospermum*	VII	Sørensen (1960)
		*Tripleurospermum*	VII	Sørensen (1960)
10	 *cis, trans*	*Anthemis*	VII	Bohlmann *et al.* (1962c); Sunde (unpublished)
	 *cis, trans*	*Chrysanthemum*	VII	Guddal and Sørensen (1959)
12	 *cis*	*Santolina*	VII	Bohlmann *et al.* (1962c)

TABLE XII

Mixed heterocycles with acetylenic side chain from Compositae

Constitution	Genus	Tribe	References
	*Chrysanthemum*	VII	Bohlmann *et al.* (1961d)
	*Matricaria*	VII	Bohlmann *et al.* (1961d)
	*Chrysanthemum*	VII	Bohlmann *et al.* (1961d)
	*Chrysanthemum*	VII	Bohlmann *et al.* (1961d)
	*Chrysanthemum*	VII	Bohlmann *et al.* (1961d)
	*Chrysanthemum*	VII	Bohlmann *et al.* (1961d)
	*Artemisia* *Chrysanthemum*	VII VII	Bohlmann *et al.* (1962c)

the Tubuliflorae. In these other tribes the flowers can either be both ligulate and tubulate, or be only tubulate, milky sap is absent, but all members instead have resin channels, which although sometimes are restricted to the root system, are often found over the entire plant (to the tip of the petals).

So far no trace of acetylenes has been found in the sub-family the Liguliflorae. It is clearly not a very rewarding task to investigate any more of the numerous members of this sub-family after about a dozen species were found to be negative, and so we have restricted our investigations to those genera (Cichorium, Hypochoeris, Scorzonera and Tragopogon) which, in some botanical investigations, have not been so well defined. We could not detect the presence of acetylenes in any of the species examined.

Acetylenic compounds have been isolated from all the other twelve tribes which constitute the sub-family Tubuliflorae. However, the different tribes have been covered to very different extents. From the Astereae (*III*), Inuleae (*IV*), Heliantheae (*V*), Anthemideae (*VII*) and Cynareae (*XI*) about 100 species or more have been investigated; from Vernonieae (*I*), Eupatorieae (*II*), Calenduleae (*IX*), Arctotideae (*X*) and Mutisieae (*XII*) only a few species have been examined. The tribe number to which a genus belongs according to Hooker is shown in Tables III–XII.

From the results shown in Table VII, it can be seen that the C_{10} methyl esters (some of them identical to those present in fungi) are very common in tribes (*III*) and (*VII*), and, if not absent, are rarely found in the other ten. These esters have, however, only been found in certain genera in tribes (*III*) and (*VII*). Other genera contain, for example, alcohols (as acetates), or products containing heterocyclic rings. So the acetylenic pattern coincides to some extent with the morphological criteria on which the botanists have based their classification.

In huge families such as the Compositae, morphological convergence or overlapping takes place in a lot of cases. As might perhaps have been expected, it appears as though the pattern of acetylenes synthesized in a sub-tribe, a genus, or a section, is quite independent of such morphological convergence. Since this aspect of the distribution of the acetylene is immediately useful to taxonomy some examples can be mentioned.

The genus *Erigeron* L. (the fleabanes) is a rather large one, containing certainly above 200 distinct species. Hoffmann (1889) divided this genus into five sections, out of which the section Caenotus approaches another Astereae genus *Conyza* L. In a modern treatment of the American Erigerons, Cronquist (1947) has moved the whole section Caenotus over to Conyza.

Of the five sections of Hoffmann, only three have been investigated as to the presence of acetylenic compounds. Altogether some fifty species have been examined, three of them belonging to the section Caenotus. All of the investigated Erigeron contain mixtures of *cis-cis*-matricaria ester and (IIIb) *cis*-lachnophyllum ester (IIIa) including the three members from section Caenotus. So far five species of *Conyza* have been investigated and none of them showed any trace of these esters. So the chemical investigations do not support the change proposed by Cronquist; it is, however, impossible for a chemist to discover what common criteria the old botanists used when sorting some fleabanes to *Erigeron*, and others to *Conyza*.

In a similar way some Erigerons approach the genus *Aster* Tourn. ex L. A typical case are two American species which were both originally described as *Asters*. In 1814 Pursh described *Aster peregrinus*; and in 1897 Greene transferred this plant to *Erigeron* (*E. peregrinus* (Pursh) Greene). The other plant was originally described in 1840 by Nuttal as *Aster glacialis*, and renamed in 1843 *Aster salsuginosus* var. *scaposus* by Asa Gray. Gray in 1884, however, moved this plant to *Erigeron* (*E. salsuginosus* var. *glacialis* Gray). Cronquist very definitely expresses the opinion that both plants belong to *Erigeron*, and that if one should be moved to *Aster* then the other must also. Contrary to this orthodox opinion, the Swedish botanist Erik Hulten kept the first species in *Erigeron*, and the second in Aster. It took some years before we, ourselves, obtained genuine material of both; the first contains the acetylenes characteristic of fleabanes, the second those common to Aster. When our investigations were finished, I asked Professor Hulten what his botanical dividing line was; his answer was, "I know them personally from 25 years in the field".

In a large genus such as *Erigeron* there is of course also a lot of morphological overlapping between *species* which all botanists agree belong to the genus. It is fortunate that some fleabanes contain mainly the *cis-cis*-matricaria ester, whilst others have lachnophyllum ester, for it has been possible to clear up a rather confused situation in the following group of species. *E. elongatus* Ledebour–*E. politus* Fr.–*E. acris** *asteroides* (Andrez. ex Bess.) D.C.–*E. acris droebachiensis* (O. F. Müll).

Chemical investigations show that the three first named "species" are identical, and the differences found scarcely exceed that expected in different populations of a circumpolar species. The remaining difficulty is one of nomenclature, since the name used by the discoverer, Ledebour, is, for other reasons, invalid. The last "species" definitely belongs to *E. acris* L. This rather variable species has sub-species or variants which very much approach the *E. elongatus–E. politus* types. One of them, originally described by Elias Fries in 1838 as *E. rigidus*, has in later years

been confused with *E. politus*. Again chemistry readily shows that it belongs to the *E. acris* group.

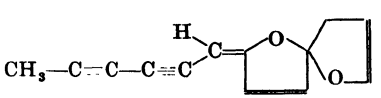
Erigeron is mainly a northern temperate circumpolar genus. Only the two first sections of Hoffmann (Oritriphium and Leptostelma) are South American. No member of these sections have been available as yet for chemical investigations. In Australia there occurs a few species which are nowadays treated as *Erigerons*. Of these *E. conyzoides* described by F. v. Mueller from the Snowy Mountains behaves, in respect of its acetylene chemistry, exactly like the northern fleabanes, and probably belongs to the rather small number of late northern intruders in the Australian flora.

In the mountains of New South Wales, Victoria and Tasmania there occur some fleabanes which at present are mainly united under the name *E. pappochromus* Labillardiere. Hooker, who first described them, divided them into half a dozen species under the genus *Aplopappus* Cass. syn. *Haplopappus* Endl. We have made preliminary investigations on material from three different types, and although they certainly all contain acetylenic compounds, neither *cis-cis*-matricaria ester (IIIb) nor *cis*-lachnophyllum ester (IIIa) is present. The American genus *Haplopappus* has so far not been investigated, so it is impossible to say if Hooker was correct. It ought, however, to be mentioned that the botanist Solbrig after a recent study of some of the South American *Erigerons* has transferred them to the antarctic genus *Celmisia*.

In 1844 Schultz-Bipontinus split a section of *Matricaria* (Tourn.) L. out as a separate genus *Tripleurospermum* because of anatomical differences in seed structure. Only three members of his genus *Tripleurospermum* have been investigated; they are all rich in *cis-cis*-matricaria ester, whereas *Matricaria* sp. has given the acetate (IV) and the ring closed acetylene (V) which so far has never been found in *Tripleurospermum*.

$$CH_3—C\equiv C—C\equiv C—CH=CH—CH=CH—CH=CH—CH_2—CH_2—O—COCH_3$$

(IV)



(V)

Another interesting observation made in Bohlmann's laboratory (Bohlmann *et al.*, 1958) is that in the cornflowers (*Centaurea* L.), the section *Centaurium cassini* is characterized by the absence of the trideca-1,11-diene-3,5,7,9-tetrayne (VI) present in more than sixty other centaureas and one of the most common acetylenes in the Compositae family. At the same time, Bohlmann and co-workers have isolated from

members of Centaurium not less than seven polyacetylenes not found in other Centaurea sections. Inspection of the formulas (VIIa) and (VIIb) at once reveals that both these compounds are derivatives of the isomeric hydrocarbon trideca-1,3-diene-5,7,9,11-tetrayne, a hydrocarbon not so far isolated. This isomeric diene-tetrayne is clearly the precursor of phenyl-heptatriyne (from *Coreopsis* and *Bidens*) and 5-methyl-5′-buta-dienyl-2,2′-dithienyl (VIII) from *Bidens*, so this isomer ought at least to exist as an intermediate in other tribes of the Compositae.

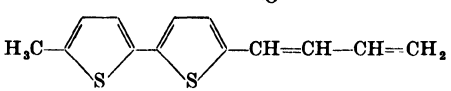
$$CH_3—CH=CH—C\equiv C—C\equiv C—C\equiv C—C\equiv C—C\equiv C—CH=CH_2$$

(VI)

$$CH_3—C\equiv C—C\equiv C—C\equiv C—C\equiv C—C\equiv C—CH=CH—R$$

(VIIa) R = $-CHCl—CH_2OH$

(VIIb) R = $-CH(O)CH_2$



(VIII)

TABLE

Acetylenic compounds from

Number of C atoms	Constitution
	Hydrocarbons
13	$H_3C—CH=CH—C\equiv C—C\equiv C—CH=CH—CH=CH—CH_2—CH_3$ *all trans*
	Alcohols
	$H_3C—CH_2—CH_2—CH(OH)—CH=CH—C\equiv C—C\equiv C—CH=CH—CH_3$ *all trans*
	$H_3C—CH_2—CH=CH—CH=CH—C\equiv C—C\equiv C—CH=CH—CH_2OH$
17	$H_{13}C_6—CH=CH—CH=CH—C\equiv C—C\equiv C—CH=CH—CH_2OH$
	$H_9C_4—CH=CH—CH=CH—CH=CH—C\equiv C—C\equiv C—CH_2—CH_2—CH_2OH$
	$H_7C_3—CH(OH)—CH_2—CH_2—CH=CH—CH=CH—C\equiv C—C\equiv C—CH=CH—CH_2OH$
	$H_7C_3—CH(OH)—CH=CH—CH=CH—CH=CH—C\equiv C—C\equiv C—CH_2—CH_2—CH_2OH$
	Ketones
17	$n\text{-}C_7H_{15}—CH=CH—CH_2—C\equiv C—C\equiv C—CO—CH=CH_2$, *cis*
	$n\text{-}C_7H_{15}—CH=CH—CO—C\equiv C—C\equiv C—CO—CH=CH_2$, *cis*
	$n\text{-}C_7H_{15}—CH=CH—CH(OH)—C\equiv C—C\equiv C—CO—CH=CH_2$, *cis*
	$H_7C_3—CO—CH_2—CH_2—CH=CH—CH=CH—C\equiv C—C\equiv C—CH=CH—CH_3$

B. Umbelliferae and Araliaceae

In 1953 Lythgoe and his co-workers (Anet *et al.*, 1953) elucidated the structure of the toxic principles of *Cicuta virosa* L. and *Oenanthe crocata* L. The early chemistry on these poisons was merely confusing. The highly toxic principles Cicutoxin and Oenanthotoxin were shown by Lythgoe *et al.* to be the diols in Table XIII. At the same time this Cambridge group elucidated the constitution of two mono-ols and one ketone accompanying the diols in these plants. These last three were found to be practically non-toxic. In recent years Bohlmann and his co-workers (Bohlmann *et al.*, 1960a, 1961b) have followed up this interesting investigation and up till now have isolated at least six new acetylenes from the Umbelliferae in addition to the five originally described by Lythgoe *et al.*

From a chemotaxonomical point of view these investigations are highly interesting. One of these compounds falcarinon (a ketone from *Falcaria vulgaris* Bernh., Table XIII) has been found by Bohlmann to occur in members of the three families Compositae, Umbelliferae and Araliaceae (cf. Table XIII). With regard to the Umbelliferae and

XIII

other plant families

Umbelliferae	Araliaceae	References
Aethusa cynapium L.		Bohlmann *et al.* (1960a)
Peucedanum verticillare Koch.		Bohlmann *et al.* (1961b)
Aethusa cynapium L.		Bohlmann *et al.* (1960a)
A. cynapium L.		Bohlmann *et al.* (1960a)
Oenanthe crocata L.		Anet *et al.* (1953)
Cicuta virosa L.		Anet *et al.* (1953)
Oenanthe crocata L.		Anet *et al.* (1953)
Cicuta virosa L.		Anet *et al.* (1953)
Carum carvi L., *Falcaria vulgaris* Bernh., *Oenanthe pimpinelloides* L., *Sium sisarum* L.	*Hedera helix* L.	Bohlmann *et al.* (1961b)
Carum carvi L., *Oenanthe pimpinelloides* L., *Sium sisarum* L.		Bohlmann *et al.* (1961b)
Carum carvi L., *Oenanthe pimpinelloides* L., *Sium sisarum* L.	*Aralia nudicaulis* L.	Bohlmann *et al.* (1961b)
Oenanthe crocata L.		Anet *et al.* (1953)

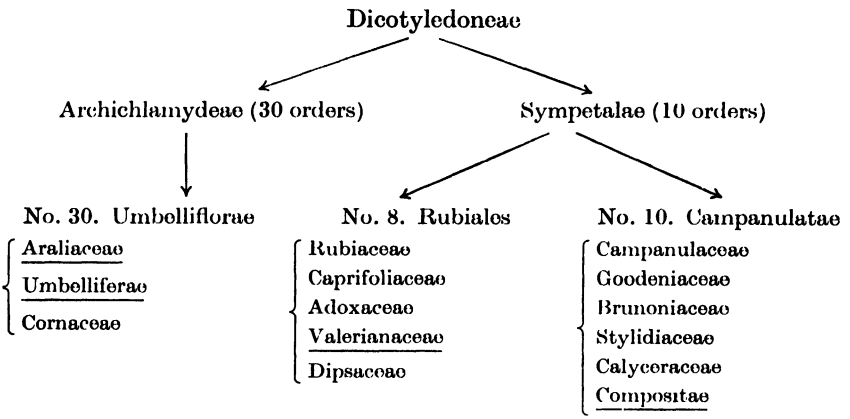


FIG. 2 (Engler and Diels, 1936).

Araliaceae this is not very surprising, since a number of leading botanists unite these two in one single family. Clearly Bohlmann's chemical investigations strengthen the botanical arguments greatly. Fossils belonging to Araliaceae are known back to early cretaceous times whereas remnants of Umbelliferae and Compositae are very scarce, or dubious, from pretertiary periods. This fact, which in botanical textbooks is used as an argument for the rather recent development of Umbelliferae and Com-

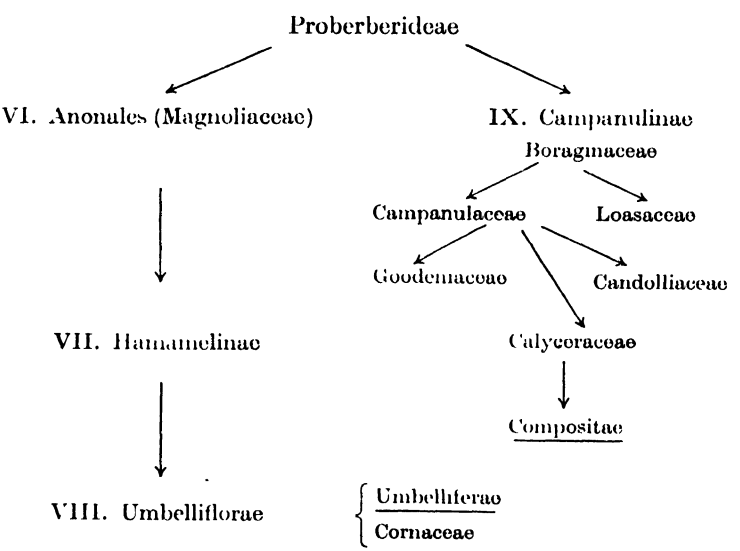


FIG. 3 (Hallier, 1912).

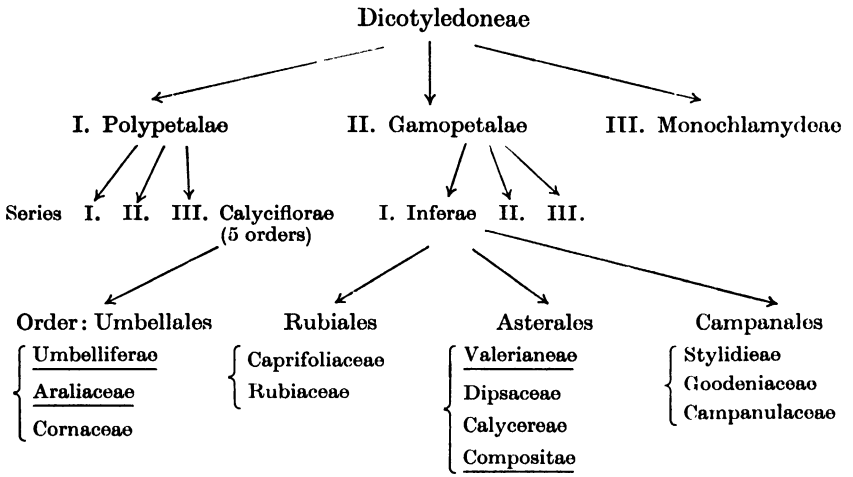


FIG. 4 (Bentham and Hooker, 1862–93).

positae, focus strong interest on the common occurrence of the very peculiar acetylene falcarinon in all three families.

In Figs. 2–7 I have selected some of the better known taxonomic or phylogenetic systems. Engler and Diels (1935) (Fig. 2) placed Umbelliflorae and Campanulatae (incl. Compositae) in different sub-classes, and the same is found in the system of Hallier (1912) (Fig. 3). The system of

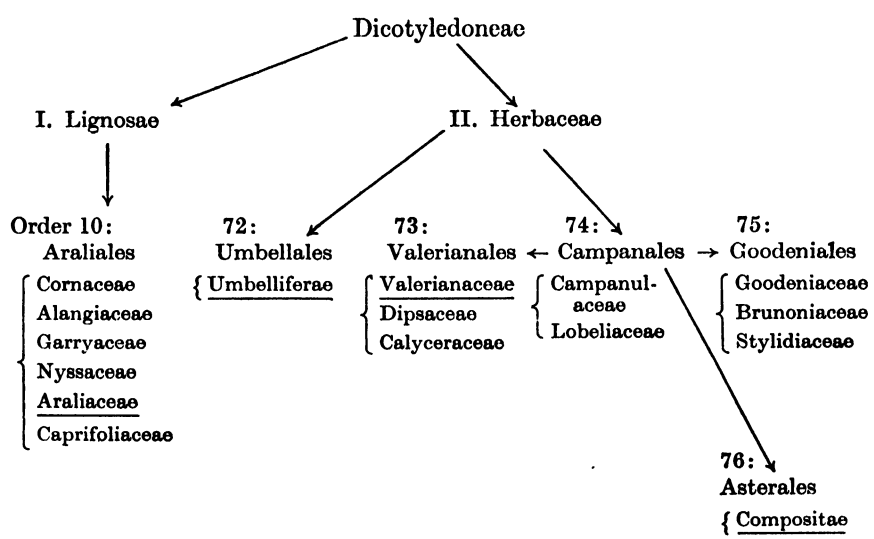


FIG. 5. (Hutchinson, 1959).

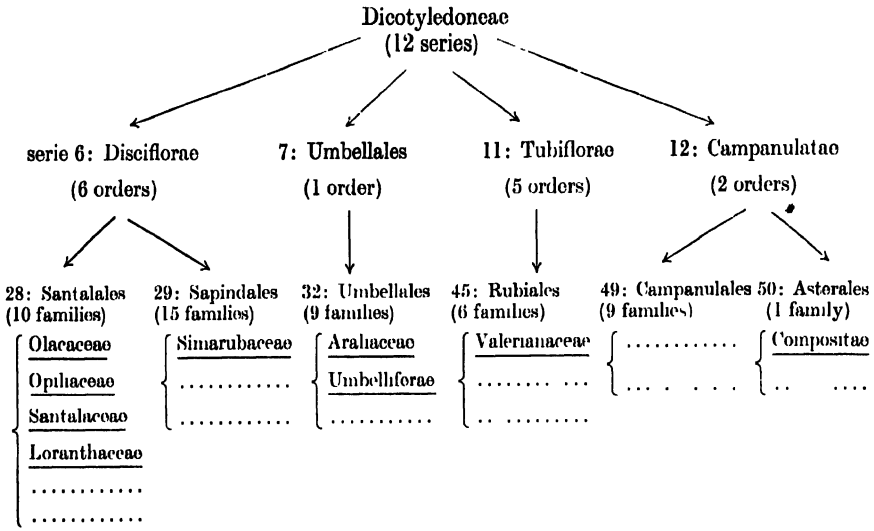


FIG. 6 (Takhtajan, 1954).

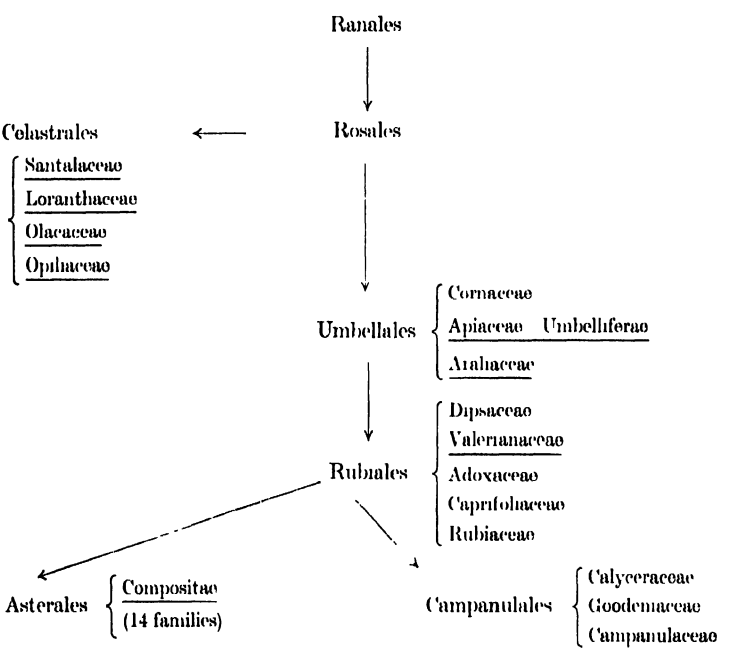


FIG. 7 (Bessey, 1915).

Bentham and Hooker (1862–93) is shown in Fig. 4, and here also Umbelliferae–Araliaceae are very distant from the Asterales. We should not of course forget that Bentham and Hooker did not intend their system to be regarded as phylogenetic, it is rather that their classification has in some cases turned out to agree with supposed phylogeny.

Hutchinson (Fig. 5) has always placed Umbelliferae rather close to Asterales, but, according to his basic idea, the Araliaceae had to be put on the other main division, the Lignosae. To those, who like myself has had the good fortune to see some representatives of large trees belonging to the Compositae (*Bedfordia*), the artificial character of the division Lignosae–Herbaceae needs no further comment. The Russian taxonomist, Takhtajian, has evolved ideas for taxonomic sub-division which in many cases agrees well with independent chemical work. As can be seen from Fig. 6, Takhtajian puts the Santalales and Sapindales close together, which is unusual, but Umbellales and Asterales are again very distant. Bessey (1915) proposed in 1915 a phylogenetic system that is partly reproduced in Fig. 7. With the exception of Simarubaceae, Bessey has managed to get the families characterized by the occurrence of acetylenes in a very simple genetic tree.

I have tried to stress in the preceding discussion a lot of the weak points in our present knowledge about naturally occurring acetylenes, especially from the point of view of their use in chemotaxonomy. I suppose it goes without saying that in a field which is showing such a tremendous and rapid growth as this one is, there is every reason to restrict conclusions to the smaller taxonomic problems mentioned above, where the chemical investigations are more complete. One must postpone any conclusions as to the more vital phylogenetical problems until the contours of the chemical picture are much more settled.

C. Observations Concerning other Families

Treibs (1947) described 1-phenyl-hex-2-en-4-yne (see Table VIII) from an essential oil isolated by Schimmel & Co. in 1922 from the root part of the grass, *Agropyron repens* (L.) P.B. The sample which gave this hydrocarbon—"old agropyren"—was, however, according to the original description in the report of the company (Schimmel, 1922) mixed with a little root of *Artemisia dracunculus*. As the compound in question is present in large quantities in roots of different *Artemisia* species (Goljmov and Afanesev, 1957), whereas pure roots of *A. repens* according to Schimmel & Co. is very low in essential oil, I do not think it permissible to accept the occurrence of acetylenes in grasses on this evidence alone.

In a recent lecture (30 January 1962) Schulte (1962) has announced

the occurrence of unspecified acetylenes in a number of medicinal plants among them *Ricinus communis* L. (Euphorbiaceae) and *Valeriana officinalis* L. (Valerianaceae). Until the structure of these acetylenes are fully described, their taxonomical importance cannot be evaluated.

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CHAPTER 10

The Distribution of Fatty Acids in Plant Lipids

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I. Introduction

The role of fats in chemical taxonomy was referred to by Hilditch (1934) in a Jubilee Memorial Lecture to the Society of Chemical Industry (London) in which he stated, "Perhaps, when in the course of time sufficiently wide and detailed data have been collected, systematic description of the natural fats will commence with those of the minute aquatic flora and fauna, will proceed to those of the larger aquatic denizens and then to the two respective branches of land flora and land fauna". This forecast was to some extent substantiated in a subsequent paper by Hilditch and Lovern (1936) entitled "The Evolution of Natural Fats". Here the fatty acid composition of fat from various species was examined against the phylogenetic scale based on morphological considerations and a number of important correlations was observed. Thus the occurrence of erucic acid was confined to the seeds of Cruciferae (p. 290) and that of petroselinic acid to the seeds of Umbelliferae (p. 287).

The extensive compilation by Hilditch (1956) covers all the then

available data on fatty acid composition of plant fats in relation to botanical classification and the subject has also been briefly reviewed by Shorland (1962).

In the present paper, therefore, the emphasis is mainly centred on work published since 1956. Owing to the advent of new techniques, notably gas chromatography, the results obtained are now expressed in a variety of forms and the data in this chapter in many instances have been taken more or less directly and expressed as "% component fatty acids" without further interpretation. This course is perhaps the only feasible one, as in some cases there will be unknown correction factors that should be applied in respect, for example, to detector response.

The data on seed fats, in particular, are so extensive that it has been necessary to restrict the text to selected examples and to provide in an appendix references to sources of detailed information. To assist in presentation abbreviations for the names of fatty acids have been used.*

The lipids from different parts, e.g. seeds and leaves of the same plant, may have a different fatty acid composition as well as different types of fatty acids. In seeds, moreover, the lipids are largely triglycerides, whereas in the leaves triglycerides are replaced by galactolipids. In mycobacteria the lipids consist largely of waxes together with lesser amounts of the so called acetone soluble "fats". In both of these fractions considerable amounts of carbohydrates (trehalose) are present but glycerides also occur (Asselineau and Moron, 1958). Despite these difficulties comparisons will be made between the fatty acids present in the different divisions of the plant kingdom.

Although much work has been carried out on varietal and environmental effects on the fatty acid composition of seed fats the results show, as a rule, only slight changes in the amounts of the various fatty acids, the most extreme example being that of safflower (*Carthamus tinctorius*) seed oil. The normal seed oil contains about 10% saturated acids with 20% oleic acid and 70% linoleic acid, whereas the variants obtained by selection of two individuals from a large number of plants contained only 4–8% saturated acids with 74–79% oleic acid and 11–19% linoleic acid (Horowitz and Winter, 1957). As far as the writer is aware seed fats from the same species almost invariably contain the same types of fatty acids. However, whereas Crossley and Hilditch (1949, 1952) found 4–5% of deca-*trans*-2,*cis*-4-dienoic acid in Stillingia (*Sapium sebiferum*, Euphorbiaceae) oils from China and Texas, Narang and Sadgopal (1958) did not find this acid in oils from India. The differences observed were also reflected in the saponification values of the oils.

* Sat. = saturated, mo. = myristoleic, po. = palmitoleic, lin. = linoleic; len. = linolenic; eicos. = eicosenoic; docos. = docosenoic.

In other parts of the plant kingdom occasional variations in fatty acid composition are observed within a given species due to environmental changes. Thus, whereas the lipids of the mycelium of *Neurospora crassa* obtained by surface culture are rich in linolenic acid, the lipids from submerged cultures yielded none of this acid (Krzeminski, White and Quackenbush, 1960). The taxonomic significance of fatty acid composition must therefore be treated with caution, pending investigation of environmental and varietal effects and the possible occurrence of physiological forms now well known to workers on essential oils but not yet encountered in the field of plant lipids.

Although lipids occur in all species of plants examined, a survey of the fatty composition has barely been started, and it is only seed fats which have received a reasonably broad coverage. Even at the present time data from other parts of the plant kingdom are fragmentary. Within a limited range, however, certain groups have received reasonably close attention including especially mycobacteria, yeasts and fungi.

As in the animal world, the lipids of aquatic species appear to have a different fatty acid make up from those of terrestrial species. It is convenient, therefore, to start with a description of the types and distribution of fatty acids in aquatic plants and then to proceed to those of bacteria, yeast, fungi, and phanerogams with special reference to the seed fats of the latter.

II. Lipids of Aquatic Plants

Although the fats of aquatic fauna are well characterized by their high content (40–60%) of highly unsaturated fatty acids, which are not present in more than trace amounts in the depot fats of terrestrial animals, the exact position with regard to the lipids of aquatic plants is much less well known. The data summarized by Hilditch (1956) include ester fractionation analyses of fats from a fresh water diatom, several classes of algae including Chlorophyceae (four fresh water species), Phaeophyceae (three marine species), Rhodophyceae (one marine species) and a higher fresh-water plant (*Anacharis alsinastrum*). Although substantial amounts of C_{22} highly unsaturated acids were found only in the Rhodophyceae lipids, C_{20} unsaturated acids (4–36%) occurred in the lipids of all of the above-mentioned species. On the other hand, Paschke and Wheeler (1954) reported the fatty acid composition of the fresh-water alga *Chlorella pyrenoidosa* to be as follows: Saturated, 17; C_{16} monoene, 4; C_{16} diene, 6; C_{16} triene, 12; C_{16} tetraene, 3; C_{18} monoene, 7; C_{18} diene, 11; C_{18} triene, 34; C_{18} tetraene, 1, and above C_{18} less than 4%. The unique feature of these results was the isolation of a C_{16} tetraenoic

TABLE I

Fatty acid composition of the lipids of algae (Rhodophyceae), methylesters as % of total (Laur, 1961)

Order and species	Saturated					Unsaturated						
	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{14} mono	C_{16} mono	C_{16} di–tri	C_{18} mono	C_{18} di	C_{18} tri	C_{20} di–penta
Fresh-water species												
NEMALIONALES												
Sacheria fucina	1·2	5·0	10·7	23·2	15·2	1·8	13·3	8·6	5·4	2·5	1·4	13·7
Lemanea nodosa	1·3	0·4	6·4	42·9	0·8	1·5	7·5	1·2	8·3	3·6		26·7
Salt water species												
RHODYMENIALES												
Rhodymenia palmata	0·5	0·1	10·7	28·3	1·5	—	3·2	—	5·6	1·4		48·7
GIGARTINALES												
Furcellaria fastigiata	1·7	2·3	5·7	40·7	3·3	2·7	15·3	1·1	11·5	0·8		15·7
CERAMIALES												
Polysiphonia elongata	0·7	0·7	3·3	47·5	1·7	0·7	10·0		5·3	2·0		28·4

hexadeca-*cis*-4,*cis*-7,*cis*-10,*cis*-13-tetraenoic acid which has not been found elsewhere.

The alkali isomerization results of Kelly, Reiser and Hood (1959) suggest that marine phytoplankton lipids have considerable amounts of pentaenoic and hexaenoic acids as do the lipids from the marine diatom *Nitzchia closterium*. In addition *Chlorella pyrenoidosa* was shown to contain small amounts of pentaenoic but no hexaenoic acids. Recently Laur (1961) has examined the fatty acid composition of the lipids of algae, including both fresh-water and salt water species (Table I).

The results in Table I provide satisfactory evidence for the occurrence of highly unsaturated C_{20} acids in Rhodophyceae in widely varying amounts which are perhaps related to the reproductive cycle. Highly unsaturated C_{22} acids were not found in more than trace amounts.

III. Lipids of Terrestrial Plants

A. Cryptogam Lipids

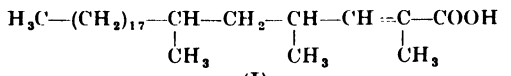
The fatty acids of cryptogam lipids have been given only limited attention. It is nevertheless already clear that some cryptogams have distinctive lipids and fatty acid types. On the other hand, there are other examples of cryptogam lipids which are disappointingly similar in their fatty acid make up to those encountered in many of the phanerogams. These aspects will now be looked at more closely in those areas of the Phylogenetic scale which have been investigated, comprising chiefly bacteria, moulds and fungi.

1. Lipids of bacteria

The pioneering efforts of Anderson and co-workers on the lipids of acid-fast bacteria, particularly mycobacteria during the period 1926–46 have been touched on by Hilditch (1956) and reviewed by Anderson (1939, 1941). These researches indicate that the main lipid constituents of mycobacteria are waxes, comprising high molecular weight branched-chain mycolic acids ($C_{88}H_{176}O_4$) combined with polysaccharides. In addition there are present the so-called acetone soluble "fats" containing the trehalose esters of palmitic, stearic, and oleic acids together with tuberculostearic (10-methylstearic) acid and the long-chain methyl branched phthioic acids. More recently Cason *et al.* (1953) (cf. Hilditch, 1956) have given the composition of the fatty acids of tubercle bacilli as palmitic 28–34, C_{18} and C_{19} acids 32–39, and acids of higher molecular weight of which about one-third were of the phthioic type as 16–21%.

The original phthioic acid of Anderson and co-workers has since been

shown to consist of a mixture of related fatty acids which includes as the main constituents

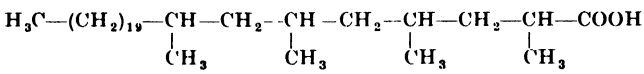


(I)

Phthienoic (mycolipenic) acid

(Chanley and Polgar, 1950; Cason and Fonken, 1952; Polgar, 1954; Asselineau, Asselineau and Stallberg-Stenhagen, 1956)

and

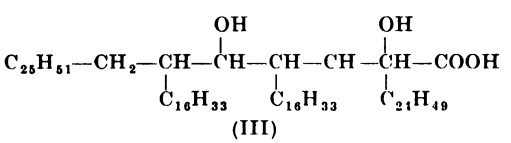


(II)

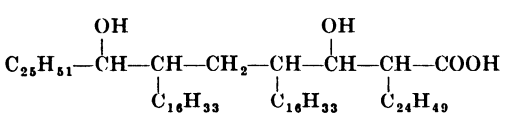
Mycocerosic acid

(Asselineau *et al.*, 1959)

Tuberculostearic (10-methylstearic) acid, phthienoic acid and mycocerosic acid are all compounds with side methyl groups. There is another class of branched-chain fatty acids in the lipids of mycobacteria wherein the branches consist of long side chains. These acids are called mycolic acids. According to Asselineau (1960) the experimental evidence for two types of mycolic acids leads to the structures (III) and (IV).



(III)



(IV)

In regard to the formation of the methyl branches of phthienoic and mycocerosic acids, Lederer (1960) considers that they result from the condensation of a normal fatty acid, e.g. stearic or arachidic, on to the α-position of propionic acid, followed by reduction of the β-keto ester thus formed. Repetition of the process provides a further side methyl group. In support of this hypothesis Lederer (1960) refers to unpublished results by Gastambide and Grisebach which show that propionic acid when incubated with growing cultures of non-virulent human strain $H_{37}Ra$ is incorporated in good yield into mycocerosic acid.

The formation of mycolic acids evidently follows a similar mechanism

as Gastambide-Odier and Lederer (1959, 1960) have shown that corynomycolic acid from *Corynebacterium diphtheriae* is produced by the condensation of two molecules of palmitic acid followed by reduction of the intermediate β-keto ester. The nocardic acids with 50 ± 3 carbons of *Nocardia asteroides* and the mycolic acids from Mycobacteria with 88 carbon atoms could, according to Lederer (1960), similarly result by the condensation of fatty acids of the appropriate chain lengths. Lederer (1960) has suggested that the biosynthesis of branched-chain compounds by Mycobacteria and Actinomycetes, in general, may be summed up as the condensation of a straight-chain acid R′—COOH on the α-C atom of an acid R″—CH_2—COOH, which gives methyl branched acids when R″ is methyl and mycolic acids when R″ is a longer alkyl group.

In addition to the branched-chain hydroxy acids of the Mycobacteria there occur in other species straight chain hydroxy-substituted acids. In *Bacillus megatherium* there is a lipid polymer consisting of the etholide of β-hydroxybutyric acid (Lemoigne, 1946; Képès and Péaud-Lenoël, 1952). The same kind of lipid has also been isolated from *B. cereus*, *B. mycoides*, and *B. anthracis* (Lemoigne, Delaporte and Croson, 1944; Williamson and Wilkinson, 1958). D(−)-β-Hydroxydecanoic acid occurs in *Pseudomonas pyocyanea* (Bergström, Theorell and Davide, 1946), D(−)-β-hydroxylauric in *Pseudomonas* sp. (Bloch *et al.*, 1961), D(−)-β-hydroxymyristic acid in *Escherichia coli* (Ikawa *et al.*, 1953), and dihydroxystearic acid in *Lactobacillus acidophilus* (Crowder and Anderson, 1932). According to Bloch *et al.* (1961) the occurrence in bacteria of D(−)-β-hydroxydecanoic and D(−)-β-hydroxylauric acids of the opposite configuration to the β-hydroxy acids in the β-oxidation cycle has a special significance in the anaerobic formation of unsaturated fatty acids. By use of labelled intermediates these are shown to arise from the condensation of acetate units to octanoic or decanoic acids, thereby resulting in the formation of Δ^{11} as well as Δ^{9} unsaturated acids. The latter is a typical feature of the aerobic process for the formation unsaturated acids in most of the other forms of plant and animal life.

The formation of *cis*-vaccenic acid (V) commonly found in anaerobic bacteria is considered by Bloch *et al.* (1961) to proceed along the lines shown in Fig. 1.

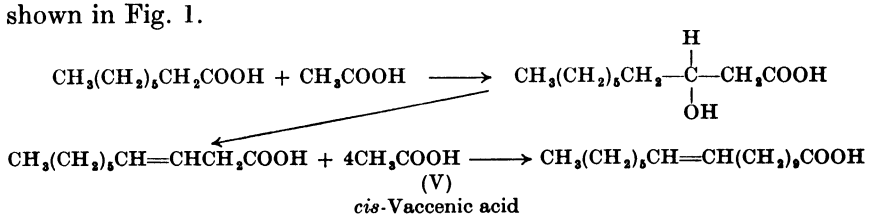


FIG. 1. Anaerobic mechanism for the formation of *cis*-vaccenic acid.

This scheme would account for the occurrence of *cis*-vaccenic acid (V) as the only C_{18} unsaturated acid in the lipids of the anaerobic bacteria, including those of *Lactobacilli* sp., *Agrobacterium* (*Phytomonas*) *tumefaciens* and *Streptococcus* sp. (Hofmann *et al.*, 1955), also *L. plantarum* (Hofmann *et al.*, 1952), *Clostridium butyricum* (obligate anaerobe), *Escherichia coli* (facultative anaerobe) and *Pseudomonas fluorescens* (obligate aerobe) (Bloch *et al.*, 1961). In *C. butyricum* and *S. faecalis* (cf. Bloch *et al.*, 1961) the C_{18} unsaturated acids contain oleic as well as *cis*-vaccenic acid. Apart from Mycobacteria in which the C_{18} unsaturated acids consist of oleic acid and *C. butyricum* and *S. faecalis* (Bloch *et al.*, 1961) it appears that *cis*-vaccenic acid (V) is the only C_{18} unsaturated acid found in bacterial lipids. In this regard Bloch *et al.* (1961) comment that the acquisition of an aerobic metabolism does not necessarily involve a shift to the aerobic mechanism for synthesizing unsaturated fatty acids.

In the lipids of some species of bacteria notably *Lactobacilli* and *Agrobacterium tumefaciens* a saturated acid containing a cyclopropane ring and known as lactobacillic acid (VI) occurs (cf. Hofmann *et al.*, 1955).

$$CH_3.(CH_2)_5CH\text{—}CH(CH_2)_9COOH$$
$$\quad\quad\quad

(1955) found that the fatty acids contained much *cis*-vaccenic but no lactobacillic acid.

More recently Bloch *et al.* (1961) have examined the composition of the fatty acids from three species of bacteria grown under anaerobic conditions (Table II).

The occurrence of such large amounts of C_{16} unsaturated acids in *C. butyricum* together with evidence for the presence of Δ^7 isomer (cf. Bloch *et al.*, 1961) in contrast to palmitoleic (Δ^9) which has hitherto been considered as the sole naturally occurring C_{16} mono-unsaturated acid is interesting and could be accounted for, according to Bloch *et al.* (1961), by the addition of acetate to decanoic acid as shown in Fig. 2.

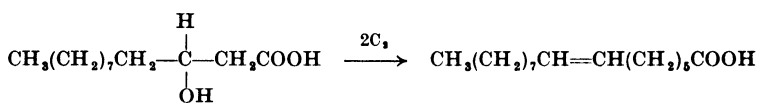


FIG. 2.

Associated with the occurrence of considerable amounts of C_{16} mono-unsaturated acids (cf. Table II) is the appearance of C_{17} cyclopropane acids. Kaneshiro and Marr (1961) found that the ethanol-soluble phospholipids of *Escherichia coli* contained 22% *cis*-9,10-methylenehexadecanoic acid together with 5% myristic, 40% palmitic, 19% octadecenoic and 7% lactobacillic.

Knowledge of the fatty acid composition of bacterial lipids has recently been extended by Saito (1960a,b) using reversed phase chromatography (Table III). It will be seen from Table III that whereas the lipids from

TABLE III

Fatty acid compositions of the lipids of various species of bacteria (Saito, 1960a,b)

Species	Saturated					Unsaturated			
	C_{12} and under	C_{14}	C_{15}	C_{16}	C_{17}	C_{15}	C_{16}	C_{17}	C_{18}
B. alcaligenes faecalis	3	3	—	43	—	—	21	—	28
S. pullorum	17	12	—	47	—	—	9	—	13
B. fluorescens	15	4	—	32	—	—	19	—	31
S. typhimurum	18	8	—	32			29	—	19
B. natto	—	—	74	11		14	—	—	—
B. subtilis	9	—	53*	10	15*	11*	—	—	—

* Iso-acids, i.e. 13-methyltetradecanoic and 15-methylhexadecanoic acids.
Results expressed as component fatty acids %.

most of the species of bacteria examined have a typical fatty distribution, those from *B. natto* and *B. subtilis* are remarkable for their high content of C_{15} acids and the absence of C_{18} unsaturated acids. In *B. subtilis* the acids were identified as consisting mainly of 13-methyltetradecanoic together with some 15-methylhexadecanoic. In the lipids of *Micrococcus lysodeikticus* the main constituent was shown to be the *anteiso* acid, 12-methyltetradecanoic acid (Macfarlane, 1961a,b). This acid is also probably the main acid in *Sarcinia* lipids (Akashi and Saito, 1960). In addition the occurrence of a range of branched acids in the lipids of *B. cereus* is indicated in Table IV.

TABLE IV

Fatty acid composition of total lipids of *B. cereus* (Kates, Kushner and James, 1962)

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}
n-Saturated	0·1	0·1	2·4	0·3	8·6	0·2	0·7
Branched	0·2	8·7	2·0	44·0	4·4	16·9	0·9
Monounsaturated	—	—	—	—	4·7	—	—
Polyunsaturated	—	—	—	—	1·0	4·8	—

Results expressed as % total area under the peaks on the gas–liquid chromatogram.

Although the bacterial lipids have not yet been widely examined there is sufficient evidence to suggest that the extraordinary differences in fatty acid types and composition have some taxonomic significance (Table V).

The presence of high molecular weight mycolic acids with long alkyl side chains together with the multibranched methyl side chains of phthienoic and mycocerosic acids still remain as striking characteristic features of Mycobacteria, although a few other species have lower molecular weight acids of the mycolic type.

In many bacteria it would appear that *cis*-vaccenic acid is the main C_{18} unsaturated acid, and in *Lactobacilli* and a few other species this is accompanied by lactobacillic acid. Oleic acid is generally absent except in Mycobacteria where it forms the characteristic C_{18} unsaturated acid. The occurrence of C_{15} branched chain fatty acids as the main fatty acid constituent of several bacterial species and the absence of these constituents in other bacteria may have taxonomic significance. The complete absence of C_{18} unsaturated acids in *B. natto*, *B. subtilis* as well as in *B. cereus* is indicated. This provides an almost unique example in the plant world apart, perhaps, from the near absence of oleic acid from the

TABLE V

Characteristic fatty acids of bacterial lipids

Source	Formula	Name
Most bacteria except Mycobacteria	*cis*-Octadec-11-enoic	*cis*-Vaccenic
Lactobacilli and some other bacteria	$CH_3(CH_2)_5CH—CH(CH_2)_9COOH$ (CH$_2$ bridge)	Lactobacillic
Escherichia coli	$CH_3(CH_2)_5CH—CH(CH_2)_7COOH$ (CH$_2$ bridge)	*cis*-9,10-Methylene hexadecanoic
Bacillus subtilis	H_3C, H_3C $CH(CH_2)_{11}COOH$	13-Methyltetradecanoic
	H_3C, H_3C $CH(CH_2)_{13}COOH$	15-Methylhexadecanoic
Micrococcus lysodeikticus	$CH_3CH_2CH(CH_2)_{10}COOH$ (CH_3)	12-Methyltetradecanoic
Mycobacteria	$CH_3(CH_2)_7CH(CH_2)_8COOH$ (CH_3)	Tuberculostearic
	$CH_3(CH_2)_{17}CHCH_2CHCH—CCOOH$ (CH_3 CH_3 CH_3)	Phthienoic
	$CH_3(CH_2)_{19}CHCH_2CHCH_2CHCH_2CHCOOH$ (CH_3 CH_3 CH_3 CH_3)	Mycocerosic
	$C_{25}H_{51}CH_2CHCHCHCHCOOH$ (OH OH; $C_{16}H_{33}$; $C_{24}H_{29}$)	Mycolic
Corynebacterium diphtheriae	$CH_3(CH_2)_{14}CHCHCOOH$ (OH; $C_{14}H_{29}$)	Corynomycolic
	$CH_3(CH_2)_5CH=CH(CH_2)_7CHCHCOOH$ (OH; $C_{14}H_{29}$)	Corynomycolenic

seed wax of *Simmondsia californica* (Green, Hilditch and Stainsby, 1936). The absence of polyenoic acids forms a special feature of bacterial lipids.

2. Lipids of yeast and fungi

The results for the fatty acid analysis of yeast fats have been recorded by Hilditch (1956) and the more recent data for *Rhodotorula graminis*

by Hartman *et al.* (1959) shown below generally confirms the earlier results, in showing an uncomplicated mixture with palmitic, oleic and linoleic acids as major constituents.

TABLE VI

Fatty acid composition of *Rhodotorula graminis* (Hartman *et al.*, 1959)

	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20-22}
Saturated	0·3	0·4	3·9	31·9	3·2	—
Unsaturated	0·1	—	1·1	0·3	37·2 oleic 10·2 lin. 4·6 len.	2·5*

Results expressed as fatty acids wt.-%.
* Also 4·3% unidentified.

Although the yeast fats lack any specific characteristics nevertheless the oil produced during the fermentation of glucose by the osmophilic yeast *Torulopsis magnoliae* was shown to be a glycolipid consisting mainly of partly acetylated 2-*O*-β-D-glucopyranosyl-β-glycopyranose units attached β-glycosidically to L-17-hydroxyoctadecanoic and L-17-hydroxy-9-octadecenoic acids (Gorin *et al.*, 1961).

Only two other hydroxy monoenoic C_{18} acids have been reported: 12-hydroxy-9-octadecenoic (ricinoleic) acid in castor oil (cf. Hilditch, 1956) with a D-configuration for the asymmetric centre (Serek-Hanssen, 1958) and 9-hydroxy-12-octadecenoic acid (Gunstone, 1952) found in the seed oils of *Strophanthus* spp.

In considering the composition of mould fats it is convenient to deal first with the fats obtained from mycelial mats and then to consider the spore fats.

(a) *Mycelial lipids*

The fatty acids of the lipids from three *Penicillium* species and from four other moulds have been recorded by Hilditch (1956). Further examples are collected in Table VII. The fungal fats shown in Table VII exhibit no unusual features and consist essentially of palmitic acid 15–32% together with oleic acid (32–45%) and linoleic acid (17–43%). Stearic acid is also present in considerable amounts (2–16%). Only in *Neurospora crassa* lipids does linolenic acid become a major component. The lipids of *Phycomyces blakesleeanus* (Bernhard and Albrecht, 1948) provide the sole exception in containing unusual fatty acids in the form

TABLE VII

The fatty acid composition (wt.-%) of fungal mycelia

Acid	*Aspergillus nidulans* (1)	*Neurospora crassa* (2)*	*Neurospora crassa* (3)‡	*Penicillium flavo-cinereum* (4)	*Penicillium lilacinum* (5)	*Penicillium spinulosum* (6)	*Penicillium soppii* (7)	*Choanephora-cucurbitarium* (8)	*Phycomyces blakesleeanus* (9)
Saturated									
Lauric	—	—	—	—	—	—	—	0·1–0·8	—
Myristic	0·7			0·3	0·1	trace	0·3	1·4–2·4	—
Palmitic	20·9	15	31	19·4	32·3	18·0	22·0	12·2–33·9	23·7
Stearic	15·9	—	2	9·9	9·4	11·9	7·6	1·2–8·9	4·7
C_{20} and above	1·4	—	—	0·7	0·7	1·4	0·9	—	5·0
Unsaturated									
Hexadecenoic	1·2	2†	2	1·5	3·4	3·8	3·3	3·4–11·2	—
Oleic	40·3	5	32	39·4	38·6	43·3	45·2	22·7–58·8	29·6
Linoleic	17·0	43	30	27·1	13·4	21·1	20·0	9·3–17·3	25·8
Linolenic	0·2	32	—	0·8	—	0·3	0·3	—	—
C_{20}–C_{22}	2·4	2	—	0·9	1·4	0·2	0·4	—	—
Unidentified	—	—	1	—	—	—	—	6·1–20·8§	‖

(1) Singh *et al.* (1955). (2) Todd *et al.* (1957). (3) Krzeminski *et al.* (1960). (4) Singh *et al.* (1956a). (5) Singh *et al.* (1956b). (6) Shimi *et al.* (1959). (7) Singh *et al.* (1957). (8) White and Quackenbush (1962). (9) Bernhard and Albrecht (1948).

* Submerged culture.

† Also 1% hexadecadienoic acid.

‡ Surface culture.

§ Results cover strain and environmental differences.

‖ Also octadeca-6,9,12-trienoic acid, 3·4%; tetracos-17-enoic acid, 2·9%; and a hexacosenoic acid, 4·9%.

of octadeca-6,9,12-trienoic acid (3·4%); tetracos-17-enoic acid (2·9%) and a hexacosenoic acid (4·9%).

In addition to the fungal lipids shown in Table VII Hartman *et al.* (1962) compared the fatty acid composition of the lipids of *Pithomyces chartarum* which have large amounts of both palmitic and linoleic acids with those of a dark coloured fungus, *Stemphylium dendriticum* from the same family—Dematiaceae—and a sample of hyaline or bright coloured fungus *Cylindrocarpon radicicola* from the related Tuberculariaceae family. All the fungi were grown on the same medium (Table VIII).

TABLE VIII

Component fatty acids of the total lipids of *Pithomyces chartarum* spores and mats, and from the mats of *Stemphylium dendriticum* and *Cylindrocarpon radicicola*

Acid	*Pithomyces chartarum*		*Stemphylium dendriticum*	*Cylindrocarpon radicicola*
	*Spores**	*Mats*†	*Mats**	*Mats**
		Saturated		
C_{11}	0·6	0·3	trace	0·2
Lauric	—	0·1	0·2	0·1
Branched-chain C_{13}	—	0·2	0·1	—
C_{13}	—	—	0·2	—
Myristic	0·8	0·6	0·6	0·3
Branched-chain C_{15}	—	0·2	—	—
C_{15}	trace	0·5	trace	0·1
Palmitic	21·6	18·5	18·6	23·8
Branched-chain C_{17}	—	0·3	—	0·1
C_{17}	—	1·7	0·1	0·2
Stearic	6·4	5·9	3·6	8·0
		Unsaturated		
Palmitoleic	1·3	1·3	1·6	0·7
Oleic	15·9	8·2	17·2	27·4
Linoleic	53·4	59·4	51·5	29·3
Linolenic	—	2·8	6·2	9·8
C_{20}	—	—	0·1	trace

Results expressed as fatty acids mole-%.
* Hartman *et al.* (1962).
† Hartman *et al.* (1960).

The results for the fungal mats of *P. chartarum* (Table VIII) have been adjusted to exclude minor constituents for closer comparison with those of the spores. It will be seen that the fatty acid compositions of the spores and mats of the two members of the Dematiaceae family are similar

and contain palmitic and linoleic acids as the principal constituents. The lipids of *Cylindrocarpon radicicola* which belongs to the same order (Moniliales) but to a different family, the Tuberculariaceae, differ in having a much lower content of linoleic acid but more oleic acid.

On the other hand, the lipids from *Penicillium* spp. and *Aspergillus nidulans* belonging to the Moniliaceae (Table VII) may be distinguished by their relatively higher oleic acid from those of the Dematiaceae and Tuberculariaceae species examined. Thus, although the fatty acids in the lipids of fungal mycelia are those commonly found in seed fats and leaf lipids, the distinctive fatty acid composition of the lipids from different families may have some taxonomic significance. Any broad generalizations in this direction, however, must also take into account the possibility of environmental factors. For example it appears that whereas the lipids of *Neurospora crassa* in submerged cultures contain much linolenic acid, those from surface cultures lack this constituent (see Table VII). Furthermore *Choanephora cucurbitarium*, a phycomycete, and therefore taxonomically distinct from the other fungi already considered, shows marked differences in the fatty acid composition of the lipids with changes in nutrient and other environmental factors. This taxonomic distinction is to some extent reflected in the fatty acid composition which generally shows a high content of palmitoleic acid, as well as in the presence of considerable amounts of unidentified constituents not noted in the lipids of the other fungi investigated.

The absence of palmitoleic acid in the lipids of *Phycomyces blakesleeanus*, another member of Phycomycetes class, may be due to the analytical technique used but the appearance of some higher molecular weight (above C_{18} constituents) in the lipids of both members of the Phycomycetes class may have some taxonomic significance.

In addition to the mycelial lipids we may consider the oil of ergot (*Secale cornutum*) sclerotium earlier examined by various investigators with conflicting results (cf. Hilditch, 1956). More recently Bharucha and Gunstone (1957) have re-examined this oil (Table IX) and confirmed

TABLE IX

The component fatty acids of ergot oil (Bharucha and Gunstone, 1957)

Saturated							
C_{14}	C_{16}	C_{18}	C_{20}	Po.	Oleic	Lin.	Ricinoleic
1·0	24·0	3·0	1·0	4·0	21·0	12·0	34·0

Results expressed as wt.-%.

Matthes and Kürschers' (1931) suggestion that the supposed epoxy acid present was ricinoleic (D(+)-12-hydroxyoctadec-*cis*-9-enoic) acid.

(b) *Spore oils*

The detailed investigations of Tulloch and Ledingham (1960) provide the main source of information on the fatty acid composition of the spore oils of plants rusts and of other fungi. Earlier information in this field is shown by Hilditch (1956) to be rather limited and somewhat uncertain.

Following the isolation by Tulloch, Craig, and Ledingham (1959) and by Tulloch (1960) of the hitherto unique *cis*-9,10-epoxyoctadecanoic acid from the uredospore oils of wheat steam rust *Puccinia graminis* var. *tritici*; this acid together with lesser amounts of (+)-*threo*-9,10-dihydroxyoctadecanoic acid was found in the spore oils of other *Puccinia* spp. as well as in those of *Phragmidium speciosum* (teliospore), *Cronartium harknessii* (aeciospore) and the uredospores of *Melampsora lini* and *Melampsora medueae* (Tulloch and Ledingham, 1960). The amount of epoxy acid in the *Puccinia* spp. varied from 4·8% to 31·3% but in *M. lini* it comprised 74·2% of the total fatty acids.

As the data dealing with the fatty acid composition of spore oils are rather extensive (cf. Appendix I), a few examples only have been selected (Table X). It will be seen that the fatty acid compositions of spore oils from the same genus grown on the same host are similar, but that with change of host there is a marked change in the composition of the oil although the types of fatty acids remain unaltered. The fatty acid compositions of the uredospore and teliospore oils of *P. hieracii* grown on the same host are shown to be similar. Likewise, as has been mentioned earlier, the spore and mycelial lipids of *Pithomyces chartarum* have almost identical fatty acid patterns. The observations suggest that the different stages of growth from the same species produce one type of oil only at least when fed on the same type of nutrient.

cis-9,10-Epoxyoctadecanoic acid was not found in the rust spore oils of *Uromyces psoraleae* (aeciospores), *Ravenelia hobsoni* (teliospores) and *Gymnosporangium juvenescens* (dried gelatinous horns), or in the chlamydospore oils of cereal smuts including *Tilletia foetens* and four *Ustilago* species, or in the oils of conidia of mildews including *Sphaerotheca humili* var. *fuliginia* and *Erysiphe graminis*. The chlamydospore oils of cereal smuts (*Ustilago* and *Tilletia*) are characterized by appreciable amounts of palmitoleic acid (3–10%) and of eicos-9-enoic acid (1–7·6%). The latter has hitherto been found only in fish oils. The oils of the conidia of mildews on dandelions are remarkable for the presence of long-chain fatty acids, the presence of 41·7% in the fatty acids from *Sphaerotheca humili* var. *fuliginia* being particularly noteworthy. The

TABLE X

Composition of oils found in spores of fungi (Tulloch and Ledingham, 1960)

Species	Saturated							Po.	Oleic	Lin.	Len.	Eicos.	*cis*-9,10-Epoxyoctadecanoic
	C_{12}	C_{14}	C_{15}	C_{16}	C_{18}	C_{20}	C_{22}						
Puccinia spp.: Uredospore oils of cereal rusts													
P. graminis var. *tritici* race 56	0·2	2·2	0·3	41·3	4·8	—	0·3	0·4	16·7	4·4	10·4	—	19·0*
Puccinia spp.: Spore oils of Compositae rusts													
P. hieracii (uredospore)	—	0·3	0·4	16·3	5·5	—	1·0	0·5	11·7	21·1	33·7	—	9·5
P. hieracii (teliospore)	—	0·3	0·4	17·1	4·6	—	0·5	0·3	15·1	19·2	34·0	—	8·5
Other rust spore oils													
Phragmidium speciosum (teliospore)	—	0·4	—	10·4	3·0	0·4	0·7	0·6	14·7	20·1	42·3	—	7·5
Chlamydospore oil of cereal smuts													
Tilletia foetens	—	0·5	—	14·6	1·0	0·3	0·4	3·0	8·8	63·2	—	1·2†	—

* Some samples also contained 5·0–11·0% of (+)-*threo*-9,10-dihydroxyoctadecanoic acid.

† Also C_{16} with two double bonds, 1·2% eicos-9-enoic acid and unidentified, 6·0%.

rust spore oils of *Ravenelia hobsoni*, *Uromyces psoraleae* and *Gymnosporangium juvenescens*, on the other hand, possess a simple fatty acid composition apart from minor amounts of unidentified components.

In conclusion it cannot be said that the spore fungal oils exhibit a uniform fatty acid composition. Throughout the saturated fatty acids amount to more than 10% and usually exceed 20%. The unsaturated acids appear to consist typically of oleic and linoleic acids accompanied sometimes with substantial amounts (up to 54%) of linolenic acid. In certain groups there appear characteristic acids such as *cis*-9,10-epoxyoctadecanoic, eicos-9-enoic and behenic (Table XI).

TABLE XI

Uncommon fatty acids found in yeast and fungal lipids

Species	Fatty acid	Amount (% total fatty acids)
	Yeasts	
Torulopsis magnoliae Oil produced during fermentation of glucose	L-17-Hydroxyoctadecanoic and L-17-Hydroxy-9-octadecenoic	Main fatty acids
	Fungi	
(a) Mycelial lipids		
Phycomyces blakesleeanus	Octadeca-6,9,12-trienoic	3·4
	Tetracos-17-enoic	2·9
	Hexacosenoic	4·9
(b) Sclerotial oils		
Ergot (*Secale cornutum*)	D(+)-12-Hydroxyoctacec-*cis*-9-enoic	34
(c) Spore oils		
Puccinia sp. (Uredospores) Also other species	*cis*-9,10-Epoxyoctadecanoic	4·8–74
Puccinia graminis var. *tritici* Race 56 (Uredospores)	(+)-*threo*-9,10-Dihydroxy-octadecanoic	Up to 5
Tilletia foetens and *Ustilago* spp. (Chlamydospores)	Eicos-9-enoic	1·0–7·6
Sphaerotheca humili var. *fuliginia* (Conidia)	Behenic	41·7

3. Other cryptogam lipids

The lipids of other Cryptogams have not been investigated to any appreciable extent but it may be significant that the oil in the spores of *Lycopodium clavatum* (order Lycopodiales) contains 30–35% palmitoleic

acid as well as some (+)-*threo*-9,10-dihydroxyoctadecanoic acid (Riebsomer and Johnson, 1933). The occurrence of substantial amounts of palmitoleic acid in *L. volubile* has also been indicated (Morice, 1962).

B. Phanerogam Lipids

Fats other than seed, fruit coat and leaf fats have not been investigated sufficiently to establish any useful taxonomic relationships. It appears (cf. Hilditch, 1956), that in general, bark, stem, root and flower fats contain only the common fatty acids. However, as will be discussed later, the unusual acetylenic acids present in the seeds of certain Olacaceae and Santalaceae species are reflected in the lipids of the roots and other parts of the plant (Chapter 9).

1. Leaf lipids

It appears that the leaf lipids often amount to some 7% of the dry matter. The main fraction is soluble in acetone at room temperature while the remainder, comprising perhaps 30% of the total lipids, is made up of phospholipids and waxes (cf. Shorland, 1953). The acetone soluble fraction was formerly believed to consist entirely of fats, but it is now clear that triglycerides are almost entirely absent, and that their place is taken by galactolipids of the type originally found by Carter, McCluer and Slifer (1956) in wheat flour lipids (cf. Shorland, 1961).

Since Hilditch (1956) reviewed the data on the fatty acid composition of leaf lipids some further analyses have appeared (see Table XII), which generally support the earlier data in showing that linolenic acid is usually a major or chief fatty acid constituent. Crombie's (1958) results (Table XIII) show that the lipids of green as compared with those of non-green leaves contain much more linolenic but less linoleic and oleic acids, and generally confirm the high levels of linolenic acid in green leaves. Although the lipids of green leaves contain, as a rule, much more linolenic acid and less amounts of linoleic acid than those of non-green leaves these differences are less marked in some species, such as *Ilex aquifolium* and *Ligustrum ovatifolium*.

The recent analyses by Debuch (1961) are interesting in showing the appearance of hexadec-*trans*-3-enoic acid as well as hexadeca-*cis*-7,*cis*-10,*cis*-13-trienoic acid in the leaf lipids of *Spinacea oleracea* and of *Antirrhinum majus*. The hexadecatrienoic acid was first reported by Heyes and Shorland (1951) as a major constituent of the acetone-soluble lipids of *Brassica napus* leaves. However, the extensive ester fractionation of the methyl esters of rye grass *Lolium perenne* lipids failed to reveal any evidence for the presence of this acid (Shorland, unpublished).

TABLE XII

Fatty acid composition of leaf lipids

Species	Saturated						Unsaturated						
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{12}	C_{14}	Po.	Oleic	Lin.	Len.	C_{20}–C_{22}
						GRAMINEAE							
Lolium perenne (1)	0·4	1·4	10·6	1·5	0·4	—	0·2	0·5	4·1	4·6	11·6	62·8	1·9
Pasture grasses (2)	—	1·1	15·9	2·0	0·2	0·3	—	—	2·5	3·4	13·2	61·3	—
						LEGUMINOSAE							
Trifolium repens (1)	—	1·1	6·5	0·5	2·0	—	—	0·8	2·5	6·6	18·5	60·7	0·8
						CHENOPODIACEAE							
Spinacea oleracea (3)	—	0·2	12·9	trace	0·6	—	—	—	7·2*	6·6	16·3	56·2	trace
						SCROPHULARIACEAE							
Antirrhinum majus (3)	—	0·1	13·4	2·4	1·8	—	—	—	1·3	1·8	17·7	57·9	3·6
Chloroplasts (3)	—	trace	9·5	1·0	—	—	—	—	1·5†	1·6	15·1	71·3	—
						URTICACEAE							
Urtica dioica (4)	—	(0–5)		—	—	—	—	—	—	82–86	13–14	(?)	—

Results expressed as % component fatty acids. The data given in references (1), (2) and (4) are on a wt.-% basis, while those given in reference (3) are on a molar-% basis.

(1) Shorland (1955) (acetone soluble fractions). (2) Garton (1960). (3) Debuch (1961). (4) Hilditch *et al.* (1944).

* Comprises 2·6% hexadec-*trans*-3-enoic and 4·6% hexadeca-*cis*-7,*cis*-10,*cis*-13-trienoic.

† Includes 0·2% hexadecatrienoic acid.

The occurrence of an unusual acid in leaf lipids is not necessarily reflected in the seed fats (Table XIV), and vice versa. Thus the hexadecatrienoic acid mentioned above is not found in the seed oils of rape or any other species so far examined. On the other hand, erucic (docos-*cis*-13-enoic) acid, the main fatty acid of the seed oils of rape and of other cruciferous species, does not occur in the leaf lipids of rape or in those of any other species so far examined. The evidence suggests that the compositions of leaf and seed lipids are generally quite unrelated.

TABLE XIII

Comparison between the fatty acid composition of the lipids of green and non-green leaves. Fatty acids expressed as % chromatographically determined acid (Crombie, 1958)

Species and type of leaf	Saturated				Unsaturated					
	C_{14}	C_{16}	C_{18}	As C_{22}	Po.*	Oleic	Lin.	Len.	Eicos.	As docos.
Vicia faba										
Aetiolated	—	16·7	4·7	4·6	—	0·0	33·5	39·4	1·0	—
Small green	—	11·7	3·2	4 0	6·9	3·4	14·3	56·4	0·4	—
Large green	—	7·1	1·4	4·4	10·1	4·6	9·7	61·1	1·6	—
Chloroplast	—	7·4	1·2	1·2	9·2	5·2	2·6	72·0	1·4	—
Acer negundo										
White	1·0	12·9	3·2	29·2	6·5	11·7	10·1	15·9	2·5	7·0
Green	0·6	11·0	1·0	5·2	3·9	6·8	11·4	51·5	2·1	6·5
Chloroplast	2·4	12·6	2·4	—	7·2	10·0	3·8	61·1	0·5	
Zea mays										
White	—	13·4	2·8	6·6	0·9	10·1	36·2	27·3	2·7	—
Green	—	8·0	1·8	2·1	2·2	6·9	7·0	69·0	1·4	1·7
Ilex aquifolium										
White	—	17·8	7·4	13·3	0·5	11·5	15·6	20·7	5·3	
Green	—	20·5	3·2	3·8	3·3	14·1	10·7	23·7	0·8	
Ligustrum ovatifolium										
Yellow	1·5	14·8	4·7	2·5	4·7	14·7	6·6	39·0	2·2	1·3
Green	1·3	11·8	2·7	3·8	5·8	20·5	6·4	43·3	0·3	4·0

* In the original table the octadecadienoic acid was determined chromographically as well as by u.v. spectroscopy. Where higher values are obtained by chromatography than by u.v. the difference has been attributed to palmitoleic acid.

Instances may be found, however, where the occurrence of an unusual acid in the seed fat is reflected in the lipids of other parts of the plant. Thus the cyclopropene acids, malvalic and sterculic (Table XXII), are found in both seed and leaf lipids of some species of Malvaceae and

Sterculiaceae. Ximenynic acids, present in seed fats of certain members of the Olacaceae and Santalaceae, also occur as such, or in a related form, in other parts of the plant. The distribution of these acids will be discussed later (p. 288). It should be remarked, however, that the occurrence of an unusual acid both in the lipids of the seeds as well as in other parts of the plants, appears to have special taxonomic significance.

TABLE XIV

The component fatty acids of the seed oil (1) and of the leaf lipids (2) of rape (*Brassica napus*)

	Saturated				Unsaturated											
	C_{16}	C_{18}	C_{20}	C_{22}	C_{14} mono	C_{16} mono	C_{16} di	C_{16} tri	C_{18} mono	C_{18} di	C_{18} tri	C_{20} mono	C_{20} di	C_{22} mono		
Seed	3	0·9	0·6	0·2	—	0·5	—	—	14	15	8	8	0·2	50		
Leaf	15				0·5			11			67			—		
	to	—	—	—	to	—	—	to	—	—	to	—	—			
	16*				0·7			17†			71‡					

(1) Mikolajczak *et al.* (1961). (2) Shorland (1945); Heyes and Shorland (1951).
* Mainly palmitic acid.
† Contains small amounts of monounsaturated.
‡ Contains small amounts of mono- and diunsaturated.

The present data along with the earlier evidence of Hilditch (1956) show that leaf lipids contain substantial amounts of linolenic acid as well as linoleic acid (or isomers), with oleic acid as a minor constituent. The content of saturated acid does not, as a rule, exceed 20%, and the main component is palmitic acid. The most substantial deviation in fatty acid distribution is to be found in the nettle (*Urtica dioica*) leaf lipids which contain mainly oleic acid (Table XII), while the seed fat, incidentally, contains 7% saturated together with 79% linoleic, 12% oleic and 2% linolenic acids (cf. Hilditch, 1956).

Part of the variations in the fatty acid composition can be ascribed to the types of lipids present. For example the phospholipids (cf. Shorland, 1962) of leaves contain somewhat more linoleic and less linolenic than do the acetone soluble fractions. The galactolipid fatty acids, on the other hand, appear to consist almost entirely of linolenic acid (cf. Weenink, 1961; Shorland, 1961).

The information on the fatty acid composition of leaf lipids is not sufficiently detailed or extensive to have any great taxonomic value. However, the occurrence of several unusual fatty acids such as hexadeca-7,10,13-trienoic as well as hexadec-*trans*-3-enoic acids offers the

hope that further work might prove fruitful. Differences between fatty acid composition of seeds and leaf lipids should also be further investigated as to their taxonomic possibilities.

2. Fruit coat fats

The fruit coat fats from sixteen families including Anacardiaceae, Burseraceae, Caprifoliaceae, Capparidaceae, Caryocaraceae, Celastraceae, Cucurbitaceae, Elaeagnaceae, Euphorbiaceae, Lauraceae, Meliaceae, Myricaceae, Oleaceae, Palmae, Sterculiaceae and Valerianaceae have been investigated. Apart from the family Cucurbitaceae no new families have been investigated since Hilditch's (1956) survey. In general, as indicated by Hilditch's (1952) simplified summary (Table XV), the fatty acid composition of fruit coat fats is simple but their consistency ranges from oils liquid at room temperature to hard wax-like substances which do not melt below about 50°. The fatty acids consist usually of palmitic and oleic acids with lesser amounts of linoleic acids. Hilditch (1956) notes the occurrence of about 5% (C_{20}–C_{21}) dibasic acids in sumach tallow, of notable amounts (up to 60%) of myristic acid in some *Myrica* spp. (Myricaceae) and of 23% linolenic acid in *Celastrus paniculatus* (Celastraceae).

TABLE XV

Composition of fruit coat fats (Hilditch, 1952)

Family	Species	Palmitic	Oleic	Linoleic
OLEACEAE	*Olea europaea* (Olive oil)	10	70–80	7–10
LAURACEAE	*Laurus nobilis* (Laurel oil)	20	63	14
PALMAE	*Elaeis guineensis* (Palm oil)	35–43	40–50	7–10
EUPHORBIACEAE	*Sapium sebiferum* (Stillingia tallow) (Syn. *Stillingia sebifera*)	66	30	—
ANACARDIACEAE	*Rhus* spp. (Sumach tallow)	75	13	—

Results expressed as fatty acids wt.-%.

As with leaf lipids, fruit coat fats bear, as a rule, little resemblance to the seed fats from the same species. Thus the seed fats of Lauraceae and Palmae are rich in lauric acid, which is found in minor amounts, if at all, in the fruit coat fats of these species (cf. Hilditch, 1956). However, in contrast, the seed oil of the olive (*Olea europaea*) does not differ materially in composition from that of the fruit coat fat.

The newer data on fruit coat fats (Table XVI) add very little to that collected by Hilditch (1956). For example, the results obtained by Prevot

and Cabeza (1962) for the fruit coat fats of *Persea gratissima* using gas–liquid chromatography agree with those obtained earlier including confirmation of the occurrence of palmitoleic and arachidic acids.

TABLE XVI

Composition of fruit coat fats

Component fatty acids (%)

Family and species	Saturated					Po.	Oleic	Lin.	Len.
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}				
CUCURBITACEAE	—	—	32	—	—	—	30·8	23·4	9·3
*Trichosanthes cucumeroides**	Also conjugated dienoic 4·0, trienoic 0·2								
EUPHORBIACEAE									
Sapium sebiferum†	0·3	4·2	62·3	5·9	—	—	27·4	—	—
LAURACEAE									
Persea gratissima‡	—	—	16·7	0·4	1·0	5·7	67·8	8·3	—

* Kato (1961).
† Narang and Sadgopal (1958).
‡ Prevot and Cabeza (1962)

The number of species in any of the families mentioned in connection with the composition of their fruit coat fats is small, but those of the four genera of Lauraceae show a degree of uniformity in their fatty acid composition. When additional data for genera of other families come to hand it is therefore possible that the composition of fruit coat oils will have some taxonomic value.

3. Seed fats

The classification of seed fats on the basis of their chief component fatty acids as developed by Hilditch (1956), is followed in outline in this review (Table XVII). In the first instance those acids which are most commonly found in seed fats are considered. These comprise palmitic, oleic, linoleic and to a lesser extent linolenic acid. Seed fats containing essentially these acids only are subdivided broadly into drying, semi-drying and non-drying oils on the basis of their mean unsaturation, as determined by the relative content of oleic, linoleic and linolenic acids. Within this broad group may be distinguished linolenic-rich and linoleic-rich seed fats each containing typically less than 10% palmitic acid. Another category of seed fats is recognized by a somewhat higher content of palmitic acid

TABLE XVII

Classification of seed fats*

Group	Major component acids	Families	Species
I	Linoleic, and/or linolenic with oleic		
	(a) Linolenic-rich	10 (5)	29 (37)
	(b) Linoleic-rich	23 (12)	66 (118)
II	Linoleic, oleic with linolenic or a conjugated polyethenoid acid (elaeostearic, licanic, etc.)	Cucurbitaceae, Euphorbiaceae and Rosaceae (4)	81 (40)
III	Palmitic, oleic and linoleic	22 (6)	85 (40)
IV	Palmitic, oleic, linoleic and specific fatty acids		
	(1) Unsaturated acids		
	(a) Petroselinic	Umbelliferae and Araliaceae	13 (10)
	(b) Ethynoid	Simaroubaceae, Olacaceae and Santalaceae	13 (5)
	(c) Cyclic	Flacourtiaceae (Malvaceae, Sterculiaceae and Styracaceae)	18 (10)
	(d) Eicosenoic†	Sapindaceae, Buxaceae (Limnanthaceae and Cruciferae)	(7)
	(e) Erucic	Cruciferae and Tropaeolaceae	35 (53)
	(f) Monosaturated n-C_{24} and above	Olacaceae (*Ximenia* spp.) (Leguminosae and Cruciferae)	3
	(2) Saturated acids		
	(g) Saturated n-C_{20} and above	Leguminosae, Moringaceae, Ochnaceae and Sapindaceae (Strychnaceae)	60 (30)
	(h) Stearic	10	61 (10)
	(i) Myristic and lauric	Lauraceae, Palmae Myristicaceae, Simaroubaceae, Vochysiaceae and Salvadoraceae	49 (8)
	(j) Capric‡	Ulmaceae (Lauraceae and Lythraceae)	2 (8)

* The data are based on Hilditch's 1956 data with the addition of the new material following the completion of this survey shown in brackets.

†‡ Seed oils from these groups are not listed separately by Hilditch (1956) but are therein discussed in relation to Groups IVe and IVi respectively.

together with substantial amounts of oleic and linoleic acids. These groups naturally embrace a considerable number of botanical families.

Other seed fats which contain as a major component, a characteristic fatty acid, other than palmitic, oleic, linoleic or linolenic acid, are grouped together under the fatty acid concerned. In this system it is convenient to list the names of families which include species whose fats fall into line with the fatty acids upon which the classification is based. This leads in many instances to the grouping together of members of the same family but does not necessarily imply that all members of a given family will invariably appear in the same group. In the Hilditch (1956) system it will sometimes happen, therefore, that the same family will appear under two different classifications if its members fall into separate categories. Thus the Ulmaceae appears under the linoleic-rich fats (Group Ib), as well as under the fats which contain n-decanoic (capric) acid as the main constituent (Group IVj). Similarly, the Malvaceae is found in the palmitic oleic, linoleic group (Group III) as well as in the cyclic acid group (Group IVc).

Table XVII summarizes the various groups (Ia–IVj) of seed fats. In the discussion which follows it is convenient to illustrate the groups by including one or two examples of seed fats and to indicate the remainder of the material by the references to the literature in the Appendix.

(i) *Group I*

Seed fats with (a) linoleic, and or (b) linolenic with oleic acids as major components; minor components, palmitic and stearic acids.

Group Ia. Linolenic-rich seed fats (Table XVIII). In this group are represented: Gymnospermae—Pinaceae, Taxodiaceae;* Angiospermae—Actinidiaceae*, Aquifoliaceae, Boraginaceae*, Celastraceae, Elaeagnaceae, Ericaceae,* Labiatae, Linaceae, Moraceae, Onagraceae (Oenotheraceae)†, Paeoniaceae*, Rhamnaceae and Valerianaceae‡.

The best-known example of the linolenic acid-rich group is that of linseed (*Linum usitatissimum*) oil (Linaceae), cf. Hilditch, 1956). This oil, together with the seed oil of *Perilla frutescens* (Labiatae) (Earle *et al.*, 1960b), illustrates the linolenic acid-rich seed fat.

The present results cover seventeen species from families already listed by Hilditch 1956 and ten species from five additional families. In connection with the additional families, the analyses by Earle *et al.* (1960a) do not clearly place *Cryptomeria japonica* (Taxodiaceae), but the high

* Additional to Hilditch's 1956 list.
† Transferred to Group Ib.
‡ Transferred to Group II.

iodine value of the oil suggests that linolenic acid is likely to be an important constituent. Similarly, *Actinidia arguta* seed oil (Actinidiaceae) has been placed in this group on account of its high content of trienoic acid (Earle *et al.*, 1960a), but the considerable content of C=O as C_{18} acid together with its 10% ROH in the infra-red could demand reconsideration when more definite evidence comes to light. The appearance of a tetraene acid in two members of the Boraginaceae family is interesting as there is no evidence of conjugation (Earle *et al.*, 1959). Hitherto the only tetraenoic acid found in seed fats has been parinaric $CH_3CH_2(CH{=}CH)_4(CH_2)_7COOH$ found in *Parinarium* spp. (Rosaceae) (cf. Hilditch, 1956).

TABLE XVIII

Group Ia: Linolenic acid-rich seed fats

Species	Saturated	Oleic	Lin.	Len.	References
Linum usitatissimum	6–16*	13–36	10–25	30–60	Hilditch (1956)
Perilla frutescens	9	21	11	55	Earle *et al.* (1960)

Results expressed as component fatty acid %.
* Mainly palmitic acid.

Hilditch (1956) placed the seed fats of the family Valerianaceae in Group Ia on the basis of results for one species (*Valerianella olitorea*) containing only 9% linolenic acid. The present example (*Valeriana officinalis* seed fat) shows 50% α-elaeostearic acid (Earle *et al.*, 1959) indicating that perhaps the seed fats from this family belong to Group II. The two members of the Celastraceae family now recorded (Simonova, 1959; Earle *et al.*, 1959) show a low content of linolenic acid, together with a relatively high content of saturated acids. Hilditch's (1956) list shows that two members of this family have appreciable amounts (16–39%) of linolenic acid, and in the third no linolenic acid is recorded. The saturated acid content of the species examined ranges from 13·1 to 32·0%. Thus this family does not fit well into Group Ia, but is also something of a misfit in Group III on account of the presence of substantial amounts of linolenic acid in two of the species. As already recorded by Hilditch (1956), the evening primrose seed oil, *Oenothera biennis* (Onagraceae), contains an isomer of linolenic acid (octadeca-6,9,12-trienoic acid), (Eibner *et al.*, 1927a, b), linolenic acid itself being absent.

In the new series of results for three other species of Onagraceae, the seed oils show less than 10% trienoic acid, and hence fit better in Group Ib. In addition the seed fats of *Clarkia elegans* (Onagraceae) contain

substantial amounts of *cis*-12,13-epoxyoctadec-*cis*-9-enoic (vernolic) acid (Smith, Jr., *et al.*, 1960b).

Apart from *Stachys lanata* (Earle *et al.*, 1959) the Labiatae seed oils are generally as rich (40–70%) in linolenic acid as linseed oil. Other members of Group Ia, however, contain less than 40% linolenic acid in their seed oils with correspondingly more linoleic acid.

With the transfer of the Onagraceae to Group Ib, and of the Valerianaceae to Group II, the remainder of the seed fats in Group Ia show no unusual fatty acids apart from the occurrence of small amounts of the tetraene acids in two members of the Boraginaceae family.

TABLE XIX

Group Ib: Linoleic-rich seed fats

Family	Species	C_{16}	C_{18}	Oleic	Lin.	Len.	
			Saturated				
Juglandaceae	Walnut (*Juglans regia*)	7·0	1·0	16	72	4	Griffiths and Hilditch (1934)
Agavaceae	*Cordyline australis*	8·1	0·8	7·3	83·8	—	Morice (1962)
Pedaliaceae	Sesame (*Sesamum indicum*)	8·2	3·6	45·3	41·2*	—	Hilditch and Riley (1945)

Results expressed as component fatty acids %.
* Also 1·1% arachidic and 0·5% hexadecenoic acid.

Group Ib. Linoleic-rich seed fats (Table XIX): In this group, the saturated acids usually form less than 12%, with less than 10% palmitic acid. Linoleic acid frequently amounts to over 50%, but sometimes oleic acid predominates. Linolenic acid is absent or present in small amounts only. Families represented are: Gymnosperms—Podocarpaceae*; Angiosperms—Agavaceae*, Amaryllidaceae*, Asclepiadaceae, Betulaceae, Caprifoliaceae†, Casuarinaceae*, Compositae, Dipsaceae, Fagaceae, Hamamelidaceae*, Helleboraceae*, Hippocastanaceae, Iridaceae*, Juglandaceae, Liliaceae;* Loasaceae*, Lobeliaceae*, Myrtaceae, Onagraceae (Oenotheraceae)‡, Olacaceae,, Oleaceae, Papaveraceae, Passifloraceae, Pedaliaceae, Plantaginaceae Polemoniaceae*, Proteaceae, Ranunculaceae*, Scrophulariaceae Solanaceae, Staphylaceae, Symplocaceae*, Theaceae, Typhaceae, Ulmaceae, and Urticaceae.

* Additional to Hilditch's (1956) list.
† Transferred from Group III (see under Group III).
‡ Transferred from Group Ia.

In the present survey, the linoleic acid-rich seed Group Ib has been increased by the addition of fourteen new families. Of these the family Agavaceae (Monocotyledonaea) provides many examples of seed fats with 75–89% of linoleic acid (Morice, 1962), which is somewhat higher than found in the families listed by Hilditch (1956). Of the remaining thirteen new families not listed by Hilditch, the Podocarpaceae and Iridaceae can only be tentatively placed in Group Ib on the rather slender evidence of iodine value, while the other families are represented in many instances by seed fats from one species only. On the other hand, the twelve species of Agavaceae provide excellent examples of linoleic-rich seed fats uncomplicated by the presence of any substantial amounts of other unsaturated acids apart from oleic acid, and containing typically less than 10% saturated (palmitic) acid.

Other seed fats in addition to those of the Agavaceae which contain more than 50% linoleic acid are to be found in the Ulmaceae, Vitaceae, Liliaceae, Onagraceae, Papaveraceae, Passifloraceae, Gramineae, Scrophulariaceae and Solanaceae. In other families included in Group Ib the seed fats typically contain 30–50% linoleic acid but in a few instances less than 20% of this acid is encountered.

Dealing with the families individually, the new results for Asclepiadaceae support Hilditch's inclusion of this family in Group Ib. The seed fats of *Asclepias syriaca* have been shown to contain substantial amounts of palmitoleic acid (10%); together with hexadeca-9,12-dienoic, 2%, and *cis*-vaccenic, 15% (Chisholm and Hopkins, 1960a). In the Compositae, earlier work (Gunstone, 1954) showed the occurrence of *cis*-12,13-epoxyoctadec-*cis*-9-enoic acid as a major constituent of *Vernonia anthelmintica* seed oil. The screening analyses by (Earle *et al.*, 1960a, c) confirm Gunstone's (1954) work, and in addition indicate a number of other seed fats from Compositae species with high epoxy values, though these are lower than found in the seed fats of *V. anthelmintica* and usually associated with a conjugated diene. The most notable example of seed oils with high epoxy values being that of *Dimorphotheca aurantiaca*. Smith, Jr., *et al.* (1960c) subsequently isolated 9-hydroxy-*trans*-10,*trans*-12-octadecadienoic acid from this seed fat. From *Tragopogon portifolius* seed fat, Chisolm and Hopkins (1959) isolated *cis*-9,10-epoxystearic acid. Thus although the Compositae in broad outline conform to Group Ib, their seed fats obviously also contain small amounts of components other than those normally associated with this Group (cf. Table XX).

In the Olacaceae, Hilditch (1956) refers to the occurrence of ricinoleic (12-hydroxyoctadec-9-enoic) acid as the main constituent of ivory wood (*Agonandra brasiliensis*) seed oil. Other members of this family whose seed oils are listed by Hilditch (1956) include *Coula edulis* with 95% oleic

acid, and *Mappia foetida* with 36·8% linolenic acid and no linoleic acid. The seed fats of the two remaining species of Olacaceae, *Ximenia americana* and *Ongokea gore*, listed by Hilditch (1956) are low in linoleic acid (10% or less) and have been placed in Group IVb on account of the presence of substantial amounts of acetylenic acids. The seed fats of the Olacaceae do not therefore comprise a uniform group and there is a general lack of correlation between fatty acid composition and botanical classification.

TABLE XX

The occurrence of new or unusual fatty acids in seed fats of Group Ib

Acid	Family and species	% Total fatty acid
	(a) Asclepiadaceae	
Octadec-*cis*-11-enoic (*cis*-vaccenic); also Hexadeca-*cis*-9,*cis*-12-dienoic (1)	*Asclepias syriaca*	15; 2
	(b) Compositae	
Octadeca-*trans*-8,*trans*-10,*cis*-12-trienoic (2)	*Calendula officinalis*	47
cis-9,10-Epoxyoctadec-*cis*-12-enoic (coronaric) (3)	*Chrysanthemum coronarium*	2·8–15·8
9-Hydroxyoctadeca-*trans*-10,*trans*-12-dienoic (dimorphecolic) (4)	*Dimorphotheca aurantiaca*	Major component
cis-9,10-Epoxyoctadecanoic (5); also 9-hydroxyoctadeca-10,12 and 13-hydroxyoctadeca-9,11-dienoic (2)	*Tragopogon porrifolius*	3; 4
cis-12,13-Epoxyoctadec-*cis*-9-enoic (vernolic) (6)	*Vernonia anthelmintica*	Major component
cis-9,10-Epoxystearic (7)	*Trapopogon porrifolius*	10
	(c) Olacaceae	
12-Hydroxyoctadec-9-enoic (ricinoleic) (8)	*Agonandra brasiliensis*	50
	(d) Proteaceae	
Hexadec-*cis*-9-enoic (palmitoleic) (8)	*Macadamia ternifolia*	20

(1) Chisholm and Hopkins (1960a). (2) Chisholm and Hopkins (1960b). (3) Smith, Jr., *et al.* (1960b). (4) Smith, Jr., *et al.* (1960c). (5) Chisholm and Hopkins (1957). (6) Gunstone (1954); Earle *et al.* (1960a). (7) Chisholm and Hopkins (1959). (8) Cf. Hilditch (1956).

The inclusion of six further seed oils from the family Papaveraceae in addition to the two already described by Hilditch (1956) confirms that the seed oils from this family conform well to Group Ib. In the Proteaceae,

Hilditch (1956) has noted the occurrence of 20% palmitoleic acid in the seed oil of *Macadamia ternifolia*.

Hilditch (1956) placed the seed fats of the Scrophulariaceae in Group Ib on the basis of the results obtained for one species only. This decision is now supported by twelve further species included in the present review, but in *Penstemon albidus* the occurrence of 20% of keto acid was noted by Earle *et al.* (1959).

In connection with the Ulmaceae, it was formerly believed by Hilditch (1956) that the seed fats of the genus *Ulmus* were unusual in containing capric acid as the main constituent. As will be noted in Group IVj, *Zelkova serrata*, also from this family, possesses seed fats rich in capric acid (Earle *et al.*, 1960a; Hopkins and Chisholm, 1959). This sharp division of the seed fats of Ulmaceae into two distinct categories comprising linoleic-rich and capric-rich may stimulate further enquiry into the botanical classification of this family.

The irregularities in composition of the Group Ib seed fats are indicated in Table XX. From this table it will be seen that there are a considerable number of unusual acids in Group Ib including *cis*-vaccenic (Asclepediaceae), ricinoleic (Olaceae), vernolic (Onagraceae and Compositae). This last family provides also examples of other epoxyacids (*cis*-9,10-epoxyoctadecanoic acid) as well as of hydroxy conjugated dienoic and of conjugated trienoic acids.

(ii) *Group II*

Seed fats containing linoleic and oleic with linolenic or a conjugated polyethenoic acid (α-elaeostearic, licanic, etc.) as major component acids. Minor components; palmitic and stearic acids. Included in this group are the following botanical families: Balsaminaceae*, Bignoniaceae*†, Cucurbitaceae, Euphorbiaceae, Punicaceae*, Rosaceae and Valerianaceae‡.

The variability in the fatty acid composition of this group does not permit the inclusion of a representative example for the fatty acid composition of a Group II seed fat. However, the unusual nature of the fatty acid pattern is perhaps indicated by that of China wood or tung (*Aleurites fordii*, or *A. montana*) seed oil, as follows: Saturated, 3–5%; oleic, 4–10%; linoleic, 8–15%; α-elaeostearic, 71–82% (cf. Hilditch, 1956).

The extended data on seed fats have revealed two further botanical families whose seed fats are classifiable under Group II. The Balsaminaceae, two species with parinaric (octacdeca-9,11,13,15-tetraenoic) acid

* Not included in Hilditch's 1956 list.
† Transferred from Group III.
‡ Transferred from Group Ia.

(Sarkar and Chakrabarty 1956b; Kaufmann and Sud, 1960) and the Punicaceae, one species with conjugated trienoic acid (Earle *et al.*, 1960a). The Bignoniaceae were placed by Hilditch (1956) in Group III on the basis of results for one seed oil only from *Crescentia cujete*. However, Hopkins and Chisholm (1962) have shown that octadeca-*trans*-9,*trans*-11,*cis*-13-trienoic acid (a stereoisomer of α-elaeostearic acid) is a major constituent of *Catalpa ovata* seed oil. In addition two more species of Bignoniaceae have been investigated, *Catalpa bignoniodes* (Markman and Bodnya, 1957*; Earle *et al.*, 1960a) and *Chilopsis linearis* (Earle *et al.*, 1960a), and in both the seed oils contained substantial amounts (21–31%) of conjugated trienoic acids. The transfer of the family Valerianaceae from Group Ia to Group II on account of the presence of α-elaeostearic acid in *Valeriana officinalis* has already been mentioned.

In the Cucurbitaceae Hilditch (1956) records some six instances from twenty-four species with seed fats containing trichosanic (octadeca-*cis*-9, *cis*-11,*trans*-13-trienoic) acid, one example with α-elaeostearic (octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic), and two examples with linolenic acid as a major constituent. However, many of the seed fats of the Cucurbitaceae conform to the linoleic-rich Group Ib. In the new series of examples, including sixteen species, there are three or four in which trichosanic acid is probably present, and one where linolenic acid constitutes the major trienoic acid. The seed fats of the Cucurbitaceae do not therefore conform well to the Group II pattern.

Most of the seed fats from thirty-three species of Euphorbiaceae recorded by Hilditch (1956), on the other hand, are rich in polyenoic acids and conform to the Group II pattern, as do most of the nine species examined since 1956. Some of the seed oils in the Euphorbiaceae, listed by Hilditch (1956), such as those from Japanese tung (*Aleurites cordata*) are rich in α-elaeostearic acid. In the seed oils of other species, such as conophor (*Tetracarpidium conophorum*,) which contains much (63–68%) linolenic acid, there are no unusual acids. Castor (*Ricinus communis*) seed oil stands apart with its high content of ricinoleic (12-hydroxy-octadec-*cis*-9-enoic) acid (Hilditch, 1956). Another exception is the seed oil of *Cephalocroton cordofanus* whose main constituent is vernolic (*cis*-12,13-epoxyoctadec-*cis*-9-enoic) acid (Bharucha and Gunstone, 1956).

Other examples of the occurrence of unusual fatty acids in the Euphorbiaceae include that of ω-hydroxyelaeostearic acid, the main constituent in Kamala (*Mallotus philippinensis*) seed oil (Calderwood and Gunstone, 1953; Gupta, Gupta and Aggarwal, 1954); *deca-trans*-2,*cis*-4-dienoic acid in minor amounts in (*Sapium sebiferum*) seed oils (Crossley and Hilditch, 1949, 1952; Devine, 1950; Crombie, 1955); and the corresponding

* Quoted by Hopkins and Chisholm (1962).

dodeca-2,4-dienoic acid which occurs in *Sebastiana ligustrina* seed oil (Hanks and Potts, 1951) (Table XXI).

The Rosaceae seed fats described by Hilditch (1956) comprise some twenty-four species and, with the exception of *Prunus* spp. and of *Prinsepia utilis*, all have polyethenoid acids including licanic (4-keto-octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic) acid and parinaric (octadeca-9,11,13,15-tetraenoic) acid, with two or three *trans*- bonds. In the nine seed fats of Rosaceae now recorded three of the four *Prunus* spp. appear to lack any substantial amounts of polyethenoid acids, as do three other species. The seed fats of *Licania rigida* and of *Parinarium* spp. conform to those reported earlier by Hilditch (1956) in containing substantial amounts of α-elaeostearic and licanic acids.

TABLE XXI

Unusual fatty acids found in Group II seed fats

Acid	Species	Family	% Total fatty acids
12-Hydroxyoctadec-*cis*-9-enoic (ricinoleic) (1)	*Ricinus communis*	Euphorbiaceae	90
cis-12,13-Epoxyoctadec-*cis*-9-enoic (vernolic) (2)	*Cephalocroton cordofanus*	Euphorbiaceae	33
18-Hydroxyoctadeca-*cis*-9,*trans*-11,*trans*-13-trienoic (α-kamlolenic) (3)	*Mallotus philippinensis*	Euphorbiaceae	Main constituent
Deca-*trans*-2,*cis*-4-dienoic (4)	*Sapium sebiferum*	Euphorbiaceae	4–5
Dodeca-*trans*-dienoic (5)	*Sebastiana ligustrina*	Euphorbiaceae	5
Octadeca-*trans*-9,*trans*-11,*cis*-13-trienoic (6)	*Catalpa ovata*	Bignoniaceae	Major constituent
Octadeca-*cis*-9,*trans*-11, *trans*-13-trienoic (α-elaeostearic) (1)	Same Cucurbitaceae, Euphorbiaceae and Rosaceae spp.		
Octadeca-*cis*-9,*cis*-11,*trans*-13-trienoic (trichosanic), also punicic (1)	*Trichosanthes* spp., also *Momordica balsamina* and *M. dioica*	Cucurbitaceae	Up to 43 64 55
4-Keto-octadeca-*cis*-9,*trans*-11,*trans*-13,trienoic (licanic) (1)	*Licania* spp. Same *Parinarium* spp.	Rosaceae	Up to 82
Octadeca-9,11,13,15-tetraenoic (parinaric) (1)	*Parinarium laurinum*	Rosaceae	Main constituent

(1) Cf. Hilditch (1956). (2) Bharucha and Gunstone (1956). (3) Calderwood and Gunstone (1953). (4) Crossley and Hilditch (1949, 1952). (5) Hanks and Potts (1951). (6) Hopkins and Chisholm (1962).

The occurrence of unusual fatty acids in Group II is to some extent implicit in terms of the system of classification which includes conjugated polyethenoid acids. In general these acids contain conjugated bonds in the 9, 11, 13 positions, and include *trans*- double bonds (cf. Table XXI). Additional unusual acids are found in the Euphorbiaceae family and include vernolic (*cis*-12,13-epoxyoctadec-*cis*-9-enoic), deca-*trans*-2,*cis*-4-dienoic, dodeca-*trans*-2,*cis*-4-dienoic and 12-hydroxyoctadec-*cis*-9-enoic (ricinoleic).

(iii) *Group III*

Seed fats with palmitic, oleic and linoleic acids as major components. In this group the following families are represented: Acanthaceae, Anacardiaceae, Annonaceae, Amaranthaceae, Apocynaceae, Berberidaceae, Bombacaceae, Buddleiaceae*, Calycanthaceae*, Capparidaceae, Caprifoliaceae†, Caricaceae, Caryocaraceae, Cobaeaceae*, Combretaceae, Daphniphyllaceae*, Fumariaceae*, Hernandiaceae, Lecythidaceae, Magnoliaceae, Malvaceae, Martyniaceae, Portulacaceae*, Rubiaceae, Tiliaceae, Zygophyllaceae.

The pattern of fatty acid composition of Group III may be illustrated by that of cotton seed *Gossypium arboreum* (Malvaceae) oil as follows (weight %): myristic 3·3; palmitic, 19·9; stearic, 1·3; arachidic, 0·6; oleic, 29·6 and linoleic, 45·3 (cf. Hilditch, 1956).

The distinction between Group III and Group IB is not always sharp, but is made largely on the basis of palmitic acid content. When this exceeds 10% the seed fat is classified in Group III. In this connection although the seed fats of the Gramineae have been classified by Hilditch (1956) in Group III, some are sufficiently low in palmitic acid to qualify for Group Ib. The seed fats examined since 1956 have revealed six further families classifiable in Group III.

A member of the Acanthaceae, *Adhatoda vasica* has been shown to contain in its seed fat considerable amounts of C_{22}–C_{26} saturated acids (Handa and Vasedu, 1956) and therefore qualifies for Group IVg. The classification of this family may have to be reconsidered in the light of further work. The infra-red absorption and gas chromatographic evidence obtained by Earle *et al.* (1959) for *Ceiba acuminata* (Bombacaceae) also indicate the presence of unusual acids requiring identification. The two species of Caprifoliaceae now recorded contain 10% or less of saturated acids, in their seed fats (Earle *et al.*, 1960a), as do three out of four

* Not included in Hilditch's (1956) classification.

† Originally included in Hilditch's (1956) classification but now transferred to Group Ib.

species recorded by Hilditch (1956). This family has therefore been transferred to Group 1b.

The regular monotony of Group III is broken by the appearance of 9-hydroxyoctadec-*cis*-12-enoic acid (7–15%) in the seeds of some twelve *Strophanthus* spp. (Apocynaceae) (Gunstone and Morris, 1959), as well as by the occurrence of vernolic (*cis*-12,13-epoxyoctadec-9-enoic) acid in some ten species of Malvaceae in amounts ranging from 1·5–7% (Hopkins and Chisholm, 1960) (Table XXII). In the Malvaceae are also found examples of the occurrence of cyclopropene acids, malvalic and sterculic (Wilson, Smith, Jr. and Mikolajczak, 1961; Shenstone and Vickery, 1961). This family will be considered further in Group IVc.

TABLE XXII

Unusual fatty acids found in Group III seed fats

Acid	Species	Family	% Total fatty acid
9-Hydroxyoctadec-12-enoic (1)	*Strophanthus* spp.	Apocynaceae	7–15
cis-12,13-Epoxyoctadec-*cis*-12-enoic (vernolic) (2)	*Hibiscus* spp.	Malvaceae	1·5–7
	Malope trifida		
	Malva moschata		
	Lavatera trimestris		
	Sidalcea hybrida		
$CH_3(CH_2)_7C{=}C(CH_2)_6COOH$ (Malvalic)	*Hibiscus syriacus* (3)	Malvaceae	16·3
	Lavatera trimestris (3)		7·7
	Gossypium hirsutum (4)		0·7–1·5
	Malva parviflora (4)		5·0*
	M. verticillata (4)		1–2*
$CH_3(CH_2)_7C{-}C(CH_2)_7COOH$ (Sterculic)	*Hibiscus syriacus* (3)	Malvaceae	3·4
	Lavatera trimestris (3)		0·6
	Gossypium hirsutum (4)		0·3–0·5
	Malva parviflora (4)		5·0*
	M. verticillata (4)		1–2*

(1) Gunstone and Morris (1959). (2) Hopkins and Chisholm (1960). (3) Wilson *et al.* (1961). (4) Shenstone and Vickery (1961).

* Total malvalic + sterculic.

(iv) *Group IV*

Seed fats containing characteristic acids other than or in addition to oleic, linoleic and palmitic acids.

Group IVa. Seed fats with petroselinic (octadec-*cis*-6-enoic) acid as a major component. Petroselinic acid is characteristic of the Umbelliferae but is also known to occur in *Hedera helix* (Araliaceae) and in *Picrasma quassioides* (Simaroubaceae) (cf. Hilditch, 1956). A well-

known example is parsley (*Petroselinum sativum*) seed oil, which has the following fatty acid composition (weight-%): palmitic, 3; oleic, 15; linoleic, 6; and petroselinic, 76 (Hilditch and Jones, 1927). The present survey includes ten species of Umbelliferae. Six of the umbelliferous seed fats as well as that of *Aralia spinosa* (Araliaceae) are not listed by Hilditch (1956).

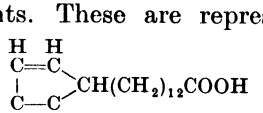
The seed oils of four species of Umbelliferae and of *Aralia spinosa* were examined by the group analysis technique (Earle *et al.*, 1959, 1960a), so that it is not possible to state whether petroselinic acid was present. The high epoxy values of the seed fats of two Umbelliferae, together with several instances of infra-red absorption suggest that some members of Group IVa are characterized by the presence of unusual acids. Hilditch (1956) concluded "that petroselinic acid has thus been observed in quantity in every umbelliferous seed fat as far analysed". The present results including those for seven umbelliferous species are not at variance with this conclusion.

Group IVb. Seed fats with tariric (octadec-6-ynoic) acid or other ethynoid acid as a major constituent. Families represented include: Simaroubaceae (*Picramnia* spp.) containing tariric acid, Olacacese and Santalaceae containing ximenynic acid (octadec-*trans*-11-ene-9-ynoic).

As noted by Hilditch (1956) the seed fats with tariric acid are confined to the genus *Picramnia* of the Simaroubaceae family. The new data for *Picramnia pentandra* support this view. The presence of tariric acid in *Ailanthus altissima* another member of the same family was not tested, but if present, it is not the main component acid (Earle *et al.*, 1960a).

The additional data on the occurrence of ximenynic acid confirms its presence in the seed fats of *Ximenia* species as well as in several other genera of Santalaceae. The interesting feature of ximenynic acid is that it is found not only in the seed fats, but in the lipids of other parts of the plant in these species either as ximenynic acid itself, or a closely related acid (cf. Hatt, Triffett and Wailes, 1959, 1960). These facts tend to emphasize that the occurrence of this acid serves a useful taxonomic role.

Group IVc. Seed fats with cyclic unsaturated acids as major or characteristic components. These are represented by the families



(VII)

Flacourtiaceae, Malvaceae, Sterculiaceae and Styracaceae. The C_{16} and C_{18} acids with a terminal cyclopentenyl ring as in chaulmoogric acid (VII)

are found in the seed fats of many members of the Flacourtiaceae* but not elsewhere. The only other known cyclic acids in seed fats are malvalic and sterculic acids (Table XXII).

The occurrence of these acids in some of the Malvaceae seed fats has been discussed under Group III. The amounts in such species appear usually to be less than 10%. In the seed oils examined by Earle *et al.* (1960a), three species of the genus *Hibiscus*, together with *Abutilon theophrasti* and *Lavatera trimestris* were recorded as giving a positive Halphen test, as were three members of the Sterculiaceae, *Brachychiton acerifolius*, *Firmiana simplex* and *Sterculia foetida*. This indicates a widespread occurrence of cyclopropene acids.

Only, however, in the seed oils of *Sterculia foetida* does the amount of cyclopropene (sterculic) acid reach a high level (70%) (Varma *et al.*, 1957). The occurrence of cyclopropene acids in seed fats apart from those of Malvaceae and Sterculiaceae has not been established but the possibility is suggested by the positive Halphen test obtained by Earle *et al.* (1960a) for the seed oil of *Styrax americana* (Styracaceae, formerly Symplocaceae, cf. Hutchinson, 1959). The insertion of the families Malvaceae (Hilditch, 1956, Group III), Sterculiaceae (Hilditch, 1956, Group IVh) and Styracaceae (not listed by Hilditch, 1956) into Group IVc may have the merit of stimulating further enquiry into the botanical classification of these species.

Group IVd. This group is not listed separately by Hilditch (1956), but in view of the increasing number of seed fats containing eicosenoic acid as a major constituent it has become desirable to distinguish these seed fats from those of other groups. The families represented include Buxaceae, Cruciferae, Limnanthaceae and Sapindaceae.

Eicos-11-enoic acid is the main fatty acid of the seed wax of *Simmondsia californica*, Buxaceae (Green, Hilditch and Stainsby, 1936). Although, in general, the seed fats of Cruciferae are characterized by the presence of erucic acid and therefore classifiable in Group IVe, nevertheless investigations of the seed fats of a large number of cruciferous species (Mikolajczak *et al.*, 1961) has brought to light five examples in which eicosenoic acid is the major constituent and erucic acid is either absent or is less than below 4%.

Minor amounts (3–9%) of eicos-11-enoic acid have been reported in the seed fats of the Sapindaceae family but in *Cardiospermum halicacabum* this acid forms 42% of the total (Chisholm and Hopkins, 1958). The

* Hilditch (1956) records the presence of chaulmoogric acid in quantity in the seed fats of seven members of the genus *Hydnocarpus*, and the complete absence in the seed fats of one member.

occurrence of eicos-*cis*-5-enoic acid in *Limnanthes douglasii* (Limnanthaceae) seed oil (Smith, Jr., *et al.*, 1960a) is noteworthy not only because of the amount present (65%) but also because the double bond occurs in the Δ^5 position. No other monoethenoid fatty acids with Δ^5 double bonds have been recorded as constituents of natural fats.

Group IVe. Seed fats with erucic (docos-*cis*-13-enoic) acid as a major component. Erucic acid is the main constituent of the seed fats of the Cruciferae (Table XXIII). It has also been found in quantity in *Tropaeolum* seed fats (cf. Hilditch, 1956).

TABLE XXIII

Group IVe: Erucic acid-rich seed fats (*Brassica napus*, family Cruciferae) (cf. Mikolajczak *et al.*, 1961)

Saturated				Unsaturated						
				C_{16}		C_{18}		C_{20}		C_{22}*
C_{16}	C_{18}	C_{20}	C_{22}	Mono	Mono	Di	Tri	Mono	Di	Mono
3	0·9	0·6	0·2	0·5	14	15	8	8	0·2	50

Results expressed as component fatty acids %.
* Erucic acid.

In the seed oils of sixteen species of Cruciferae, Hilditch (1956) notes that, with the exception of *Hesperis matronalis*, erucic acid occurs in substantial (30–60%) amounts along with linoleic and linolenic acids.

In the further work now recorded the number of cruciferous seed fats examined has been greatly increased, and the extensive investigations by Mikolajczak *et al.* (1961) have revealed a few examples, including *Hesperis matronalis* mentioned above, with no erucic acid, and some already noted in Group IVd which contain eicosenoic acid to the exclusion, or near exclusion, of erucic acid. Notwithstanding these findings erucic acid still remains a striking characteristic of cruciferous seed oils.

An interesting deviation is provided by *Lesquerella* seed fats (Mikolajczak, Earle and Wolff, 1962). Of the fourteen species examined twelve contained 45–74% 14-hydroxy-*cis*-11-eicosenoic acid, the remaining two contained about 40% of a C_{18} hydroxy unsaturated acid (Table XXIV). Another member of the Cruciferae family, *Lunaria biennis*, is also unusual in containing in addition to erucic acid, substantial amounts (21%) of tetracos-15-enoic acid (Wilson *et al.*, 1962). The

occurrence of traces of epoxy acids in cruciferous seed oils has been reported. Gunstone and Morris (1959a,b) isolated *cis*-15,16-epoxyoctadeca-*cis*-9,*cis*-12-dienoic acid from the seed oil of *Camelina sativa*.

Group IVf. Seed fats with unsaturated acids of higher molecular weight than erucic acid. Families represented include: Cruciferae and Olacaceae. The occurrence in seed fats of unsaturated acids of higher molecular weight than that of erucic acid is rare. However, as noted by Hilditch (1956), the seed fats of *Ximenia* species (Olacaceae) contain C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} acids of the general formula

$$CH_3(CH_2)_7CH{=}CH(CH_2)_nCOOH$$

amounting collectively to more than 20% of the total fatty acids. The occurrence of substantial amounts of tetracos-15-enoic *Lunaria biennis* (Cruciferae) has already been referred to under Group IVe.

TABLE XXIV

The occurrence of unusual fatty acids in the seed fats of Cruciferae

Acid	Species	% Total fatty acids
cis-15,16-Epoxyoctadeca-*cis*-9,*cis*-12 enoic (1)	*Camelina sativa*	1
14-Hydroxy-*cis*-11-eicosenoic (2)	*Lesquerella* spp.	45–74
Tetracos-15-enoic (3)	*Lunaria biennis*	21

(1) Gunstone and Morris (1959a,b). (2) Mikalajczak, Earle and Wolff (1962). (3) Wilson *et al.* (1962).

Group IVg. Seed fats with saturated acids of higher molecular weight than stearic acid. These are represented by Leguminosae, Moringaceae, Ochnaceae, Sapindaceae and Strychnaceae.* The composition of the seed fats of this group may be illustrated by that of ground-nut oil (Table XXV).

TABLE XXV

Fatty acid composition (wt.-%) of groundnut (*Arachis hypogaea*) oil (Longenecker, 1937)

Saturated						
C_{14}	C_{16}	C_{18}	C_{20}–C_{24}	Po.	Oleic	Lin.
0·4	9·4	3·1	5·1	0·9	54·9	26·2

* Not included in Hilditch's (1956) list.

In this group Earle *et al.* (1959, 1960a) have examined, by their screening analysis technique, the seed fats of some fourteen species of Leguminosae. Generally speaking, no evidence was obtained for the occurrence of unusual constituents, apart from the indication of high epoxy values for the seed oils of *Cercidium floridium* and of *Calliandra eriophylla*. Throughout the content of saturated fatty acids is shown to be high (18–38%). In other instances where the detailed fatty acid composition is available the new data provide evidence for the occurrence of C_{20}–C_{24} saturated acids, though the total amounts involved are generally less than 10%.

The seed oil of one species of Moringaceae represented, *Moringa concanensis* (Patel *et al.*, 1958), on the other hand, contained C_{20}–C_{24} saturated acids amounting to 12·4% and accords with the results for *Moringa oleifera* (Hilditch, 1956). Hilditch (1956) records 5·9–31·1% arachidic acid in the seed fats of four species of Sapindaceae which he lists and the two examples now given (Subrahmanyan and Achaya, 1957a; Chisholm and Hopkins, 1958) conform to this pattern.

Group IVh. Seed fats of which stearic acid is a major component. These are represented by Burseraceae, Clusiaceae (formerly Guttiferae, cf. Hutchinson, 1959), Convolvulaceae, Dipterocarpaceae, Gnetaceae, Meliaceae, Menispermaceae, Sapotaceae, Sterculiaceae and Verbenaceae.

The fatty acid composition of this Group may be represented by that of Kokum butter from *Garcinia indica* (Clusiaceae) seeds. Component acids (Hilditch, 1956) (weight %): palmitic, 2·5; stearic, 56·4; oleic, 39·4 and linoleic, 1·7. The new data covering the seed fats of four families generally support the results given in Hilditch's 1956 survey. However, the seed fats of two species of Verbenaceae which have been examined suggest that these fats are not notably rich in stearic acid compared with those of other families classified in this Group. The occurrence of cyclopropene acids—Sterculic and Malvalic in the seed fats of the Sterculiaceae has been referred to in Group IVc.

Group IVi. Seed fats of which myristic and lauric are major components. In this group are represented the following botanical families: Irvingiaceae,* Lauraceae, Myristicaceae, Palmae, Salvadoraceae, Simaroubaceae† and Vochysiaceae. In Table XXVI are shown respectively examples of lauric-rich and myristic-rich seed fats.

* Not included in Hilditch's (1956) list.

† As noted in the text there is no longer justification for inclusion of this family in Group IVi.

The new analyses for the seed oils of Lauraceae, Myristicaceae and Palmae species generally support the earlier results given by Hilditch (1956). However, as will be noted under Group IVj one species of Lauraceae (*Sassafras albidum*) has mainly capric acid in its seed fat (Earle *et al.*, 1960a). The sharp distinction between the Myristicaceae seed oils and those of the Lauraceae could perhaps justify the inclusion of two separate groups comprising the myristic-rich and the lauric-rich seed fats respectively.

TABLE XXVI

Composition (wt.-%) of seed fats containing lauric and myristic acids as major components

Species	Saturated							Oleic	Lin.
	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}		
Elaeis guineensis (Palmae)									
Dale and Meara (1955)	2·4	3·7	45·2	18·6	8·5	2·5	1·9	15·1	2·1
Myristica fragrans (Myristicaceae)									
Collin and Hilditch (1929)	—	—	1·5	76·6	10·1	—	—	10·5	1·3

In the Simaroubaceae, two species, *Ailanthus altissima* and *Picramnia pentandra*, whose seed fats have been listed, do not qualify for inclusion in Group IVi. The *Picramnia* seed fats have been earlier listed under Group IVb. In Hilditch's (1956) list eleven species of the family Simaroubaceae are noted, but only in the genus *Irvingia* is there definite evidence of lauric and myristic acid appearing in quantity. *Irvingia* is now placed by Hutchinson (1959) in the Irvingiaceae. As *Irvingia* was the only genus to justify the inclusion of Simaroubaceae in Group IVi, the Simaroubaceae should be removed from Group IVi. The members of the family Simaroubaceae thus show such wide variations in the composition of their seed fats as to prompt further enquiry into their botanical classification.

Group IVj. Many more capric-rich seed fats are now known to justify a separate group which includes the following botanical families: Lauraceae, Lythraceae and Ulmaceae. An example of a capric acid-rich seed fat is given in Table XXVII.

It will be noted that the seed fats of Ulmaceae appear also under (Linoleic-rich) Group Ib. In 1956 the seed fats of *Ulmus* spp. appeared to possess a unique fatty acid composition with capric acid as the main component. Now, however, the discovery of capric acid-rich seed fats in

other genera of Ulmaceae (*Zelkova serrata*) as well as in the seed fats of *Sassafras albidum*, Lauraceae (Hopkins and Chisholm, 1959b; Earle *et al.*, 1960a) and that of *Cuphea llavea*, Lythraceae (Wilson *et al.*, 1960) points to a wider distribution of this acid than was formerly suspected.

TABLE XXVII

Fatty acid composition (wt.-%) of the seed fats of *Ulmus americana* (cf. Hilditch, 1956)

Saturated						
C_8	C_{10}	C_{12}	C_{14}	C_{16}	Oleic	Lin.
5·3	61·3	5·9	4·6	2·9	11·0	9·0

IV. General Observations on the Occurrence of Fatty Acids in Plant Lipids

In view of the diversity of the fatty acids in plant life it is desirable to examine the main features of their distribution in relation to the phylogenetic scale as a whole rather than from the point of view of differentiating between families in the same order. It is already clear that the lipids of aquatic plants may be distinguished from those of land plants by the presence of highly unsaturated acids, particularly of the C_{20} series.

Within plants of terrestrial origin, lipids from species in different parts of the phylogenetic scale may also be distinguished in some instances by the distribution and types of fatty acids present, as shown in Table XXVIII.

The data summarized in Table XXVIII on the distribution of fatty acids indicate that bacterial lipids contain specific fatty acids not found in other forms of plant life, and which could be of taxonomic value in distinguishing between different types of bacteria. Thus the high molecular weight mycolic, phthienoic and mycocerosic acids are peculiar to mycobacteria. 2-Hydroxy-decanoic acid occurs in some bacteria and is considered an intermediate in the formation of *cis*-vaccenic acid, the latter generally regarded as characteristic of anaerobic bacteria which lack oleic acid, the most commonly occurring constituent of other plant lipids. *cis*-Vaccenic acid is not generally found in other forms of plant life but has been reported as occurring in the seed lipids of *Asclepias syriaca* (Asclepiadaceae). With *cis*-vaccenic acid is associated lactobacillic acid in *Lactobacillus* and some other species of bacteria. In addition to the above-mentioned acids, there occur in some bacterial lipids notable

TABLE XXVIII

Distribution and types of fatty acids present in terrestrial plants

Type of fatty acid present	Sub-division	Distribution
n-Saturated fatty acids	Palmitic-rich (> 40%)	Some anaerobic bacteria, some fungi (spores), some fruit-coat fats and some Group III seed fats.
	Stearic-rich (> 10%)	Some fungi (spores), some fruit-coat fats and Group IVh seed fats.
	Rich in higher (C_{20} and above) saturated acids	Mycobacteria, some species of fungi (spores) and Group IVg seed fats
Branched-chain acids	High molecular weight (mycolic, phthienoic and mycocerosic)	Mycobacteria
	Iso- and anteiso-C_{15} and C_{17}	Some anaerobic bacteria
n-Unsaturated acids	Hexadecenoic	Δ^3, *trans*, *Spinacea oleracea*, (leaves); Δ^7, some anaerobic bacteria; Δ^9, widespread occurrence
	Octadecenoic	Δ^6, Umbelliferae (seeds); Δ^9, most forms of plant life, except anaerobic bacteria; Δ^{11}, anaerobic bacteria and seeds of *Asclepias syriaca* (Asclepediaceae)
	Eicosenoic	Δ^5, *Limnanthes douglasii* (Limnanthaceae) seeds; Δ^9 some spore oils of cereal rusts (fungi); Δ^{11}, Group IVd seed fats
	Docos-*cis*-13-enoic	Group IVe seed fats
	Linoleic (octadeca-*cis*-9,*cis*-12-dienoic)	Most plant lipids except those of bacteria
	Linolenic (octadeca-*cis*-9,*cis*-12,*cis*-15,trienoic)	Many plant fats except those of bacteria
	Octadeca-*cis* 6,*cis*-9,*cis*-12-trienoic	*Phycomyces blakesleeanus* (fungi) and *Oenothera biennis* (Onagraceae) seeds
	Hexadeca-*cis*-7,*cis*-10,*cis*-13-trienoic	*Brassica napus* (Cruciferae) leaf, and *Spinacea oleracea* (Chenopodiaceae) leaf
Conjugated acids	*d*-elaeostearic (octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic) and related acids	Group II seeds, and *Calendula officinalis* (Compositae) seeds
Hydroxy acids	9-Hydroxyoctadec-*cis*-12-enoic	*Strophanthus* sp. (Apocynaceae) seeds

TABLE XXVIII—*cont.*

Type of fatty acid present	Sub-division	Distribution
Hydroxy acids	D(+)-12-Hydroxyoctadec-*cis*-9-enoic	Ergot (*Secale cornutum*), fungi; *Ricinus communis* (Euphorbiaceae), seeds and *Agonandra brasiliensis* (Olacaceae) seeds
	17-L-Hydroxyoctadec-9-enoic and 17-L-hydroxyoctadecanoic	In oil from fermentation of glucose by *Torulopsis magnoliae*
	14-Hydroxyeicos-*cis*-11-enoic	*Lesquerella* spp. (Cruciferae) seeds
	9-Hydroxyoctadeca-*trans*-10,*trans*-12-dienoic	*Dimorphotheca aurantiaca* (Compositae)
	(+)-Threo-9,10-dihydroxyoctadecanoic	*Puccinia graminis* (fungi) spores, *Lycopodium clavatum* (lycopod) spores
Epoxy acids	*cis* 9,10-Epoxyoctadecanoic	Spores of *Puccinia* spp. and other species of fungi; *Tragopogon porrifolius* (Compositae) seeds
	cis-9,10-Epoxyoctadec-*cis*-9-enoic (coronaric)	Compositae (some species) seeds
	cis-12,13-Epoxyoctadec-*cis*-9-enoic (vernolic)	Seeds of *Clarkia elegans* (Onagraceae), *Cephalocroton cordofanus* (Euphorbiaceae), and of some species of the families Compositae and Malvaceae
	cis-15,16-Epoxyoctadeca-*cis*-9,*cis*-12-dienoic	*Camelina sativa* (Cruciferae) seeds
Cyclic acids	Acids with terminal cyclopentenyl ring (chaulmoogric acid)	Group IVc seed fats of the family Flacourtiaceae
	Acids with a cyclopropane ring:	
	cis-9,10-Methylenehexadecanoic and *cis*-11,12-methyleneoctadecanoic	Some anaerobic bacteria
	Acids with a cyclopropene ring:	
	Sterculic $CH_3(CH_2)_7C{=}C(CH_2)_7COOH$ (with CH_2 bridge)	Seeds of some species of Malvaceae and Sterculiaceae
	Malvalic $CH_3(CH_2)_7C{=}C(CH_2)_6COOH$ (with CH_2 bridge)	
Acetylenic acids		Group IVb seed fats

amounts of iso- and anteiso-C_{15} and C_{17} acids not reported in more than trace amounts elsewhere in plant life. The complete absence of di- and polyenoic C_{18} acids in bacterial lipids and their presence in other forms of plant life serves further to characterize the bacterial lipids.

In the Phanerogamae the seed fats of some families contain in some instances fatty acids not reported as occurring in the lipids of other divisions of the plant kingdom. In this regard may be mentioned the occurrence of acids with acetylenic linkages, such as in ximenynic acid, cyclopentenyl derivatives, such as chaulmoogric acid, and conjugated trienoic acids such as α-elaeostearic acid.

Epoxy acids have been found in the spore oils of fungi as well as in seed fats. Whereas, however, the spore oils contain the saturated *cis*-9,10-epoxy-octadecanoic acid seed fats contain as a rule unsaturated epoxy acids. In this connection similar contrasts are to be found between the saturated cyclopropane acids (lactobacillic) of certain bacteria and the cyclopropene (sterculic) acids of seed fats of Sterculiaceae and Malvaceae. The occurrence of (+)-threo-9,10-dihydroxyoctadecanoic acid in the spores of *Puccinia* sp. (fungi) and in the spores of *Lycopodium clavatum* (lycopods) but not elsewhere may have some taxonomic significance. Although the seed fats of species within a given family have been shown in a number of instances to be similar in regard to the nature and distribution of their fatty acids the seed fats of families belonging to the same order are commonly unrelated. Moreover, using as a basis the phylogeny of the orders as outlined by Hutchinson (1959), there is no generally readily discernible relationship between the composition of the seed fats and evolutionary trends. It is, however, possible that the phylogenetic proximity of the orders Bixales, Tiliales and Malvales and the occurrence of cyclic acids in the seed fats of families from these orders is significant.

In conclusion, although the data on the types and distribution of fatty acids do not provide an unequivocal guide to the classification of plants, many correlations of taxonomic significance have become apparent in spite of the small number of species examined up to now. It is believed that the results so far obtained justify more extensive investigations in this field and that the study of fats has a role in the chemical taxonomy of plants.

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Appendix A

I. Fatty acid composition of oils found in spores of fungi

(Tulloch and Ledingham, 1960)

Puccinia graminis, P. triticina, P. coronata, P. hieracii, P. helianthi, P. carthami, Phragmidium speciosum, Cronatium harknessii, Melampsora lini, M. medusae, Uromyces psoraleae, Ravenelia hobsoni, Gymnosporangium juvenescens, Tilletia foetens, Ustilago zeae, U. tritici, U. nigra, U. levis, Sphaerotheca humili and *Erysiphe graminis.*

II. Fatty acid composition of seed oils

FAMILIES INCLUDED IN HILDITCH'S (1956) CLASSIFICATION

Group Ia (Linolenic-rich seed fats)

CELASTRACEAE: *Euonymus verrucosa* (Simonova, 1959), *E. alatus* (Earle *et al.*, 1959).

LABIATAE: *Majorana hortensis, Monarda fistulosa, Nepeta mussinii, Ocimum basilicum, Perilla frutescens, Salvia columbariae, Satureja hortensis, Thymus vulgaris* (Earle *et al.*, 1960b), *Stachys lanata* (Earle *et al.*, 1959). *Hyptis suaveolens, Leonurus cardiaca, Lycopus asper, Mentha arvenensis, Mentha* spp., *Nepeta cataria, Pycnanthemum muticum* (Earle *et al.*, 1960a).

LINACEAE *Linum usitatissimum* (Earle *et al.*, 1960b).

ONAGRACEAE: *Oenothera lamarckiana* (Mazhdrakov and Popov, 1957; Earle *et al.*, 1959), *Clarkia elegans* (Earle *et al.*, 1959), *Oenothera biennis, O. rhombipetala* (Earle *et al.*, 1960a).

PINACEAE: *Pinus griffithii* (Prakash, Sharma and Sutter, 1957).

RHAMNACEAE *Rhamnus cathartica, R. davurica, R. purshiana* (Earle *et al.*, 1960a).

VALERIANACEAE: *Valeriana officinalis* (Earle *et al.*, 1959).

Group Ib (Seed fats rich in linoleic acid)

ASCLEPIADACEAE: *Asclepias syriaca* (Chisholm and Hopkins, 1960a), *A. engelmanniana, A. incarnata, Marsdenia edulis* (Earle *et al.*, 1960a).

COMPOSITAE: *Carthamus tinctorius* (Grynberg, Rutkowski and Szczepanska, 1959), *Xanthium italicum, X. spinosum, X. strumarium*

(Rankov and Popov, 1957), *Helianthus annuus* (Popov and Ivanov, 1957), *Ageratum houstonianum, Artemisia absinthum, Chrysanthemum coronarium, C. leucanthemum, Dimorphotheca aurantiaca, Echinops exaltatus, Encelia farinosa, Eupatorium rugosum, Gaillardia aristata, G. pulchella, Grindelia squarrosa, Heliopsis helianthoides, Liatris spicata, Rudbeckia bicolor, Vernonia anthelmintica, V. baldwini, V. missurica, Centaurea cyanus, Cosmos bipinnatus, Helichrysum bracteatum* var. *monstrosum* (Earle *et al.*, 1960c), *Ambrosia trifida, Anthemis sancti-johannis, Arctium minus, Arctotis grandis, Aster alpinus, A. ericoides, Boltonia asteroides, Brachycome iberidifolia, Chrysanthemum coronarium, Cirsium altissimum, Cnicus benedictus, Cynara cardunculus, Echinacea angustifolia, Helenium autumnal, Helianthus annuus, H. maxmiliani, H. petiolaris, H. rydbergi, H. scaberrimus, H. tuberosus, Hulsea heterochrome, Iva xanthifolia, Kuhnia glutinosa, Lactuca scariola, Liatris punctata, L. pycnostachya, Ratibida columnaris, Rudbeckia laciniata, Solidago canadensis, S. serotina, Tithonia speciosa, Vernonia deppeana, Viquiera laciniata, Wyethia helenioides, Ximenesia encelioides* (Earle *et al.*, 1960a).

DIPSACEAE (Earle *et al.*, 1959): *Scabiosa atropurpurea.*

JUGLANDACEAE (Aizenberg, 1956): *Juglans regia.*

MYRTACEAE (Subrahmanyan and Achaya, 1957a): *Psidium guajava.*

OLACACEAE (Earle *et al.*, 1960a); *Ximenia americana.*

PAPAVERACEAE: *Argemone hispida, A. intermedia* (Earle *et al.*, 1960a): *Dicentra ochraleuca, Macleaya cordata, Papaver rhoeas* (Earle *et al.*, 1959).

PASSIFLORACEAE (Brooker, 1960): *Tetrapathea tetrandra.*

OLEACEAE (Jart, 1959): *Olea europaea.*

SCROPHULARIACEAE: *Alonsoa warscewiczii, Antirrhinum majus, Paulownia tomentosa, Penstemon albidus, P. grandiflorus, Nemesia suttoni, Veronica spicata* (Earle *et al.*, 1960a): *Chelone barbata, Digitalis purpurea, Linaria maroccana, Penstemon murraya* × *grandiflora, P. spectabilis* (Earle *et al.*, 1959).

SOLANACEAE: *Nicotiana tabacum* (Chakrabarty and Chakrabarty, 1953), *Datura metel* (Earle *et al.*, 1960a).

Group II (Seed fats with linoleic, oleic, linolenic or conjugated polyethenoid acids as major components)

CUCURBITACEAE: *Citrullus colocynthis* (Patel, Patel and Rabari, 1961), *C. fistulosis* (Aggarwal, Dhingra and Gupta, 1959), *Cucurbita maxima* (Tewari and Gupta, 1954), *Luffa graveolens* (Sehdayl *et al.*, 1961), *Momordica dioica, Trichosanthes cucumerina* (Chakrabarty *et al.*, 1956), *Cayaponia* spp., *Citrullus vulgaris, Echinocystis fabacea, E. oregana* (Earle *et al.*, 1960a), *Cucurbita pepo, Luffa acutangula, Marah gilensis, M. macrocarpa, Momordica balsamina* (Earle *et al.*, 1959).

EUPHORBIACEAE: *Antidesma diandrum*, *Bischofia javanica* (Sarkar and Chakrabarty, 1956b), *Cephalocroton cordofanus* (Bharucha and Gunstone, 1956), *Euphorbia heterophylla*, *E. marginata* Earle *et al.*, 1960b), *Phyllanthus maderaspatensis* (Bhakuni, 1959), *Croton texensis*, *Daphniphyllum humile*,* *Mercurialis annua*, *Phyllanthus* spp. (Earle *et al.*, 1960a).

ROSACEAE: *Licania rigida*, *Parinarium annamense* (Kaufmann and Sud, 1960), *P. macrophyllum* or *senegalense*, *Prunus amygdalus* var. *dulcis*, *P. armeniaca* (Prevot and Cabeza, 1962), *P. cerasus* (Weckel and Lee, 1960), *Prunus amygdalus* (Subrahmanyan and Achaya, 1957a), *Crataegus mollis*, *Geum chiloense* (Earle *et al.*, 1960a), *Sanguisorba minor* (Earle *et al.*, 1959).

Group III (Seed fats with palmitic, oleic and linoleic acids as major components)

ANACARDIACEAE: *Mangifera indica* (Mackie and Mieras, 1961).

ANNONACEAE: *Monodora myristica*, *Xylopia aethiopica* (Mackie and Mieras, 1961).

APOCYNACEAE (Earle *et al.*, 1960a): *Apocynum cannabinum*, *Thevetia* spp.

BOMBACACEAE (Earle *et al.*, 1959): *Ceiba acuminata*.

CAPPARIDACEAE: *Gynandropsis pentaphylla* (Gupta and Chakrabarty 1957), *Cleome pungens* (Earle *et al.*, 1959), *C. serrulata*, *C. spinosa*, *Isomeris arborea*, *Polanisia trachysperma*, *P. viscosa* (Earle *et al.*, 1960a).

CAPRIFOLIACEAE: *Lonicera tatarica*, *Viburnum dentatum* (Earle *et al.*, 1960a).

CARICACEAE: *Carica papaya* (Subrahmanyan and Achaya, 1957a).

GRAMINEAE: *Zea mays* (Burquette, 1955–6; Prevot and Cabeza, 1962).

LECYTHIDACEAE: *Bertholletia excelsa* (Ojida, 1955).

MALVACEAE: *Gossypium* spp. (Artamanov and Mamedov, 1959), *Hibiscus cannabinus* (Hopkins and Chisholm, 1959a; de Castro Ramos, 1958), *H. esculentus* (Hopkins and Chisholm, 1959a), *H. mutabilis* (Kato, 1961), *H. syriacus*, *Lavatera trimestris* (Wilson *et al.*, 1961).

RUBIACEAE: *Coffea arabica*, *C. robusta* (Subrahmanyan and Achaya, 1957b), *Oldenlandia biflora* (Bhakuni, 1959), *Cinchona* spp., *Gardenia jasminoides* (Earle *et al.*, 1960a).

RUTACEAE: *Citrus sinensis* (Subrahmanyan and Achaya, 1957b), *C. aurantium dulcis* (Weerakoon, 1960), *Ptelea trifoliata*, *Skimmia japonica* (Earle *et al.*, 1960a).

Group IVa (Petroselinic acid-rich seed fats)

ARALIACEAE: *Aralia spinosa* (Earle *et al.*, 1959).

UMBELLIFERAE: *Ammi visnaga* (Skellon and Spence, 1954), *Anethum*

* According to Hutchinson (1959) this species belongs to the Daphniphyllaceae.

sowa, Cuminum cyminum, Ptychotis ajowan (Menon and Raman, 1953), *Carum carvi, Pimpinella anisum* (Zaraiskaya and Broisyuk, 1956), *Daucus carota* (Prakash, Ram and Gupta, 1957), *Anthriscus cerefolium, Daucus carota, D. pusillus, Heracleum lanatum* (Earle *et al.*, 1960a).

Group IVb (Seed fats with tariric or other ethynoid acid as a major component)

SIMAROUBACEAE: *Ailanthus altissima, Picramnia pentandra* (Earle *et al.*, 1960a).

SANTALACEAE: *Exocarpus cupressiformis, E. strictus* and *Leptomera aphylla* (Hatt *et al.*, 1959, 1960).

Group IVc (Seed fats with substantial amounts of cyclic fatty acids)

MALVACEAE: *Abutilon theophrasti, Gossypium hirsutum, Hibiscus cannabinus, H. moscheutos, H. syriacus, Lavatera trimestris* (Earle *et al.*, 1960a).

STERCULIACEAE: *Sterculia foetida* (Varma *et al.*, 1957), *Brachychiton acerifolius, Firmiana simplex, Sterculia foetida* (Earle *et al.*, 1959, 1960a).

Group IVd (Seed fats containing eicosenoic acid as a major constituent)

This group is not specifically mentioned in Hilditch's 1956 classification but the occurrence of eicosenoic acid along with erucic acid is mentioned in connection with erucic acid-rich seed fats.

SAPINDACEAE: *Cardiospermum halicacabum* (Chisholm and Hopkins, 1958).

CRUCIFERAE: *Camelina sativa, Capsella bursa-pastoris, Lobularia maritima, Nerisyrenia camporum, Selenia grandis* (Mikolajczak *et al.*, 1961).

Group IVe (Seed fats with erucic acid as a major component)

CRUCIFERAE: *Camelina sativa, Crambe abyssinica* (Grynberg, Rutkowski and Sczepanska, 1959; Niewiadomski, Drozdowski and Zwierzykanski, 1959), *Eruca sativa* (Popov and Mazhdrokov, 1958), *Lepidium ibekis* (Joshi and Tewari, 1957), *Lunaria biennis* (Wilson *et al.*, 1962), *Matthiola incana* (Rahman and Khan, 1961), *Sisymbrium loeselii* (Choudhari, Singh and Handa, 1957), *Alyssum saxatile, Arabis alpina, A. virginica, Brassica campestris, B. carinata, B. juncea, B. napus, B. nigra, B. oleracea, Cakile edentula, Cheiranthus cheiri, Crambe abyssinica, Descurainia sophia, Eruca sativa, Erysimum perofskianum, Hesperis matronalis, Iberis amara, I. umbellata, Isatis tinctoria, Lepidium lasiocarpum, L. montanum, L. sativum, L. virginicum, Lunaria annua, Malcomia maritima, Matthiola bicornis, Nasturtium officinale, Raphanus*

* Tropaeolaceae.

sativus, *Sisymbrium irio*, *Sophia ochroleuca*, *Stanleyella texana*, *Thlaspi arvense*, *Tropaeolum majus** (Mikolajczak *et al.*, 1961), *Lesquerella angustifolia*, *L. argyraea*, *L. densipila*, *L. engelmannii*, *L. fendleri*, *L. globosa*, *L. gordonii*, *L. gracilis*, *L. grandiflora*, *L. lasiocarpa*, *L. lescurii*, *L. lindheimeri*, *L. ovalifolia*, *L. pinetorum* (Mikolajczak *et al.*, 1962).

Group IVf (Seed fats containing substantial amounts of unsaturated acids of higher molecular weight than erucic)

Hilditch (1956) records that the seed fats of *Ximenia* species (Olacaceae) contain small amounts of monoethenoid acids of the C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} series. Apart from small amounts of C_{24} monoethenoid acids found in *Pongamia glabra* (Leguminosae), the more recent work shows that certain Cruciferae species, notably *Lunaria annua* and *Lunaria biennis* (cf. under Group IVe), have substantial amounts of C_{24} monoethenoid acids.

Group IVg (Seed fats of which arachidic, behenic or lignoceric acids are major components)

LEGUMINOSAE:

Sub-family Caesalpiniaceae: *Caesalpinia digyna* (Gupta, Iyengar and Chakrabarty, 1957), *Cassia tora* (Tewari and Gupta, 1954), *Bauhinia purpurea*, *Cercidium floridum* (Earle *et al.*, 1960a).

Sub-family Mimosaceae: *Albizzia amara* (Chandra, Sud and Handa, 1956), *A. lobek* (Sen Gupta and Chakrabarty, 1958), *Acacia willardiana* (Earle *et al.*, 1959), *Calliandra eriophylla* (Earle *et al.*, 1960a).

Sub-family Papilionaceae: *Arachis hypogaea* (Gattanao, 1957), *Clitoria ternatea* (Tewari and Gupta, 1954), *Dipteryx odorata* (Dominguez and Canales, 1954), *Erythrina indica* (Pathak and Dey, 1956), *Pongamia glabra* (Pathak and Dey, 1957), *Soya hispida* (Collins and Sedgwick, 1959), *Sophora secundiflora* (Dominguez and Canales, 1954), *Coumarouna alata*, *Erythrina* spp., *Gliricidia* spp., *Machaerium rosescens*, *Medicago tribuloides*, *Milletia ovalifolia*, *Pongamia pinnata*, *Stylosanthes gracilis*, *Tephrosia* spp., *Trifolium subterraneum* (Earle *et al.*, 1960a).

MORINGACEAE: *Moringa concanensis* (Patel, Patel and Patel, 1958).

SAPINDACEAE: *Cardiospermum halicacabum* (Chisholm and Hopkins, 1958), *Sapindus trifoliatus* (Subrahmanyan and Achaya, 1957a), *Koelreuteria formosana*, *S. mukorossi* (Earle *et al.*, 1960a).

Group IVh (Seed fats of which stearic acid is a major component)

CONVOLVULACEAE: *Ipomea palmata* (Handa, Paul and Vasedu, 1956).

DIPTEROCARPACEAE: *Shorea robusta* (Prakash, Gupta and Rai, 1956).

MELIACEAE: *Aglaia odorotissima* (Baslas, 1959), *Carapa guianensis* (Pinto, 1956), *C. procera* (Mackie and Mieras, 1958), *Dysoxylum spectabile* (Brooker, 1960), *Swietenia macrophylla* (Chakrabarty and Chowdhury, 1957), *Trichilia* spp. (Earle *et al.*, 1960a).

MENISPERMACEAE: *Menispermum canadense* (Earle *et al.*, 1960a).

VERBENACEAE: *Tectona grandis* (Subrahmanyan and Achaya, 1957b), Indian lantanas (Nigam and Kaul, 1958).

Group IVi (Seed fats of which myristic and lauric acids are major components)

LAURACEAE: *Cinnamomum camphora* (Narung and Puntambekar, 1957), *Laurus nobilis* (Krajčinović and Filajdić, 1957).

MYRISTICACEAE: *Myristica fragrans* (Pathak and Ojha, 1957); *M. beddomei* (Kartha and Narayanan, 1958).

PALMAE: *Acrocomia mexicana* (Giral and Peralta, 1956), *Areca catechu* (Mackie and Mieras, 1961).

SIMAROUBACEAE: *Ailanthus altissima*, *Picramnia pentandra* (Earle *et al.*, 1960a).

Group IVj (Seed fats of which capric (decanoic) acid is a major component)

LAURACEAE: *Sassafras albidum* (Earle *et al.*, 1960a).

ULMACEAE: *Ulmus carpinifolia*, *U. fulva*, *U. glabra*, *U. laevis*, *U. procera*, *U. pumila* (Sørenson and Søltoft, 1958), *Zelkova serrata* (Earle *et al.*, 1960a; Hopkins and Chisholm, 1959b).

Appendix B

Families not included in Hilditch's (1956) Classification

Group Ia (Linolenic-rich seed fats)

Gymnosperms

TAXODIACEAE: *Cryptomeria japonica* (Earle *et al.*, 1959, 1960a).

Angiosperms

ACTINIDIACEAE: *Actinidia arguta* (Earle *et al.*, 1960a).

BORAGINACEAE: *Anchusa capensis, A. italica, Borago officinalis, Cynoglossum amabile, Onosmodium occidentale* (Earle *et al.*, 1959, 1960a).

ERICACEAE: *Arctostaphylos glauca* (Earle *et al.*, 1960a).

PAEONIACEAE: *Paeonia brownii* (Earle *et al.*, 1960a).

Group Ib (Linoleic-rich seed fats)

Gymnosperms

PODOCARPACEAE: *Podocarpus nagi* (Earle *et al.*, 1960a).

Angiosperms

AGAVACEAE: *Cordyline australis, C. banksii, C. indivisa, C. kaspar, C. pumilio, Phormium colensoi, P. tenax* (Morice, 1962), *Agave schottii, Dasylirion wheeleri, Yucca constricta, Y. glauca* (Earle *et al.*, 1960a), *C. australis, Y. elata* (Earle *et al.*, 1959).

AMARYLLIDACEAE: *Allium porrum* (Earle *et al.*, 1960a).

CASUARINACEAE: *Casuarina torulosa* (Earle *et al.*, 1960a).

CHENOPODIACEAE: *Kochia scoparia, Salsola pestifer* (Earle *et al.*, 1960a).

HAMAMELIDACEAE: *Liquidambar styraciflua* (Earle *et al.*, 1960a).

HELLEBORACEAE: *Nigella hispanica* var. *damascona* (Earle *et al.*, 1959).

IRIDACEAE: *Iris germanica* (Earle *et al.*, 1960a).

LILIACEAE: *Asparagus officinalis* (Hopkins and Chisholm, 1957), *Asphodelus fistulosus* (Khan *et al.*, 1961), *A. officinalis, Hemerocallis* (hort. spp.) (Earle *et al.*, 1960a).

LOASACEAE: *Mentzelia decapetala* (Earle *et al.*, 1960a).

POLEMONIACEAE: *Polemonium caeruleum* (Earle *et al.*, 1959).

RANUNCULACEAE: *Anemone coronaria, Thalictrum polycarpum* (Earle *et al.*, 1959), *T. revolutum* (Earle *et al.*, 1960a).

SYMPLOCACEAE: *Symplocos paniculata* (Earle *et al.*, 1960a).

Group II (Seed fats with linoleic, oleic, linolenic or conjugated polythenoid acid as major components)

BALSAMINACEAE: *Impatiens balsamina* (Sarkar and Chakrabarty, 1956a; Earle *et al.*, 1960a), *I. glanduligera* (Kaufmann and Sud, 1960).

BIGNONIACEAE: *Catalpa bignoniodes*, *C. ovata* (cf. Hopkins and Chisholm, 1962); *Chilopsis linearis* (Earle *et al.*, 1960a).

PUNICACEAE: *Punica granatum* (Earle *et al.*, 1960a).

Group III (Seed fats with palmitic, oleic and linoleic as major component acids)

BUDDLEIACEAE: *Buddleia davidi* (Earle *et al.*, 1960a).

CALYCANTHACEAE: *Calycanthus floridus*, *Chimonanthus praecox* (Earle *et al.*, 1960a).

COBAEACEAE: *Cobaea scandens* (Earle *et al.*, 1959).

DAPHNIPHYLLACEAE: *Daphniphyllum humile* (Earle *et al.*, 1960a).

FUMARIACEAE: *Dicentra ochraleuca* (Earle *et al.*, 1959).*

PORTULACACEAE: *Portulaca oleracea* (Handa *et al.*, 1956).

Group IVc (Seed fats containing cyclic acids)

STYRACACEAE: *Styrax americana* (Earle *et al.*, 1960a).

Group IVd (Seed fats containing eicosenoic acid as a major component)

LIMNANTHACEAE: *Limnanthes douglasii* (Smith *et al.*, 1960a).

Group IVg (Seed fats containing arachidic, behenic or lignoceric acids as major components)

STRYCHNACEAE: *Strychnos nux-vomica* (Subrahmanyan and Achaya, 1957a).

Group IVj (Seed fats of which capric (decanoic) acid is a major component)

LYTHRACEAE: *Cuphea llavea* (Wilson *et al.*, 1960).

* See under Papaveraceae, p. 305.

CHAPTER 11

Distribution of Aliphatic Polyols and Cyclitols

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I. Introduction

The aim of this chapter is to examine the distribution of polyalcohols in plants, especially cyclitols in Phanerogamae, and to point out relationships between their distribution and plant systematics.

The results which we shall consider have been obtained from two quite different types of research: firstly, from research on natural products present in an individual species; secondly, from a study of the comparative biochemistry of numerous species carried out with a view to defining the limits of polyol distribution within a botanical group. These two types

of research are separated in the present chapter, the first being mentioned only in connection with the presence of a polyalcohol in a family.

Research of the second type, which clearly has a taxonomic purpose, is relatively recent, and has increased rapidly during the last ten years. However, it should be remembered that as long ago as 1893, Monteverde examined 797 species in 199 genera of Scrophulariaceae with the aid of a simple test for the presence of mannitol and dulcitol. He established that the presence of these two compounds was constant at the levels of genus, sub-tribe and tribe, and even suggested a division in the genera *Cordylanthus* and *Orthocarpus* depending on whether the species contain one or other of the two polyols. Thus Monteverde, both by the width of his survey and by the conclusions he reached, can be regarded as a father of chemical taxonomy.

II. Isolation and Identification of Polyols

Polyols have been isolated from many different parts of higher plants. Many authors, interested in the active principles of medicinal plants, have used native drugs, but polyols have also been isolated from the latex of rubber-containing plants, and from gums and exudates from many other species. Quercitol has been obtained from the seeds of members of the Sapotaceae, sorbitol from the fruits of the Rosaceae, and pinitol and sequoyitol from the heartwoods of many gymnosperms. However, although polyols are found in all organs, the main sources are the leaves and green stems. Although the concentration is not always highest in these tissues, they are the material of choice for taxonomic purposes. In this chapter, the organ of the plant is only indicated when it is not leaf or leafy shoot or stem.

The presence of polyols has usually been established by their isolation in a pure state. Although, in general, this is satisfactory, there must always be some doubt about negative results. For example, the material may contain too little of the polyol to isolate, or other constituents present may hamper crystallization, or interfere with methods of purification. Undoubtedly some negative results which appear aberrant, may be attributed to such causes.

The method of choice for studying the comparative biochemistry of polyols is undoubtedly paper chromatography. Although it has been used successfully by several workers studying the Cryptogamae, it has not as yet been applied fully to higher plants.

Finally, it should be noted, that the value to taxonomy of the results obtained depends on both the systematic position and the number of species examined. These should be chosen so that each taxonomic unit

is represented as far as possible, and especially those that are near the limit of the zones of distribution. Usually, however, many species which are critically required are often not available.

In view of the foregoing, it is not surprising that the results presented below are really only a starting point for the study of chemical plant taxonomy. The families and genera are classified according to Engler.

III. Classification of Natural Polyols

The 35 polyols which have been examined can be classed as either *aliphatic polyols* (alditols or glycitols) which have a straight chain of carbon atoms, or *cyclitols*, which are derivatives of cyclohexane. These can be further sub-divided on the basis of the number of alcoholic hydroxyl groups which they contain (Table I).

TABLE I

Classification of natural polyols

Aliphatic polyols	
Tetrols (tetritols) $C_4H_{10}O_4$	erythritol (I), D-threitol (II)
Pentols (pentitols), $C_5H_{12}O_5$	adonitol (ribitol) (III), D-arabitol (IV)
Hexols (hexitols), $C_6H_{14}O_6$	allitol (V), dulcitol (galactitol) (VI), D-sorbitol (VII), D-mannitol (VIII), L-iditol (IX)
Anhydrides of sorbitol and mannitol, $C_6H_{12}O_5$	D-polygalitol (X), L-styracitol (XI)
Heptols (heptitols), $C_7H_{16}O_7$	D-perseitol (XII), D-volemitol (XIII)
Cyclitols	
Tetrols, $C_6H_{12}O_4$ (saturated)	betitol
$C_6H_{10}O_4$ (unsaturated)	conduritol (XIV), L-leucanthemitol (XV)
Pentols $C_6H_{12}O_5$	D- and L-quercitols (XVI), L-viburnitol (XVII)
Hexols or inositols, $C_6H_{12}O_6$	*myo*-inositol (XVIII), L-inositol (XIXa) (D-inositol (XIXb), DL-inositol, scyllitol (XX)
Monomethyl ethers of *myo*-inositol, $C_7H_{14}O_6$	sequoyitol (XXI), D- and L-bornesitols (XXII), D-ononitol (XXIII)
Dimethyl ethers of *myo*-inositol, $C_8H_{16}O_6$	dambonitol (XXIV), L-liriodendritol (XXV)
Monomethyl ethers of D- and L-inositols, $C_7H_{14}O_6$	D- and L-pinitols (XXVI) L-quebrachitol (XXVII)
C-Methylinositols $C_7H_{14}O_6$	L-laminitol (XXVIII), mytilitol (XXIX)

IV. Distribution of Aliphatic Polyols

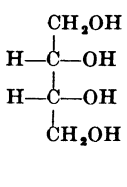
1. Erythritol (I)

Constitution by de Luynes (1862), only found in Cryptogamae—Algae, Chlorophyceae: *Protococcus vulgaris, Trentepohlia iolithus* (in the free state); Lichens: *Roccella, Parmelia, Aspicilia, Dendrographa* (ordinarily in the form of an ether of orsellinic acid called erythrin). It is also present in moulds (*Ustilago, Penicillium, Aspergillus*) and yeasts.

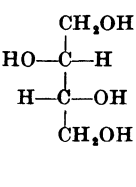
2. D-Threitol (L-erythritol) (II)

Synthesized by Maquenne (1900), only found in the fungus *Armillaria mellea*, where it forms 13% of the weight of the dry mycelium (Birkinshaw *et al.*, 1948).

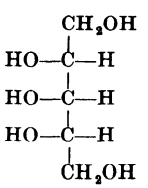
A. Tetrols



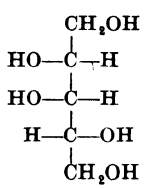
(I)
Erythritol



(II)
Threitol



(III)
Adonitol
(Ribitol)



(IV)
Arabitol

B. Pentols

1. Adonitol (ribitol) (III)

Constitution by Fischer (1893). Adonitol phosphate forms 20–30% of the cellular membranes of *Lactobacillus arabinosus* and *Bacillus subtilis* (Baddiley *et al.*, 1958). Free adonitol has been found in two families of Dicotyledoneae—Ranunculaceae: *Adonis vernalis* (Merck, 1893), roots of *A. amurensis* (Santavy and Reichstein, 1948); Umbelliferae: roots of *Bupleurum falcatum* (Wessely and Wang, 1939), and of Manchurian "saiko" (?) (Sato, 1950).

2. D-Arabitol (arabinitol) (IV)

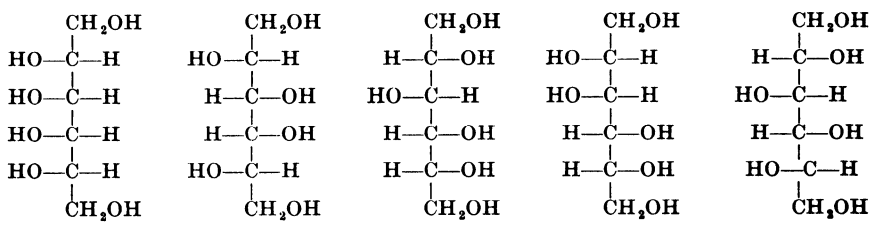
Constitution by Ruff (1899). Only found in Cryptogamae—Basidiomycetes: *Fistulina hepatica, Boletus bovinus* (other *Boletus* only contain mannitol (Frèrejacque, 1939, 1943)); Lichens: *Sticta, Cladonia, Lecanora, Umbilicaria, Parmelia, Alectoria, Ramalina, Usnea.* (Most of these genera are in the order Lecanorales.) Arabitol has also been shown, by paper chromatography, to be present in all species of the order

Gymnocarpeae which have been examined (50 species, 22 genera), but not in the order Pyrenocarpeae (genera *Dermatocarpon* and *Endocarpon*) (Lindberg *et al.*, 1953). Arabitol has also been found in a yeast (*Rhodotorula graminis*).

C. Hexols

1. Allitol (allodulcitol) (V)

Synthesized by Lespieau and Wiemann (1932), and so far only found in two members of the Saxifragaceae *Itea ilicifolia* and *I. virginica*. Closely related genera to *Itea* do not contain allitol (Plouvier, 1959).



(V) Allitol (VI) Dulcitol (Galactitol) (VII) Sorbitol (VIII) Mannitol (IX) Iditol

2. Dulcitol (VI)

Structure by Michael (1899). It was discovered in "manna of Madagascar" by Laurent in 1850, and has since been found in yeasts (*Torula*), Algae, and Dicotyledoneae Algae, Rhodophyceae: *Bostrychia scorpioides* (Haas and Hill, 1931); *Iridaea laminarioides* (Hassid, 1933); Lauraceae: *Cassytha filiformis* (Huzikawa *et al.*, 1941); Saxifragaceae: *Brexia madagascariensis* (Plouvier, 1956a); Celastraceae: several species of *Euonymus* (by different authors), *Celastrus* and *Schaefferia* (Czapek, 1913), bark of *Lophopetalum toxicum* (Dieterle *et al.*, 1934), *Tripterygium wilfordii* (Chou and Mei, 1937), *Gymnosporia senegalensis* (Dussy, 1947), *Maytenus vitis-idaea* (Orazi and Corral, 1960). Since dulcitol has been shown to be present in all 19 species of the Celastraceae which have been examined (in *Euonymus*, *Celastrus*, *Catha*, *Maytenus*), it is probably generally present in this family (Plouvier, 1948a, 1949c). Hippocrateaceae: *Tontelea brachypoda* (Almeida Costa and Lazzarini Peckolt, 1935), roots of *Pristimera indica* (Bhatnagar and Divekar, 1951), roots of *Salacia prinoides* (Pillay and Lekshmi, 1958); Scrophulariaceae: *Melampyrum*, *Rhinanthus*, *Scrophularia* (by different authors). Monteverde (1893) only found dulcitol in 4 genera (26 species) out of 199 species examined.

The presence of this compound in the Hippocrateaceae (formerly regarded as the tribe Celastrinae Benth. and Hook., and elevated to the rank of family) confirms their parentage with the Celastraceae. *Brexia* also has affinities with the latter family and, in agreement with Perrier de la Bâthie, this genus would perhaps be better placed there, rather than in the Saxifragaceae. It is also interesting to note that the Scrophulariaceae have botanical affinities with both Celastraceae and Hippocrateaceae through the intermediate family, the Stackousiaceae.

3. D-Sorbitol (VII)

Constitution by Vincent and Delachanal (1889). It has been obtained in 13·6% yield from the red algae *Bostrychia scorpioides* (Haas and Hill, 1932) and is also produced by the fermentation of sugars by anaerobic micro-organisms. In higher plants it has been found mainly in the Rosaceae.

In this family it was isolated from the fruits of *Sorbus aucuparia* (Boussingault, 1872); fruits of numerous species (Vincent and Delachanal, 1889), fruits of 15 species and leaves of six others (Strain, 1937); in the leaves of 55 species (out of 73 examined) in the following 30 genera Spiraeoideae: *Physocarpus*, *Neillia*, *Stephanandra*, *Spiraea*, *Sibiraea*, *Aruncus*, *Sorbaria*, *Exochorda*, *Holodiscus*; Pomoideae: *Cotoneaster*, *Osteomeles*, *Chaenomeles*, *Cydonia*, *Malus*, *Pyrus*, *Sorbus*, *Raphiolepis*, *Photinia*, *Amelanchier*, *Stranvaesia*, *Mespilus*, *Crataegus*, *Pyracomeles*, *Pyracantha*; Rosoideae: *Rhodotypos*, *Kerria*, *Neviusia*; Prunoideae *Nuttallia*, *Prunus*, *Prinsepia*.

Sorbitol is present in all the genera of the Spiraeoideae, Pomoideae and Prunoideae which have been examined. Its presence in the Rosoideae, on the other hand, is confined to the three genera shown. Some systematists (de Candolle, Bentham and Hooker) have placed these genera (*Rhodotypos*, *Kerria* and *Neviusia*) along with *Spiraea*, and the presence of sorbitol is an argument in favour of this. The genus *Ulmaria*, on the contrary, do not contain this polyol, and this confirms its new position in the Rosoideae, rather than as part of the *Spiraea* as considered formerly. Thus the distribution of sorbitol is in complete accordance with taxonomy in this family (Plouvier, 1955d).

4. D-Mannitol (VIII)

Constitution by Combes and Le Bel (1892). Mannitol was discovered in the manna of the ash by Proust in 1806, and is the most widely distributed aliphatic polyol in both higher and lower plants.

Basidiomycetes: found in 60 genera by Inagaki and Toki (1951);

shown by chromatography in the majority of species examined (Paris *et al.*, 1957). The amount present is often very large, *Agaricus integer* for example contains over 20% on a dry weight basis (Thörner, 1879).

Mannitol is also found in moulds and yeasts, and in a host of fermented products (formed by the action of anaerobic micro-organisms on sugars).

Algae: it has been isolated from many species especially in the Pheophyceae (*Cladostephus*, *Desmarestia*, *Laminaria*, *Macrocystis*, *Fucus*, *Pelvetia*) and Chlorophyceae (*Chlorella*, *Enteromorpha*) where it is sometimes present as a glucoside (Lindberg, 1955a; Lindberg and Paju, 1954; Lindberg and McPherson, 1954; Bouveng and Lindberg, 1955). It has also been obtained by hydrolysis of laminarin (Peat *et al.*, 1955).

Lichens: shown, by chromatography, to be present in all 55 species examined (Lindberg *et al.*, 1953).

Mannitol has been found in over 50 families of higher plants distributed in many diverse groups. The main families are the Umbelliferae (*Apium*, *Oenanthe*, *Aethusa*, *Meum*, *Daucus*) and above all the Oleaceae and Scrophulariaceae.

Oleaceae: mannitol has been isolated from 55 species (out of 57 examined) in the following 12 genera. Oleoideae: *Fraxinus*, *Forsythia*, *Syringa*, *Phillyrea*, *Osmarea*, *Osmanthus*, *Forestiera*, *Chionanthus* *Notelaea*, *Olea*, *Ligustrum*; Jasminoideae: *Jasminum*. Only *Fontanesia* does not contain it. The presence of mannitol shows the link between the three tribes of the Oleoideae, and between this sub-family and the Jasminoideae. It thus confirms the homogeneity of the Oleaceae and distinguishes this family from neighbouring ones where mannitol is rare (Plouvier, 1948c, 1952b, 1954a).

Scrophulariaceae: shown to be present in 272 species in 36 genera (out of 797 species in 199 genera examined) (Monteverde, 1893). These plants are distributed in the three sub-families.

5. L-Iditol (IX)

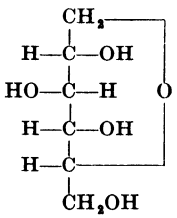
Constitution by Fischer and Fay (1895). It has only been isolated so far from the fruits of *Sorbus aucuparia* (Rosaceae) (Vincent and Meunier, 1898).

D. Anhydrohexitols

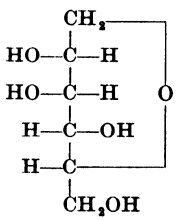
1. D-Polygalitol (1,5-anhydrosorbitol)

Constitution by Freudenberg and Rogers (1937). Found in two families. Polygalaceae: *Polygala amara* (Chodat, 1888), *P. vulgaris* (Picard, 1927), *P. tenuifolia* (Shinoda *et al.*, 1932), *P. senega* (Freudenberg and

Rogers, 1937). Aceraceae: obtained by hydrolysis of a crystalline tannin from the leaves of *Acer ginnala* (Perkin and Uyeda, 1922).



(X)
Polygalitol



(XI)
Styracitol

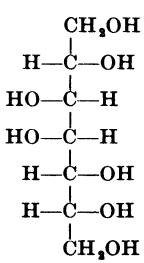
2. L-Styracitol (1,5-anhydromannitol)

Constitution by Freudenberg and Rogers (1937) and Zervas and Papa dimitriou (1940). Only found in the fruits of *Styrax obassia* (Styraceae) (Asahina, 1907).

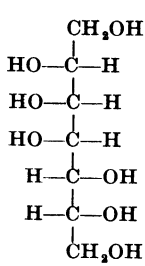
E. Heptols

1. D-Perseitol (D-α-mannoheptitol) (XII)

Constitution by Peirce (1915). Only found in *Laurus persea* (Lauraceae) (Muntz and Marcano, 1884).



(XII)
Perseitol



(XIII)
Volemitol

2. D-Volemitol (D-β-mannoheptitol) (XIII)

Constitution by Ettel (1933). Found mainly in Cryptogamae and only in one genera of Phanerogamae. Basidiomycetes: *Lactarius volemus* (Bourquelot, 1895). Other *Lactarius* only contain mannitol. Algae: Rhodophyceae: *Porphyra umbilicalis* (Lindberg, 1955b); Pheophyceae: *Pelvetia canaliculata* (as mono- and diglucosides) (Lindberg and Paju, 1954); Lichens: only in the Pyrenocarpeae (*Dermatocarpon* and *Endocarpon*) (Lindberg *et al.*, 1953); Primulaceae: roots of *Primula grandiflora*, *P. elatior*, and *P. officinalis* (Bougault and Allard, 1902).

V. Distribution of Cyclitols

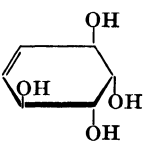
A. Tetrols

1. Betitol (1,2,3,4-tetrahydroxycyclohexane)

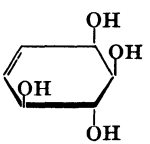
Only found in the molasses from *Beta vulgaris* (Chenopodiaceae) (Lippmann, 1901).

2. Conduritol (XIV)

Structure by Dangschat and Fischer (1939). Only found in the bark of *Gonolubus condurango* (Asclepiadaceae) (Kubler, 1908).



(XIV)
Conduritol



(XV)
Leucanthemitol

For clarity H-atoms attached to C are not shown

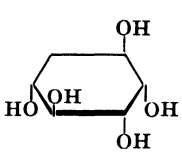
3. L-Leucanthemitol (XV)

Only found in *Chrysanthemum leucanthemum*, *C. Maximum* and *C. corymbosum* (Compositae). These three species are all in the same section, Pyrethrum Gaertn. (Plouvier, 1962a).

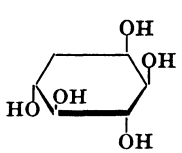
B. Pentols

1. D-Quercitol (XVI)

Configuration by Posternak (1932). Found in 9 families of Phanerogamae. Palmae: *Chamaerops humilis* (Müller, 1907), but not found in



(XVI)
Quercitol



(XVII)
Viburnitol

other genera examined (Plouvier, 1955a). Fagaceae: fruits of *Quercus robur* (Dessaignes, 1851), cork of *Q. suber* (Bräutigam, 1898). Quercitol has been isolated from the leaves of all *Quercus* examined (35 species), in each of the three sub-genera Cyclobalanopsis Prantl (East Asian oaks),

Erythrobalanus Spach (North American oaks) and Lepidobalanus Endl. (European oak). It established a link between *Pasania*, Cyclobalanopsis and Euquercus. Other Fagaceae (*Fagus*, *Castanea*) do not contain quercitol (Plouvier, 1955a, 1961).

Menispermaceae: bark of *Tiliacora acuminata* (van Itallie and Steenhauer, 1922), *Triclisia gilletii* (Castagne, 1934), roots of *Cissampelos pareira* (Bhattacharji *et al.*, 1953), roots of *Legnephora moorii* (Hughes *et al.*, 1953), *Cocculus trilobus* and *C. laurifolius* (Plouvier, 1955a), roots of *Cyclea burmanni* (Chaudhry and Dhar, 1958). All these species except *Triclisia*, are placed in the tribe Cocculeae. Magnoliaceae: *Talauma mexicana* (Sodi Pallares and Garza, 1947); Leguminosae: *Pterocarpus lucens* (Plouvier, 1955e); Myrtaceae: fruits of *Eugenia Jambolana* (Pottiez, 1899); Myrsinaceae: fruits of *Myrsine africana*, *M. semiserrata* and *Embelia ribes* (Krishna and Varma, 1936 and 1943); Sapotaceae: seeds of *Achras sapota* and *Mimusops elengi* (Van der Haar, 1922 and 1929); seeds of *Butyrospermum parkii* (Bauer and Moll, 1942), Loganiaceae: from native poison from *Strychnos toxifera* (Boehm, 1897).

Quercitol is thus distributed in species belonging to very diverse families. However, it appears to be uniformly present in *Quercus* and is widespread in the Menispermaceae.

2. L-Quercitol

Only found in *Eucalyptus populnea* (Myrtaceae) (Plouvier, 1961), although it has sought for in other members of this family.

3. L-Viburnitol (XVII)

Configuration by Posternak (1950). Found in four families of Dicotyledones—Menispermaceae: *Stephania hernandifolia* (Ewing *et al.*, 1950), *Menispermum canadense* (Plouvier, 1956b); Asclepiadaceae: *Gymnema sylvestre* (Power and Tutin, 1904); Caprifoliaceae: *Viburnum tinus* (Hérissey and Poirot, 1936). Other *Viburnum* which have been examined, do not contain viburnitol. Compositae: *Achillea millefolium*, *A. tournefortii*, *Tanacetum vulgare*, *Chrysanthemum leucanthemum*, *C. maximum* and *C. corymbosum* (Plouvier, 1960). These six species are in the tribe Anthemideae; neighbouring species do not contain it.

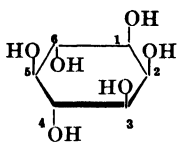
C. Hexols

1. *myo*-Inositol (*meso*-inositol) (XVIII)

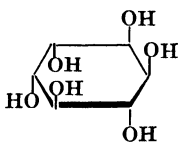
Configuration by Dangschat and Fischer (1942) and by Posternak (1942). *myo*-Inositol was discovered in beef muscle by Scherer (1850) and appears to be widespread in higher animals. In 1856, Vohl found it in

the fruits of *Phaseolus vulgaris*, and subsequently it was found in several other plants, both Cryptogamae and Phanerogamae (Marmé, 1864; Meillère, 1908). The berries of *iscum Valbum* (Loranthaceae) are exceptionally rich, containing 1·2% on a fresh weight basis (Tanret, 1907).

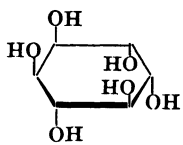
myo-Inositol is a normal constituent of living cells, a growth factor for yeasts, and a vitamin of group B (Schopfer, 1951), and thus is of little taxonomic value.



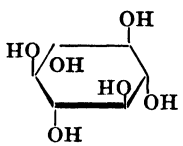
(XVIII)
myo-Inositol



(XIXa)
L-Inositol



(XIXb)
D-Inositol



(XX)
Scyllitol
(*scyllo*-Inositol)

This compound has also been found in a combined state: as a glucoside in *Beta vulgaris*; as mono- and diphosphates in the tubercle bacillus; and as the hexaphosphate, phytic acid, in many plants, especially seeds. The latter compound is only exceptionally found in animals (Courtois, 1951).

2. L-Inositol (XIXa)

Configuration by Posternak (1936). Only found in two families of Angiospermae—Euphorbiaceae: *Euphorbia pilulifera* (Hallett and Parks, 1951), latex of *Hevea* (Smith, 1954); Compositae: latex of *Sonchus arvensis* (Stern and Zellner, 1925), *Vernonia altissima* (Rowe *et al.*, 1955), *Helichrysum arenarium* (Vrkoč *et al.*, 1959). L-inositol has been isolated from the following 9 species (out of 27 examined): *Eupatorium cannabinum*, *Erigeron ramosum* (flowers), *Inula helenium*, *Pulicaria dysenterica*, *Anthemis nobilis*, *Chrysanthemum uliginosum*, *C. arcticum*, *Serratula coronata*, *Centaurea jacea* (Plouvier, 1962a,b).

Sonchus is a member of the Liguliflorae, the other species are spread throughout the six tribes of the Tubuliflorae. This distribution indicates that a link exists between these different groups, and points to a common metabolism that has remained unchanged in spite of the gross morphological evolution which is a character of this large family.

3. D-Inositol (XIXb)

Only found in the wood of *Pinus lambertiàna* (Pinaceae) (Ballou and Anderson, 1953). It exists perhaps (in traces) in numerous pinitol-containing plants.

4. DL-Inositol

Found in the fruits of *Viscum album* (Loranthaceae) (Tanret, 1907) and the stems of *Triclisia gilletii* (Menispermaceae) (Castagne, 1935). The presence of such racemic compounds in plants is very rare.

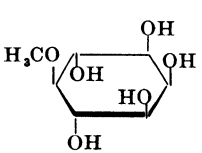
5. Scyllitol (XX)

Configuration by Posternak (1942). Scyllitol was discovered in the kidney and liver of plagiostomous fishes in 1858, and has been since isolated from Algae and 7 families of angiosperms—Algae, Rhodophyceae: *Porphyra umbilicalis* (Lindberg, 1955b), *Polysiphonia fastigiata* (Wickberg, 1957); Palmae: *Cocos nucifera* and *C. plumosa* (Müller, 1907); Fagaceae: fruits of *Quercus robur* (Vincent and Delachanal, 1887); Calycanthaceae: *Calycanthus floridus*, *C. glaucus* (Manske, 1941), *C. occidentalis*, *Chimonanthus fragrans* (Plouvier, 1956*b*). Scylitol is thus generally present in this small family. Rhamnaceae: *Helinus ovatus* (Goodson, 1920); Tiliaceae: *Tilia tomentosa* (Mokranjac and Medakovic, 1955); Cornaceae: *Cornus florida* (Hann and Sando, 1926); Compositae: *Verniona altissima* (Rowe *et al.*, 1955).

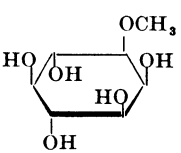
D. Monomethyl Ethers of *myo*-Inositol

1. Sequoyitol (5-*O*-methyl-*myo*-inositol) (XXI)

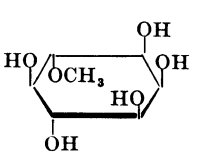
Configuration by Anderson *et al.* (1957). Found only in the gymnosperms. Wood of *Sequoia sempervirens* (Sherrard and Kurth, 1929), seeds of *Macrozamia riedlei* (Riggs, 1949), wood of *Taxus baccata* (King *et al.*, 1952), wood of *Pinus lambertiana* (Ballou and Anderson, 1953), pollen of *P. montana* (Nilsson, 1956), *Metasequoia glyptostroboides* (Kariyone *et al.*, 1958), wood of *Podocarpus spicatus* (Briggs *et al.*, 1959), bark of *Dacrydium cupressinum* (Cambie and Cain, 1960), *Glyptostrobus pensilis*



(XXI)
Sequoyitol



(XXII)
Bornesitol



(XXIII)
Ononitol

(Takahashi *et al.*, 1960). Sequoyitol has been isolated from 38 species (out of 52 examined) in the 19 following genera—Ginkgoaceae: *Ginkgo*; Taxaceae: *Torreya*, *Taxus*; Podocarpaceae: *Podocarpus*; Araucariaceae: *Araucaria*; Cephalotaxaceae: *Cephalotaxus*; Pinaceae: *Picea*, *Tsuga*, *Pseudo-tsuga*, *Abies*, *Larix*, *Pseudo-larix*, *Pinus*; Taxodiaceae: *Sciadopitys*, *Sequoia*, *Taxodium* Cupressaceae: *Thuja*, *Cupressus*, *Juniperus*.

It should be noted that as *Macrozamia* is in the Cycadaceae, all families of the gymnosperms contain sequoyitol. Although its distribution appears to be sporadic, it still suggests that the families are linked phylogenetically especially as it has been looked for without success in primitive Angiospermae.

However, most botanists, on the basis of morphological, palaeontological and other studies, believe that the Gymnospermae are polyphyletic. It must be concluded, therefore, that this apparent polyphyletism conceals a true monophyletic situation which perhaps is shown in the Cordaitales and Pteridophyta of palaeozoic times, and for which sequoyitol is a present-day symbol. It is probable that there is a link between this compound and the fundamental metabolism of the Gymnospermae which has allowed this biochemical character to be retained in spite of divergences in morphological evolution (Plouvier, 1957b, 1958b, 1960, 1962b).

2. D-Bornesitol (1-*O*-methyl-*myo*-inositol) (XXII)

Configuration by Foster and Stacey (1953). Found in two families which are closely related according to Hutchinson—Apocynaceae: in the latex of species yielding Borneo rubber: *Urceola elastica*, *U. esculenta* (Girard, 1871), *Dyera costulata* and *D. lowii* (Angyal *et al.*, 1957), *Amsonia angustifolia*, *A. Tabernaemontana*, *Vinca difformis* and *V. major* (Plouvier, 1961). These species occur in both sub-families, which suggested that D-bornesitol is perhaps universally present in this family. Rubiaceae: wood of *Sarcocephalus diderrichii* (King and Jurd, 1953).

3. L-Bornesitol

Found in five different families of Dicotyledoneae—Proteaceae: *Banksia integrifolia*, *Macadamia ternifolia*, *Stenocarpus sinuatus* (Plouvier, 1958b, 1962b). Leguminosae: 10 species of *Lathyrus* distributed in the two sections Archilathyrus Taub. and Orobus L., and it is therefore probably general in this genus (Plouvier, 1955c, 1958a). Rhamnaceae: L-bornesitol was found in all 23 species of *Rhamnus* examined occurring in both subgenera (Eu-rhamnus Dipp. and Frangula Dipp.) and in European, Asiatic and American species. Among 9 other genera of Rhamnaceae which have been examined only *Berchemia racemoas* yielded L-bornesitol (Plouvier, 1958a, 1962b). Apocynaceae: *Apocynum androsaemifolium*, *A. cannabinum* (Plouvier, 1961). Boraginaceae: *Lithospermum ruderale* (Bien and Ginsburg, 1958). L-Bornesitol has been isolated from 28 species (out of 31 examined) of the Borraginoideae occurring in the genera: *Omphalodes*, *Cynoglossum*, *Lindelofia*, *Solenanthus*, *Symphytum*, *Borrago*, *Anchusa*, *Lycopsis*, *Alkanna*, *Pulmonaria*, *Myosotis*, *Lithospermum*, *Onosma*, *Cerinthe*, *Echium*, and seems to be a constant

feature of this sub-family; the other two sub-families (Ehretioideae and Heliotropioideae) do not contain it (Plouvier, 1958a, 1962b).

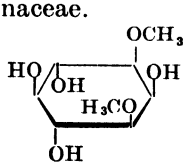
4. D-Ononitol (4-*O*-methyl-*myo*-inositol) (XXIII)

Found in two families—Leguminosae: *Ononis natrix*, *O. alopecuroides*, *Dolichos lablab*, *Vigna catjang*, *Leucaena glauca* (Plouvier, 1955c, 1962b). The first four species are in the Papilionatae (Trifolieae and Phaseoleae), *Leucaena* is in Mimosoideae. Flacourtiaceae: *Kiggelaria africana* (Plouvier, 1958b).

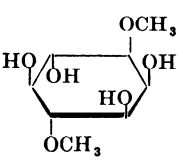
E. Dimethyl Ethers of *myo*-Inositol

1. Dambonitol (1,3-di-*O*-methyl-*myo*-inositol) (XXIV)

Configuration by Kiang and Loke (1956). Discovered in the sap of lianes of the Gabon rubber tree (Girard, 1868) and found in two related families. Moraceae: latex of *Castilloa elastica* (Weber, 1903). Looked for without success in many *Ficus* which are close to *Castilloa*. Apocynaceae: latex of *Dyera lowii* and *D. costulata* (de Jong, 1908; Comollo and Kiang, 1953). *Nerium oleander*, *Vinca minor*, *V. major*, *Trachelospermum jasminoides* (Plouvier, 1960, 1961). These species are distributed in the two sub-families which suggests that the compound is generally present in the Apocynaceae.



(XXIV)
Dambonitol



(XXV)
Liriodendritol

2. Liriodendritol (1,4-di-*O*-methyl-*myo*-inositol) (XXV)

Configuration by Engyal and Bender 1961. Only found in *Liriodendron tulipifera* and *L. chinense*. (Magnoliaceae) (Plouvier, 1955b) and not in any other species of the same family so far examined.

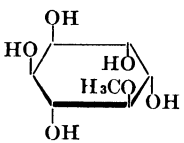
F. Monomethyl Ethers of D- and L-Inositol

1. D-Pinitol (3-*O*-methyl-D-inositol) (XXVI)

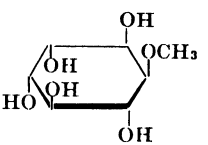
Configuration by Angyal and Macdonald (1952) and by Anderson *et al.* (1952). This compound is the most widely distributed methyl ether of the inositols, being found in 6 families of gymnosperms and 13 families of Angiospermes.

(A) Gymnospermae—resin from *Pinus lambertiana* (Berthelot, 1856), wood of *P. strobus* and *P. cembra* (Erdtman, 1943, 1946), wood of *P.*

monticola and *P. excelsa* (Lindstedt, 1949), wood of *Sequoia sempervirens* (Sherrard and Kurth, 1928). D-Pinitol has also been found in the green parts of 75 species (out of 117 examined) occurring in the 19 following genera. Cycadaceae: *Cycas*; Ginkgoaceae: *Ginkgo*; Cephalotaxaceae: *Cephalotaxus*; Pinaceae: *Picea*, *Tsuga*, *Pseudotsuga*, *Abies*, *Larix*, *Pseudolarix*, *Cedrus*, *Pinus*; Taxodiaceae: *Cunninghamia*, *Sequoia*, *Cryptomeria*; Cupressaceae: *Thujopsis*, *Libocedrus*, *Thuja*, *Cupressus*, *Chamaecyparis*.



(XXVI)
Pinitol



(XXVII)
Quebrachitol

All the Pinaceae which have been examined have been shown to contain D-pinitol, but it is not present in *Sciadopitys*, *Taxodium*, *Metasequoia* (Taxodiaceae), *Callitris* and *Juniperus* (Cupressaceae), nor in any of the Taxaceae, Podocarpaceae and Araucariaceae. It should be noted that species without pinitol, contain sequoyitol instead, and the absence of both cyclitols in *Ephedra* is in accordance with its position outside the gymnosperms (Plouvier, 1952a, 1953a, 1957b, 1958b).

(B) Angiospermae—Olacaceae: *Ximenia americana* (Finnemore *et al.*, 1938); Loranthaceae: *Viscum album* (Plouvier, 1953b); Aristolochiaceae: *Aristolochia clematitis*, *A. sipho*, *A. tomentosa* (Plouvier, 1956b); Nyctaginaceae: *Mirabilis jalapa*, *M. longiflora*, *Bougainvillea glabra*, *Oxybaphus viscosus*, and is undoubtedly widely distributed in this family (Plouvier, 1954b and 1957a); Phytolaccaceae: *Phytolacca americana* (Plouvier, 1954b); Aizoaceae: *Tetragonia expansa* (Plouvier, 1954b).

Caryophyllaceae: Pinitol has been isolated from 83 species (out of 85 examined) in the following 22 genera. Paronychioideae: *Paronychia*, *Polycarpon*, *Herniaria*, *Spergula*, *Telephium*, *Spergularia*; Alsinoideae: *Stellaria*, *Cerastium*, *Scleranthus*, *Sagina*, *Minuartia*, *Arenaria*, *Moehringia*; Silenoideae: *Silene*, *Heliosperma*, *Lychnis*, *Gypsophila*, *Tunica*, *Dianthus*, *Saponaria*, *Vaccaria*, *Velezia*. It is thus generally present in this family, and in particular establishes a link between the Paronychieae (apetales) and the Caryophylleae (dialypetales) which some botanists consider to be separate families (Plouvier, 1954b, 1957a, 1962).

Magnoliaceae: Pinitol has only been isolated from *Magnolia* species (10 out of 11 examined) and links the two sub-genera Magnoliastrum D.C. and Gwillimia Rottler, and also Asiatic and American species of

this genus. It is not present in other genera or in nearly related families (Plouvier, 1956b, 1957a).

Leguminosae: *Cassia angustifolia* (Dragendorff and Kubly, 1866), manna of *Ceratonia siliqua* (Charaux, 1922), *Acacia stolonifera* (Rimington, 1936), *Lotus australis*, *Trifolium repens* (Finnemore *et al.*, 1938), *Lotononis laxa* (de Waal, 1939), *Astragalus earlei*, *Oxytropis lambertii* (Pease *et al.*, 1940), *Lupinus caudatus* (Soine and Jenkins, 1941), *Astragalus wootoni* (Knowles and Elderfield, 1942), *Erythrophleum guineense* (Sannié and Dussy, 1947), wood of *Acacia mollissima* (Keppler, 1957).

D-Pinitol has been isolated from 181 species (out of 216) in the following 75 genera. Mimosoideae: *Albizzia*, *Acacia*, *Leucaena*, *Mimosa*, *Piptadenia*; Caesalpinioideae: *Schotia*, *Bauhinia*, *Cercis*, *Cassia*, *Ceratonia*, *Gymnocladus*, *Gleditschia*, *Haematoxylon*, *Caesalpinia*; Papilionatae: Sophoreae: *Sophora*, *Cladrastis*, *Maackia*; Podalyrieae: *Anagyris*, *Baptisia*, *Thermopsis*, *Chorizema*; Genisteae: *Crotalaria*, *Lupinus*, *Laburnum*, *Petteria*, *Genista*, *Genistella*, *Spartium*, *Ulex*, *Cytisus*, *Cytisanthus*; Trifolieae: *Ononis*, *Trigonella*, *Medicago*, *Melilotus*, *Trifolium*; Loteae: *Anthyllis*, *Hymenocarpos*, *Securigera*, *Lotus*, *Dorycnium*; Galegeae: *Psoralea*, *Amorpha*, *Indigofera*, *Galega*, *Wistaria*, *Robinia*, *Carmichaelia*, *Colutea*, *Halimodendron*, *Caragana*, *Astragalus*, *Bisserula*, *Oxytropis*, *Glycyrrhiza*; Hedysareae: *Scorpiurus*, *Ornithopus*, *Coronilla*, *Hippocrepis*, *Hedysarum*, *Onobrychis*, *Arachis*, *Desmodium*, *Lespedeza*, *Campylotropis*; Dalbergieae: *Dalbergia*, *Pterocarpus*, *Lonchocarpus*; Vicieae: *Cicer*, *Vicia*, *Lens*; Phaseoleae: *Amphicarpa*, *Glycine*, *Mucuna*, *Pueraria*.

The exceptions, which do not contain pinitol, are one member of the Mimosoideae (*Pithecolobium*), Dalbergieae (*Pterocarpus*, *Lonchocarpus*, *Andira*) and above all the Vicieae (*Vicia*, *Lathyrus* and *Pisum*) and Phaseoleae (*Erythrina*, *Apios*, *Phaseolus*, *Vigna*), and in certain of these another cyclitol (ononitol or L-bornesitol) is encountered.

Pinitol has not been found in other species apart from some families close to the Leguminosae and placed with them in the order Rosales (Engler). It does establish a link between the three sub-families of Leguminosae, and suggests a monophyletic origin of this large and diverse family. Indeed, its isolation from so many different types of plants (woody and herbaceous) from various geographical localities, is almost unique (Plouvier, 1949a, 1950a, 1955e, 1962b). Zygophyllaceae: *Zygophyllum fabago* (Plouvier, 1954b); Euphorbiaceae: *Euphorbia* sp. ("kan-zui") (Yanagita, 1943); Cistaceae: *Halimium umbellatum*, *Helianthemum apenninum*, *H. grandiflorum*, *H. nummularium* and several hybrids. It is not, however, present in *Cistus*, or in species from

neighbouring families in the order Parietales (Engler) (Plouvier, 1958b). Apocynaceae: latex of *Landolphia madagascariensis* (Girard, 1873).

Among these thirteen families which contain D-pinitol, Olacaceae, Loranthaceae and Aristolochiaceae are related, according to Engler, although Hutchinson separates the last one. Similarly Engler unites the Nyctaginaceae, Phytolaccaceae, Aizoaceae, and Caryophyllaceae in the order Centrospermae, whereas Hutchinson splits off the first of this group and places it in the Lignosae.

2. L-Pinitol

Found only in *Artemisia dracunculus* (Compositae). Its presence in a genus which contains quebrachitol (see below) is anomalous in regard to the position of the methoxyl group in L-inositol (Plouvier, 1956c).

3. L-Quebrachitol (2-*O*-methylinositol) (XXVII)

Configuration by Angyal and Macdonald (1952). Found in 11 families of angiosperms. Ulmaceae: all 10 species of *Celtis* examined; *Pteroceltis tatarinowii*; it has not been found in any other genera (in particular *Ulmus*) which have been examined (Plouvier, 1958b).

Moraceae: Only found in two genera in the sub-family Cannabinoideae (*Cannabis sativus*, Adams *et al.*, 1940, *Humulus lupulus*, Plouvier, 1960). Seven other members of the Moraceae occurring in the other two sub-families do not contain quebrachitol.

Proteaceae: *Grevillea robusta* (Bourquelot and Fichtenholz, 1912), *Hakea laurina* (Bourquelot and Hérissey, 1919); Loranthaceae: *Viscum album* (Plouvier, 1953b); Euphorbiaceae: latex of *Hevea brasiliensis* (de Jong, 1906); *Acalypha indica* (Rimington and Roets, 1938). A dozen other species have been examined without success. Aceraceae: *Acer pseudoplatanus*, *A. platanoides* (Malmy and Bouvet, 1942). Quebrachitol has been isolated from 20 species of *Acer*, *Negundo aceroides and N. californicum*; only *Acer carpinifolium* does not contain it (this species has different shaped leaves from the others). *Dipteronia sinensis* also appears to lack this compound (Plouvier, 1947, 1948b, 1953a). Hippocastanaceae: *Aesculus hippocastanum*, *A. pavia*, *A. flava*, *A. californica*, *A. parviflora* (Plouvier, 1949c, 1960); Sapindaceae: *Heterodendron oleaefolium* (Petrie, 1918). Quebrachitol has been also isolated from 15 species (out of 16) ranging as follows. Eusapindaceae: *Paullinia pinnata*, *Cardiospermum halicacabum*, *Sapindus saponaria*, *S. drummondii*, *Nephelium leiocarpum*, *Alectryon excelsum*, *Guioa villosa*, *Arythera arcuata*; Dyssapindaceae: *Koelreuteria paniculata*, *K. bipinnata*, *Dodonaea attenuata*, *Hippobromus alatus*, *Harpullia pendula*, *Xanthoceras*

sorbifolia, *Ungnadia speciosa*. As these species are well distributed in the different tribes of the two sub-families one expects that quebrachitol is present in all Sapindaceae (Plouvier, 1947, 1948a, 1949c, 1962b).

Elaeagnaceae: 6 species of *Elaeagnus*, *Hippophae rhamnoides*, *H. salicifolia*, *Shepherdia argentea*. Quebrachitol, thus characterizes this small family, especially as it is not present in the plants of neighbouring families (in the order Myrtiflorae) (Plouvier, 1951).

Apocynaceae: bark of *Aspidosperma quebracho* (Tanret, 1889), shoots of *Haplophyton cimicidum* (Clark, 1936), *Conopharyngia durissima* (Plouvier, 1961), both of which are placed in the sub-family Plumieroideae.

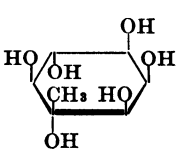
Compositae: inflorescence of *Artemesia afra* (Goodson, 1922); out of nine other species of this genus, only *A. dracunculus* (which contains L-pinitol) did not contain quebrachitol. The other genera of the tribe Anthemideae also do not contain it (Plouvier, 1949b, 1960).

These 11 families are well distributed among the Dicotyledoneae. However, Ulmaceae and Moraceae are nearly related, and certain systematists place the Celtideae and Cannabineae as sub-families of Urticaceae. Quebrachitol also confirms the morphological affinities of the three families present in the order Sapindales (Aceraceae, Hippocastanaceae and Sapindaceae).

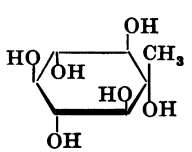
G. *C*-Methylinositols

1. L-Laminitol (4-*C*-methyl-*myo*-inositol) (XXVIII)

Structure by Lindberg and Wickberg (1959), and only found in Algae. Pheophyceae: *Laminaria cloustoni* (Lindberg and McPherson, 1954), *Fucus spiralis* and *Desmarestia aculeata* (Bouveng and Lindberg, 1955); Rhodophyceae: *Porphyra umbilicalis*, *Gelidium cartilagineum* (Lindberg, 1955b), *Polysiphonia fastigiata* (Wickberg, 1957).



(XXVIII)
Laminitol



(XXIX)
Mytilitol

2. Mytilitol (2-*C*-methylscyllitol) (XXIX)

Configuration by Posternak (1944). It is found only in invertebrates (*Mytilus*, *Patella*, *Ciona*) and red algae (*Gelidium cartilgineum*) (Lindberg, 1955b), and *Polysiphonia fastigiata* (Wickberg, 1957).

VI. Conclusions

The aliphatic polyols are well distributed in Cryptogamae, but certain of them, notably mannitol, are also found in Phanerogamae. In the case of the cyclitols, on the other hand, only *myo*-inositol, scyllitol, laminitol, and mytilitol, have been found in lower plants. Although the Monocotyledoneae have not been well studied, none of the methylinositols has ever been isolated from them. Hardly any investigation has been made of the Bryophyta and Pteridophyta.

Among the 35 polyols which have been discussed, some are only found in rare species and only of interest therefore to the phytochemist. Others are widespread, and attract the attention of physiologists as to the rôle they play in metabolism. Between these two extremes are those polyols which are present in some botanical groups and not in others, and which are of great interest in taxonomy. Some are generally distributed in all species of one group (e.g. pinitol in Caryophyllaceae), others only sporadically found (L-inositol in Compositae), and the extent of distribution thus extends from a single species to an entire family, and often characterizes phyla which are morphologically very diverse. Often the presence of a polyol has served to confirm the individuality of unique species, and in extensive groups allows a comparison between different systems of botanical distribution.

In any one botanical group, the polyols are generally present in the same enantiomorphic form. For example bornesitol is present as the L-form in all *Rhamnus*, and pinitol as the D-form in all Caryophyllaceae. There are exceptions, however: both D- and L-bornesitols being found in the same sub-family (Apocynaceae–Echitoideae), D-pinitol and L-inositol in the same genus (*Euphorbia*), and both D- and L-forms of inositol in the same species (*Viscum album*).

It is not rare to find more than one polyol in the same species: *Polysiphonia fastigiata* contains at least four (mannitol, scyllitol, laminitol, mytilitol). The fact that polyols of similar configuration exist in one and the same plant, leads us to believe that they are formed sequentially. Thus, traces of *myo*-inositol (XVIII) accompanies sequoyitol (XXI) in *Macrozamia*, bornesitol (XXII) in *Sarcocephalus*, and ononitol (XXIII) in *Ononis* (Angyal *et al.*, 1957) and is probably the direct precursor of these methyl ethers. Similarly the dimethyl ether, dambonitol (XXIV), is probably formed from bornesitol (XXII) which accompanies it in several members of the Apocynaceae. The tetrahydroxycyclohexene derivative, leucanthemitol (XV), is probably similarly formed from the corresponding pentol, viburnitol (XVII), in *Chrysanthemum* where they occur together.

In some cases, a biochemical homogeneity is conferred on a botanical group by the fact that several polyols which are similar in configuration occur. Thus, the tribe Anthemideae (Compositae) has yielded five corresponding compounds, L-inositol, L-quebrachitol, L-pinitol, L-viburnitol, and L-leucanthemitol.

Several cases exist, however, where polyols of different configuration co-exist, even in the same species (pinitol and sequoyitol in Gymnospermae; scyllitol and laminitol in *Porphyra umbilicalis*), and it is probable that they are formed by different routes from one common precursor. Nevertheless, the simple inversion of one hydroxyl group changes pinitol (XXVI) into sequoyitol (XXI) and such isomerizations are perhaps perfectly possible in some organisms, especially since *myo*-inositol (XVIII) is known to be transformed into scyllitol (XX) in the rat (Posternak *et al.*, 1959).

In view of this, polyols of different configuration found in the same botanic group can only have a limited taxonomic value. For example, *Lathyrus* should not be separated from the Vicieae because it contains L-bornesitol rather than pinitol; nor should one divide the Proteaceae-Grevilleoideae into species with L-bornesitol or those with quebrachitol. On the other hand, the presence of pinitol and sequoyitol in the Gymnospermae shows the homogeneity and probable monophyletic origin of the 9 families in which these compounds occur.

Numerous groups of Phanerogamae are known in which the distribution of aliphatic polyols or cyclitols is in excellent agreement with botanical taxonomy. Some homogeneous groups are recognizable even though they contain only one polyol (dulcitol in the Celastraceae), others by the sporadic distribution of chemically related compounds (the five methyl ethers of inositol in Apocynaceae). All the groups are outlined by the fact that nearly related plants contain no polyalcohols.

The polyalcohols are distributed throughout the Dicotyledoneae; in the Archichlamydeae and Metachlamydeae of Engler and in the Lignosae and Herbaceae of Hutchinson. The same compound is found in primitive as well as in advanced families (pinitol in Magnoliaceae and Apocynaceae, viburnitol in Menispermaceae and Compositae) and it would appear that the capacity to produce the cyclitols has not changed during the course of evolution.

The polyols do not allow us to differentiate between the higher divisions of the Dicotyledoneae. For examples, pinitol is present in the Leguminosae but not in other Rosales, and sorbitol which is found in the Rosaceae, is not present in the phylogenetically related Magnoliales or Dilleniales, or in the derived Hamamelidales. Again, bornesitol in the Boraginoideae, is not found in other Labiales. We can conclude there-

fore, that the Angiospermae, unlike the Gymnospermae, must have rather rapidly lost the chemical character of their more primitive ancestors. In fact this flexibility in metabolism is one probable cause of their development.

We may conclude by stating that our present knowledge is insufficient to make many definite conclusions about the relationship between the distribution of polyols and taxonomy. One point has been established, however, and that is that the occurrence of a hydroxyl group, or a methoxyl group in this or that position is a sufficiently precise character to be of use in taxonomy.

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CHAPTER 12

The Distribution of Plant Glycosides

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I. Introduction

Before proceeding to discuss the distribution of glycosides in the different plant taxa, and the link that may exist between such distribution and the systematic position of the plants concerned, it will be useful to define what is meant by the term glycoside and to describe briefly the different types found in nature (McIlroy, 1951; Paris, 1954; Tyler and Abonchaar, 1960).

Glycosides are organic compounds in which there is usually a semi-acetal linkage between the reducing group of a sugar and an alcoholic or phenolic hydroxyl group of a second molecule called an aglycone. This link, being effected through oxygen, gives rise to the *O*-glycosides which are the most common in plants. These compounds are easily hydrolysed to the parent sugar and the aglycone by either enxymes or acids. The oxygen bridge of the sugar ring is retained, and thus one can have furanose or pyranose glycosides, the latter being the more common. Also, since the

sugar moiety can exist in either α- or β-forms, one can obtain both α- and β-glycosides.

Two other groups of glycosides are known which involve a semi-acetal type of linkage. If the reducing group of the sugar is linked to a thiol, one obtains *S*-glycosides (e.g. I), which are less common than those mentioned above, and are somewhat restricted to particular families (Cruciferae, Tropaeolaceae, Resedaceae). The second group comprises the *N*-glycosides, which involve linkage to an amino group such as the nucleosides from ribose and purines, vicine (divicine-3-β-D-glucoside, II) and crotonoside (isoguanine-*N*-riboside).

Several glycosides are known where the sugar moiety is not a true sugar, but a derivative such as uronic acid. For example, glycyrrhizic acid (IIIa), obtained from certain leguminous plants, yields on acid hydrolysis glycyrrhetinic acid (IIIb) and two molecules of glucuronic acid.

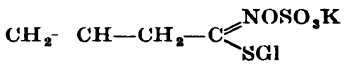
Finally, there is a group of compounds known as *C*-glycosides, which resist normal acid hydrolysis but have i.r. spectra and yield alkaline degradation products that indicate the presence of a sugar-like (or at least polyhydroxy) chain. In this category are aloin (IV) from various *Aloe* species, and several flavonoid derivatives such as vitexin (Va), orientin (Vb) and scoparoside (Vc) (Hörhammer and Wagner, 1961; Paris, 1962), which are distributed in many very different families of both monocotyledons and dicotyledons.

Several different sugars are found in glycosides; the most common being the aldohexoses (especially D-glucose), some pentoses, or methyl pentoses (rhamnose). Many glycosides contain more than one sugar; in some cases the sugars are linked to different hydroxyl groups of the aglycone (e.g. diglucosides), but more often the sugars are linked together to form di- or trisaccharides as in the case of rutinose [6-(α-L-rhamnosido)-D-glucose] or primeverose [6-(β-D-xylosido)-D-glucose].

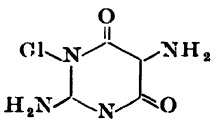
The nature of the aglycone is even more diverse and varies from simple alcohols and phenols to complex substances such as the steroids and triterpenes (e.g. III).

From the point of view of nomenclature it appears logical to restrict the term *glucoside* to those compounds which yield glucose on hydrolysis; those giving rhamnose or galactose being called likewise rhamnosides or galactosides. The suffix *-ine*, which is often used, should be reserved for nitrogen-containing compounds, and to avoid confusion with alkaloids, it is recommended that the suffix *-oside* be used instead, the rest of the name being related to the botanical origin of the substance in question (e.g. aucuboside (aucubin) from *Aucuba japonica*, franguloside (frangulin) from *Rhamnus frangula*).* However, compounds whose names have

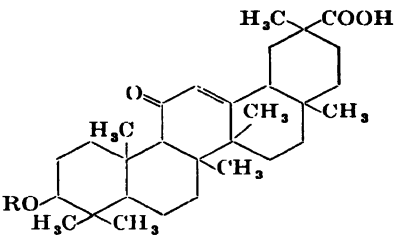
* Traditional names are retained in this Chapter.



Sinigrin
(I)

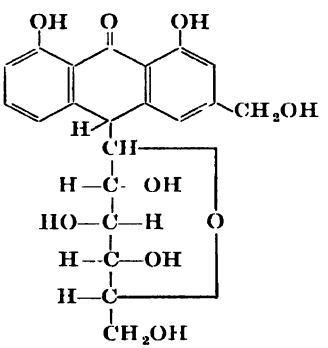


(II)
Vicine

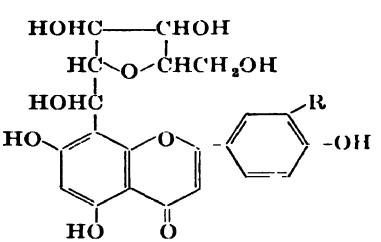


(IIIa, R = diglucuronide)
Glycyrrizin

(IIIb, R = H)
Glycyrrhetinic acid



(IV)
Aloin



(Va, R = H)
Vitexin

(Vb, R = OH)
Orientin

(Vc, R = OCH_3)
Scoparoside

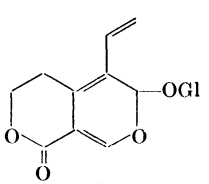
been well established, such as digitalin, ouabain, strophanthin, should retain such names. When the same glucoside has been isolated from several plants, it should be denoted by the earliest given name; for example, strophanthin G would better be called ouabain.

II. Localization and Distribution of Glycosides

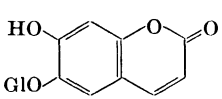
Since the discovery of salicin (salicoside) in the bark of the willow in 1830, many hundreds of glycosides have been isolated. They not only occur in Angiospermae, certain families of which (Apocynaceae, Rhamnaceae) are particularly rich sources, but also in lower plants as shown by the complex *N*-glycosides in *Streptomyces* species. Often several related glycosides are found in the same plant (e.g. a dozen cardiac glycosides in *Digitalis purpurea*, and more than twenty in *D. lanata*). They are found in all parts of the plant roots (gentiopicrin, VI, in gentian roots), bark (aesculin, VII, in horse chestnut), leaves (sennosides, VIII, in senna), flowers (anthocyanins, Chapter 16), fruits (flavanones of *Citrus* species) and seeds (sinigrin, I, in mustard).

They are generally present in the vacuole, and are sometimes localized in particular cells. They often show large variation in concentration during the growth of the plant (hydrolysis during germination, disappearance after flowering, accumulation in leaves or bark, and occasionally seeds). Their concentration often diminishes or they disappear completely, leaving only aglycones, due to enzyme action during drying of plant tissue; these phenomena greatly complicate the study of their distribution in plants.

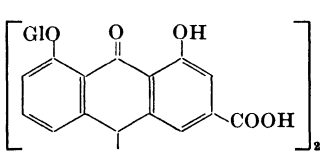
The distribution of the enormous number of glycosides that have been isolated cannot be clearly visualized unless the different sub-groups are



(VI)
Gentiopicrin



(VII)
Aesculin



Sennosides A and B
(VIII)

treated separately. The classification can be based on the nature of either the sugar or the aglycone.

III. Types of Sugar Found in Glycosides

As mentioned above, a great many different sugars are found in glycosides, but they are, on the whole, not specific to any particular group of plants except in a few cases that merit special attention.

Apiose, a branched chain tetrose, occurs in the form of apiin, which is only found in two members of the Umbelliferae, *Petroselinum sativum* and *Apium graveolens*.

D-Arabinose is also somewhat uncommon, being found in aloin (IV) mentioned earlier and in a saponin from *Sapindus* species, and a few flavonoids. The same is true of xylose, which has been obtained from some flavonoid glycosides (e.g. in *Adonis vernalis*) and certain saponins.

Glycosides containing the methyl pentose rhamnose are very numerous and occur in widely separated families. Some other sugars of this class, however, are uncommon; D-antiarose from antiarin (*Antiaris toxicaria*, Moraceae) and cheirotoxin (*Cheiranthus cheiri*, Cruciferae); D-fucose from convolvulin and turpethin (Convolvulaceae); D-quinovose from quinovin (*Cinchona calisaya*, Rubiaceae) and convolvulin; D-thevetose (methylquinovose) from hongheloside (*Adenium honghel*, Apocynaceae), bovoside (*Bowiea volubilis*, Liliaceae) and condurangin (*Gonolobus condurango*, Asclepiadaceae). Some have so far only been found in a single species: corchsularose from corchsularoside (*Corchorus capsularis*, Tiliaceae); L-acofriose (methyl ether of a methyl pentose) from acofrioside (*Acokanthera friesiorum*, Apocynaceae), L-acovenose, an isomer of the last sugar, from acovenoside.

The desoxypentoses are found in a number of cardiac glycosides; digitoxose and cymarose, which are found in several families (Apocynaceae, Scrophulariaceae, Liliaceae, etc.), are of little interest from the taxonomic point of view, but some other sugars of this class are more specific in their distribution, D-boivinose from stroboside (*Strophanthus boivinii*); D-oleandrose from oleandrin (*Nerium oleander*, Apocynaceae), urechitoxin (*Urechites subcreta*, Apocynaceae) and divaricoside (*Strophanthus divaricatus*); and D-sarmentose obtained from several glycosides of the Apocynaceae (*Strophanthus sarmentosus*, *S. boivinii*) and Asclepiadaceae (*Cryptostegia grandiflora*).

Among the hexoses, glucose is, of course, the most frequently encountered; D-mannose and D-galactose (in the flavonoids robinin, chaerophyllin) are much less common. Hamamelose, a branched chain sugar, has been found only in the tannin from *Hamamelis virginiana*.

Some sugars (streptose, mycaminose, hoviose, mycarose, cladinose) are only found in the complex, often nitrogenous, glycosides which have antibiotic properties (streptomycin, novobiocin, carbomycin, erythromycin) and are produced by various fungi. These special sugars may therefore be of interest in the taxonomy of such organisms.

Several glycosides yield on gentle hydrolysis, generally enzymic, di- or more rarely trisaccharides. Leaving aside the more common sugars of this class, primeverose and rutinose, which are of little interest in taxonomy, there are some which, at present, are only known to be present in one or two species: Neohesperidose (rhamnosidoglucose) in neohesperidin from *Citrus aurantium*, Rutaceae; scillabiose (glucosidorhamnose) in scillaren A from *Scilla maritima*, Liliaceae; strophanthobiose (glucosidocymarose) in strophanthin K from *Strophanthus kombe*, Apocynaceae; gentiobiose [6-(β-D-glucosidg)-D-glucose] in amyodalin from *Prunus amygdalus* var. *amara*, Rosaceae, and in crocin from *Crocus sativus*, Iridaceae; and finally robinobiose [6-(β-L-rhamnosido)-D-galactose] in robinin from *Robinia pseudacacia*, Leguminosae.

In the case of trisaccharides there are rhamninose (2 rhamnose + galactose) in xanthorhamnin (flavonoid from *Rhamnus infectoria*, Rhamnaceae), scillatriose (L-rhamnose-glucose-glucose) in scillaren A (*Scilla maritima*, Liliaceae), strophanthotriose in strophanthin K (*Strophanthus kombe*, Apocynaceae) as well as lycotriose (glucose-glucose-galactose) and solatriose (glucose-galactose-L-rhamnose) in certain alkaloids of the Solanaceae.

IV. Types of Aglycone

With few exceptions, then, the sugars are probably of little value in plant taxonomy. This is certainly not true for the aglycones, which are often specific to definite groups of plants, and which are normally used for the classification of the glycosides.

Since, as mentioned above, hundreds of glycosides are already known and their number increases daily, no attempt will be made to present an exhaustive review, which, in any case, would require a whole book to itself. Leaving aside the flavonoids and thioglycosides, which are the subject of separate chapters, we will confine our attention to typical representatives of the other types of *O*-glycosides (Karrer, 1958; McIlroy, 1951; Paris, 1954).

A. Simple Alcohols and Phenols

Very few glycosides of alcohols are known. One of the most typical is floridoside (2-galactosylglycerol) which so far has been isolated only

from red algae (Florideae). First found in *Rhodymenia palmata*, it has also been shown to be present in several species of *Corallina*, *Batrachospermum*, *Porphyra*, *Gelidium*, *Gigartina* and *Diginea*. It would obviously be interesting to extend this survey to verify that this glycoside is specific to the Florideae. Isofloridoside (1-galactosylglycerol) has been reported in one other red alga, *Porphyra umbilicalis*.

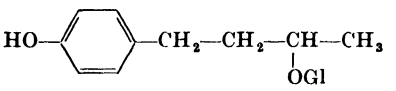
Echinacoside, a complex glycoside containing two molecules of glucose, and one each of rhamnose, caffeic acid and dihydroxyphenylethanol, has been found only in the bark of *Echinacea angustifolia* (Compositae). Betuloside (IX), on the other hand, has been found in two widely different families, Betulaceae (*Betula alba*) and Ericaceae (*Rhododendron chrysanthum*).

Phenolic glycosides (Bate-Smith, 1960; Pridham, 1960) have been known for over a century. Coniferin (Xa), which was first found in conifers (*Larix*, *Abies*), has also been recorded from Compositae (*Scorzonera hispanica*) and Oleaceae (*Fraxinus quadrangulata*) and, as it is probably involved in lignification, is of little taxonomic value. The same is true of many other phenolic glycosides; salicin (phenolic β-D-glucoside of salicyl alcohol, saligenin) was first found in Salicaceae (*Salix* and *Populus* species) but has also been isolated from *Viburnum prunifolium*, which is a member of a very distant family (Caprifoliaceae). Derivatives of salicin appear to be more specific: populin, in which a benzoyl group is attached to the sugar, has only been found in *Populus tremula*, and glycosmin (a veratroyl derivative of salicin) in *Glycosmis pentaphylla* (Rutaceae).

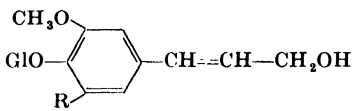
Salireposide, the β-D-glucoside of gentisic alcohol, has been found in several *Salix* species (*S. repens*, *S. purpurea*, *S. koriyanasi*). The parent alcohol has been isolated from *Penicillium*, and as an α-glucoside (xylosmoside) from *Xylosma apactis* (Flacourtiaceae).

Syringin (Xb), is widely distributed in the various tribes of the Oleaceae (Jasmineae, Syringeae, Fraxineae and Oleineae). In the genus *Fraxinus* it has been isolated from 15 species out of 25 examined (Plouvier, 1952, 1954), but it also occurs in other Sympetalae, Scrophulariaceae (*Paulownia imperialis*) and Caprifoliaceae (*Lonicera*) and since, like coniferin, it is probably a precursor of lignin, it is of little taxonomic value. Picein, β-D-glucoside of *p*-hydroxyacetophenone, has been found not only in conifers (*Picea*, *Pinus*) but also in *Salix*. Arbutin, glucoside of hydroquinone, likewise occurs in widely separated families: Ericaceae (*Arbutus unedo*, *Arctostaphylos uva-ursi*, *Vaccinium*, *Calluna*, *Pyrola*, *Azalea*, etc.), Leguminosae (*Orobus niger*), Ptoreaceae (*Persoonia salicina*), Saxifragaceae (*Bergenia cordifolia*) and Rosaceae (*Pyrus*), often accompanied by its methyl ether. Recently its monoacetate has been found in *Pyrus communis*.

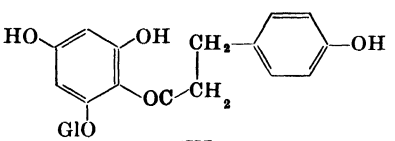
Among the glycosides which yield methyl salicylate on hydrolysis, monotropitin (primeveroside) is found in certain Ericaceae (*Gaultheria*, *Monotropa*), Betulaceae (*Betula*) and Rosaceae (*Spiraea*), whilst violutoside (vicianoside) has been isolated from *Viola*. Phloridzin (XI) is found in *Malus*, *Pyrus* and *Prunus* (Rosaceae), whilst the corresponding rhamnoside (glycophyllin) has been extracted from a member of the Liliaceae (*Smilax glycyphylla*).



(IX)
Betuloside



(Xa, R = H)
Coniferin
(Xb, R = OCH_3)
Syringin



(XI)
Phloridzin

However, there are a number of phenolic glycosides which are more localized from the taxonomic point of view than those described above. Glucovanillin has only been obtained as yet from Monocotyledoneae, Gramineae (*Avena sativa* and *Triticum repens*) and Orchidaceae (*Vanilla planifolia*). The glucoside of salicylaldehyde has only been found in the seeds of *Spiraea*; geoside (eugenol vicianoside) only from several members of the genus *Geum* (*G. urbanum*, *G. coccineum*, *G. rivale*), and lusitanicoside (chavicol rutinoside) occurs uniquely in *Cerasus lusitanica* Lois (*Prunus lusitanica* L.) (Hegnauer, 1954).

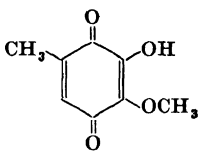
The phenolic glycosides, therefore, appear to be either very widely distributed or to be specific to a certain family or sub-group.

B. Anthraquinone Glycosides

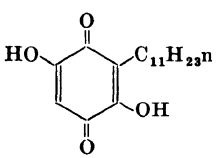
Quinones are fairly common in the Vegetable Kingdom and a few occur as glycosides. Unfortunately, such compounds are difficult to obtain in a crystalline state, and only a little is known about them (Fairbairn, 1959; Paris and Moyse, 1959). Usually they have been identified only by colour reactions, and it is difficult from such data to draw any conclusions about their distribution.

No benzoquinone has been obtained as a glycoside, and 2,6-dimethoxyquinone (*Adonis vernalis*), fumigatin (XII, *Aspergillus fumigatus*),

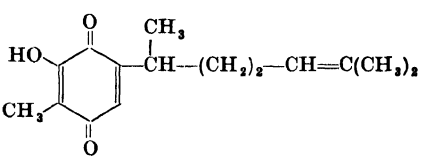
embelin (XIII, *Myrsine africana*), perezone (XIV, *Perezia adnata*, Compositae) all occur in the free state. It is the same with naphthoquinones, plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) from *Plumbago*, *Drosera* and *Diospyros*, and lapachol (2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone), from Bignoniaceae (*Bignonia*, *Tecoma*) verbenaceae (*Avicennia*) and Sapotaceae (*Illipe*). Only one naphthoquinone in the reduced state has been isolated: hydrojuglone glucoside (from juglone, 5-hydroxy-1,4-naphthoquinone) from *Juglans regia*.



(XII)
Fumigatin



(XIII)
Embelin



(XIV)
Perezone

On the other hand a large number of anthraquinone glycerides are known, some of which occur as derivatives of the reduced form (anthranol or anthrone) in the fresh tissue. The distribution of these compounds is somewhat strange (Hegnauer, 1959). They have been isolated from fungi, lichens and Angiospermae, but not from Bryophyta, Pteridophyta and Gymnosperms. In the Angiospermae they occur both in Monocotyledoneae (Liliaceae) and Dicotyledoneae (Polygonaceae, Rhamnaceae, Leguminosae and Rubiaceae), although not in all genera of these families.

Alizarin (1,2-dihydroxyanthraquinone) occurs as the primveroside (ruberythric acid) *Rubia tinctorum*, accompanied by the 3-glucoside of rubiadin (1,3-dihydroxy-2-methylanthraquinone); the corresponding primeveroside of this latter compound has been found in *Galium vernum*.

Among the well known glycosides of this class are chrysophanein (glucoside of chrysophanic acid, 4,5-dihydroxy-2-methylanthraquinone) from several species of *Rheum* and *Rumex*; the free acid has also been isolated from *Cassia occidentalis* (Leguminosae) and the mould *Penicillium islandicum*. Morindin (6-primeveroside of morindone, 1,5,6-trihydroxy-2-methylanthraquinone) occurs in *Morinda citrifolia* and *M. persicaefolia* (Rubiaceae), frangulin (rhamnoside of emodin, 1,3,8-tri-

hydroxy-6-methylanthraquinone) in the bark of the ash (*Rhamnus frangula*) and other *Rhamnus* species (e.g. *R. purshiana*). In the latter plant it is accompanied by the rhamnoside of the reduced form (emodin-anthranol) and other related glycosides containing extra sugar residues (frangulosíde and glucofrangulosíde).

Glucochrysaron (glucoside of chrysarone 1,2,7-trihydroxy-6-methyl-anthraquinone) has been found so far only in *Rheum rhaponticum*. Rheochrysin (glucoside of 3-*O*-methylemodin) also occurs in *Rheum* species, but the aglycone (physcion) has been isolated from moulds (*Penicillium*, *Aspergillus glaucus*) and lichens (*Xanthoria fallax*, *Teloschistes flavicans*) glucosides of rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) have been obtained from species of *Rheum*, *Rumex*, *Cassia* and *Fistula*. The leaves of senna (*Cassia angustifolia* and *C. acutifolia*) yield the sennosides A and B (stereoisomers, VIII) which appear to be specific to this species. Pseudopurpurin (1,3,5-trihydroxy-anthraquinone-2-carboxylic acid) on the other hand has been found in *Galium verum*, *Rubia peregrina*, *Crucianella maritima* and *Relbunium tetragonum*.

Finally, a special group of anthraquinone derivatives is aloin and its congeners whose structure is still under discussion. Barbaloin (aloin) from Cape, Barbadoes, and Curaçao aloes (*Aloe ferox*, *A. vera*, *A. africana*) is a *C*-glycoside (IV) which was believed to be specific to these species, but an analogous compound has been found in *Rhamnus purshiana*. This species also contains casanthranol, a glucoside of the anthranol corresponding to (IV). Natal aloes have been shown to contain another *C*-glycoside corresponding to aloin.

In conclusion, it appears that the anthraquinones, like the simple glycosides, have little taxonomic value except in one or two specific cases such as the sennosides and aloin.

C. Cyanogenetic Glycosides

The problem of determining the distribution of this class of glycoside is similar to that discussed for the quinones. Many plants yield hydrocyanic acid on treatment with enzymes or with acid, and although it is possible that they contain cyanogenetic glycosides, it is by no means certain. Only cases where actual glycosides have been isolated, therefore, will be dealt with here (Dilleman, 1954; Hegnauer, 1960).

These compounds are really derivatives of hydroxynitriles, the link to the sugar being through oxygen. The liberation of hydrocyanic acid occurs by a secondary reaction of the aglycone. Three types of glycosides are known: those like amygdalin (XV), which yields mandelonitrile or

a homologue on hydrolysis; the latter compound then giving benzaldehyde and hydrocyanic acid (e.g. vicianin, vicianoside of mandelonitrile; prunasin, glucoside; sambunigrin, L-isomer of prunasin; and zierin, glucoside of *m*-hydroxymandelonitrile. The second type is based on linamarin, glucoside of α-hydroxyisopropyl cyanide (which yields acetone on hydrolysis) (e.g. lotaustralin, which gives methyl ethyl ketone; and acacipetalin, XVI). The third type is gynocardoside which gives on hydrolysis a diketone of unknown structure.

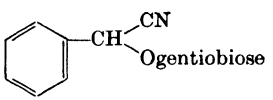
One of the glycosides, zierin, has only been found in *Zieria laevigata*, and vicianin only in species of the genus *Vicia*. Similarly (XVI) has only been isolated from the genus *Acacia*. Two others are restricted to one family; amygdalin (XV) itself in the Rosaceae and lotaustralin in the Leguminosae (*Lotus australis* and *Trifolium repens*). Four of the other cyanogenetic glycosides are found in several different families: dhurrin (glucoside of *p*-hydroxymandelonitrile) in Gramineae (*Sorghum vulgare*) and Euphorbiaceae (*Phyllanthus gastroemi*); sambungrin in Caprifoliaceae (*Sambucus nigra*), Olacceae (*Ximenia americana*) and Leguminosae (*Acacia glaucescens* and *A. chelii*); prunasin in numerous species of Rosaceae, Myoporaceae (*Eremophila maculata*), Myrtaceae (*Eucalyptus corynocalyx*), Scrophulariaceae (*Chaenorrhinum minus*, *Linaria stricta*); linamarin from five families, Linaceae (*Linum usitatissimum*), Leguminosae (*Phaseolus lunatus*, *Lotus australis*, *Trifolium repens*), Euphorbiaceae (*Manihot usitatissima*, *Hevea brasiliensis*), Ranunculaceae (*Thalictrum aquilejifolium*) and Compositae (*Dimorphotheca*).

Certain species contain two or three different glycosides. In some members of the Rosaceae one finds amygdalin in the seeds, prunasin in the leaves, and perhaps also prulaurasin (the DL-form amygdalin). Similarly in the Leguminosae, linamarin and lotaustralin are both present in *Lotus australis* and *Trifolium repens*.

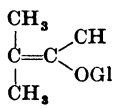
Two compounds of different structure can be added to this group of compounds, since they yield hydrocyanic acid on hydrolysis. Karakin (3 molecules of β-nitropropionic acid and glucose) from the fruit of *Corynocarpus laevigata* (Sapindaceae) and a related compound hiptagin from *Hiptagene madablota* (Malpighiaceae).

Finally, there is a group of azoxy-compounds which yield hydrocyanic acid and which are specific to the Cycladaceae. These include macrozamin (XVIIa) from various species of *Macrozamia*, and cycasin (XVIIb) (there are other derivatives in which gentiobiose and laminaribiose are present). A large number of other species are known which yield hydrocyanic acid (900 Spermatophyta, 30 Pteridophyta, 20 Fungi). Certain families are especially rich (e.g. the Rosaceae with 150 such species, Gramineae with 100, Leguminosae with 80). Some genera (*Acacia*, *Lotus*,

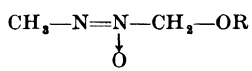
Linum) contain a number of cyanogenetic species and others which are not, and neighbouring species can often be differentiated in this way (e.g. *Ribes aureum* without HCN, and *R. odoratum* with Dillemann, 1954). Similarly, some races or varieties of the same species (*Trifolium repens*, *Lotus corniculata*, *Sorghum vulgare*, *Amygdalus communis*) are called *dulcis* or *amara* depending on whether they contain amygdalin or not.



(XV)
Amygdalin



(XVI)
Acacipetalin



(XVIIa, R = primeverosyl)
Macrozamin

(XVIIb, R = glucosyl)
Cycasin

There appears to be little direct relationship, then, between the presence of this glycoside and the position of the plant in the system of classification. However, the distribution of particular compounds such as macrozamin (XVIIa) is perhaps of taxonomic interest, and at the species level, one can obviously use the presence of cyanogenetic glycosides for differentiation.

D. Coumarin Glycosides

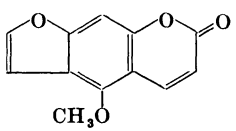
These compounds (e.g. VII) are most frequently found in higher plants especially Dicotyledons, although phenylcoumarins have been isolated from a fungus *Alternaria tenuis*. Besides the simple coumarins, furocoumarins such as bergaptene (XVIII), xanthotoxin and pimpinellin are found, mainly in Umbelliferae and Rutaceae and always in the free state, and also isocoumarins such as hydrangenol (XIX).

Coumarin itself, which is responsible for the agreable smell of many plants, is usually present in both free and so-called bound forms. This latter form which yields coumarin only after hydrolysis, has been shown to be the glucoside of *cis-o*-coumaric acid (coumarinic or melilotic acid), and has only been isolated from *Melilotus* species. Coumarin itself has been isolated from ferns (*Polypodium hastatum*), Gramineae (*Hierochloë odorata*, *Anthoxanthum odoratum*), Orchidaceae (*Angraecum fragrans*),

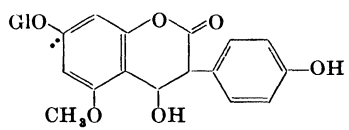
Moraceae (*Ficus radicans*), Berberidaceae (*Achlys triphylla*), Umbelliferae (*Levisticum officinale*), Labiatae (*Melittis melissophyllum*), Rubiaceae (*Asperula odorata*), Leguminosae (*Coumarouna odorata, Melilotus officinalis*), Compositae (*Liatris odoratissima* and *Chrysanthemum segetum*).

Umbelliferone (7-hydroxycoumarin) has been isolated as a glucoside (skimmin) from *Skimmia japonica* (Rutaceae) and diglucoside (neohydrangin) from *Hydrangea paniculata* (Saxifragaceae). It occurs free in many Umbelliferae (*Apium, Angelica, Daucus, Ferula, Heracleum, Pimpinella*), Rutaceae (*Citrus, Aegle*), Saxifragaceae (*Skimmia, Dichroa*), Solanaceae (*Atropa*) and Compositae (*Hieracium, Matricaria*).

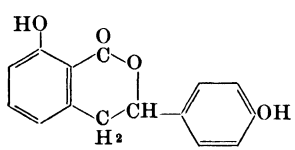
The distribution of aesculin (VII) is rather peculiar, being reported in horse-chestnut (*Aesculus hippocastanum*), in *Crataegus oxyacantha* (doubtful!), *Bursaria spinosa* (Pittosporaceae) and *Fraxinus ornu* (Oleaceae). The aglycone (aesculetin) has been found in *Euphorbias lathyris* (Euphorbiaceae) and *Symphoricarpus occidentalis*



(XVIII)
Bergaptene



(XX)
Isoshehkanin*



(XIX)
Hydrangenol

(Caprifoliaceae.) Cichoriin (7-β-glucoside of aesculetin) is restricted to the Sympetalae, occurring in *Cichorium intybus* (Compositae) and *Fraxinus ornus* (Paris and Stambouli, 1960, 1961). Fraxin (8-β-glucoside of fraxetin, 7,8-dihydroxy-6-methoxycoumarin) has been isolated from the bark of a number of *Fraxinus* species, and also from *Aesculus turbinata* (Hippocastanaceae) and *Diervilla lutea* (Caprifoliaceae). The distribution of this glycoside in *Fraxinus* has been studied by Plouvier (1954). In the sub-section Bumeloideae, the species contained fraxetin and no syringin, in Meliodeae syringin and no fraxetin, whilst the third sub-section Sciadantheae, occupied an intermediate position. The author and his colleagues have studied the coumarins in the leaves and bark of *Fraxinus excelsior*, *F. excelsior*

* Structure not correct, see Gilbert *et al.* (1957).

var. *pendula*, *F. ornus* and *F. oxyphylla* (Paris and Stambouli, 1960, 1961). All the barks contained aesculin and fraxetin, the bark of *F. ornus* being particularly rich in the former compound; the leaves only contained traces of glycosides but instead had fraxetin, except for *F. ornus*, in which they contained cichoriin, thus distinguishing it from the *excelsior* group.

The other common coumarin glycosides are distributed in a number of different dicotyledons, though very rarely in monocotyledons. Fabiatrin (primeveroside of scopoletin, 7-hydroxy-6-methoxycoumarin) has only been obtained from *Fabiana imbricata*, whilst the corresponding glucoside (scopolin) not only occurs in another member of the Solanaceae (*Scopolia japonica*) but also in *Murraya exotica* (Rutaceae) and *Nerium odorum* (Apocynaceae). The aglycone scopoletin has been isolated from Solanaceae (*Atropa*, *Nicotiana*), Convolvulaceae (*Ipomea*, *Convolvulus*), Ebenaceae (*Diospyros maritima*), Rutaceae (*Casimiroa edulis*) and also from a monocotyledon (*Avena sativa*).

The distribution of daphnetin (7,8-dihydroxycoumarin) and its 7-glucoside (daphnin) is restricted to Thymeleaceae (*Daphne gnidia*) and some Euphorbiaceae (*Euphorbia lathyris*) in the same group of Apetalae. Calycanthoside (glucoside of isofraxidin, 7-hydroxy-6,8-dimethoxycoumarin), which is closely related to fraxetin, has not been isolated from Oleaceae in which the latter occurs, but from a distant family, Calycanthaceae.

Finally, several other coumarin glycosides are distributed in certain specific plants; vellein, glucoside of osthenol (7-hydroxy-8-prenylcoumarin) in *Velleia discophrora* (Goodeniaceae), isoshehkanin (XX) in *Iris watti* (Iridaceae), nodokenin (glucoside of a furocoumarin) in *Peucedanum decursivum* (Umbelliferae), and the glucoside of hydrangenol (XIX) in the Saxifragaceae.

On the whole, therefore, there does not seem to be a close connection between the presence of coumarin glycosides and the systematic position of the plant, although in some cases they can be used to differentiate between sub-species.

E. Cardiac Glycosides

These compounds are related to the steroids, having in addition a lactone ring and a sugar (often a tetrasaccharide) attached to carbon 3 of the cyclopentanophenanthrene skeleton (e.g. XXI, XXII). In contrast to the anthraquinones and coumarins, the aglycones are rarely found in the free state but, since the sugar moiety may vary widely, a large number of glycosides has been found. These sugars are usually specific

derivatives of deoxymethylpentoses (e.g. antiarose, acovenose) occasionally acetylated, and only rarely are normal hexoses like glucose encountered.

The aglycones can be divided into two classes depending on the size of the lactone ring: cardenolides (e.g. XXI) with a five-membered ring, and bufanolides (e.g. XXII) with a six-membered ring.

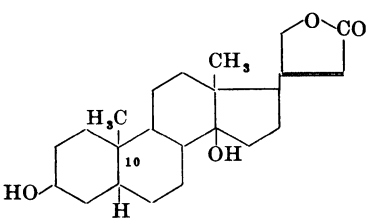
Cardiac glycosides have only been isolated from Angiospermae, bufanolides (e.g. XXII) from Liliaceae (*Scilla maritima*) and Ranunculaceae (*Helleborus niger*), and cardenolides in Liliaceae (*Convallaria*), Ranunculaceae (*Adonis*), Moraceae (*Antiaris*), Cruciferae (*Cheiranthus*), Sterculiaceae (*Mansonia*), Tiliaceae (*Corchorus*), Celastraceae (*Euonymus*), Leguminosae (*Coronilla*) and Scrophulariaceae (*Digitalis*). They are particularly abundant in Apocynaceae and Asclepiadaceae.

Even in the Apocynaceae they do not appear to have a taxonomic value as they are distributed unequally in the three tribes of this family. *Acokanthera* in Carisseae, *Cerbera*, *Tanghinia*, *Thevetia* in the Plumiereae, and *Apocynum*, *Nerium* and *Strophanthus* in the Nerieae. It is not known if all the species in these genera contain cardiac glycosides since, in spite of the outstanding work of Reichstein, the list is incomplete. For example, although all *Strophanthus* species appear to contain these compounds, a dozen or more, some of them present in more than one species, have also been isolated from other taxa (cymaroside, periplocymarine, strospeside, panstroside, odoroside), whilst others appear to be restricted to the species in which they occur (amboside, stroboside, boistroside, ledenioside, thollonoside). It is likely, however, with the advances in phytochemistry, that these latter substances will be subsequently found in other plants. The genus *Strophanthus* has been divided into two sub-sections; Eutrophanthus, comprising *S. hispidus*, *S. kombe*, *S. nicholsonii*, etc., and Roupellia, in which is placed *S. gratus*, which contains ouabain (XXIII). Unfortunately, ouabain is not confined to this species; first isolated from the nearly related *Acokanthera oubaio*, it has also been found in other *Strophanthus*, *S. tholloni* and *S. gardeniflorus*. Nevertheless, Bush (1952) proposed a chemical classification of the genus depending on whether the species contained either ouabain or strophanthidin (XXIV) and periplogenin or sarverogenin. In *S. sarmentosus*, which has been extensively studied on account of the presence of sarmentocymarin, used in cortisone synthesis, only one out of four morphological varieties contained the glycoside in large amounts (Schnell and Reichstein, 1953); it may be mentioned that this compound is also present in *S. gerrardi* and *S. tholloni*.

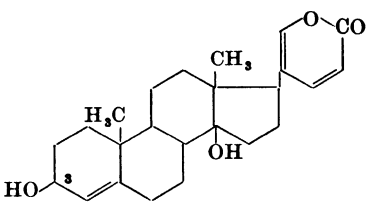
Let us now turn to the digitalis glycosides. It is strange that in the family Scrophulariaceae only the genus *Digitalis* contains cardiac

glycosides. One member, *Digitalis canariensis*, requires further chemical work to be done on it as some botanists prefer to class it in the genus *Isoplexis*. The glycosides in the other *Digitalis* are distributed in a somewhat similar manner to those of *Strophanthus* described above.

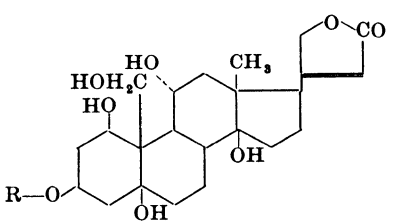
Certain glycosides are common to more than one species (*Digitalis purpurea*, *D. lanata*) or are found in other families or genera (e.g. stropeside in *Strophanthus speciosus*, odoroside in *Nerium odorum*). Others appear to be specific (digoxin and lanatoside D from *D. lanata*). It should be noted that the compounds present in *Digitalis*, although related to those found in the Apocynaceae, show some differences in structure. Thus whereas a typical *Digitalis* glucoside (e.g. XXI) has an angular methyl at C_{10}, the others often have a hydroxymethyl or aldehyde group in this position (e.g. XXIII and XXIV).



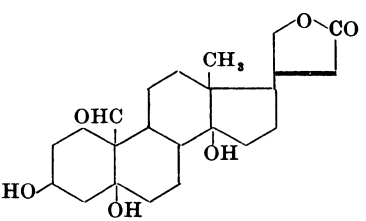
(XXI)
Digitoxigenin



(XXII)
Scillarenin



(XXIII, R = rhamnosyl)
Ouabain



(XXIV)
Strophanthidin

In conclusion, the distribution of the bufanolides (e.g. XXII) is not so different from that of the compounds discussed above, at least from the family point of view. The scillarens have been found in *Scilla* and one or two neighbouring genera (*Bowiea*), and these compounds have the same sort of distribution as the strophanthidins (XXIV). Both hellebrin (*Helleborus niger*) cardenolides and (*Adonis vernalis*) are found in Ranunculaceae.

Less common glycosides occur in many other families. The Liliaceae contain many of type (XXI); convallatoxin (rhamnoside of strophanthidin) in *Convallaria majalis*, and rhodexine (rhamnoside of sarmento-

genin) in *Rhodea japonica* as well as bufanolide in *Scilla maritima* (glucorhamnoside of XXII) and *Bowiea volubilis* (bovosides A and B).

In the *Antiaris* (Moraceae) are found α- and β-antiarin (glycosides of a congener of strophanthidin, XXIV). In the Ranunculaceae, apart from hellebrin mentioned above, *Adonis vernalis* contains cymarine (also found in *Apocynum* and *Strophanthus*), which is a cymaroside of strophanthidin, and adonitoxin (a rhamnoside of the related adonitoxigenin).

Cheiranthus cheiri (Cruciferae) contains a specific glycoside, cheirotoxin (D-gulomethylose, D-glucose and strophanthidin); *Euonymus* (Celastraceae) contains a derivative of digitoxigenin, evonoside. *Coronilla glauca*, the only genus in the Leguminosae with cardiac glycosides, contains a compound having the same aglycone (corotoxigenin) as glycosides from *Gomphocarpus fructicosus* and *Xysmalobium* (Asclepiadaceae). Compounds of the strophanthidin (XXIV) type are also present in *Mansonia altissima* (Sterculiaceae) and *Corchorus capsularis* (Tiliaceae).

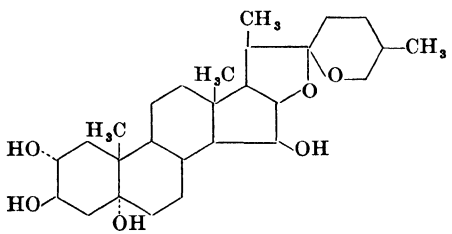
Until the chemistry of this class of glycosides is better known, the relation between their presence and the systematic position of the plant concerned must remain somewhat unclear. However, in contrast to the other types of glycoside which have been discussed, the sugar moiety of these compounds may be of help taxonomically, since some may be specific to one genus (e.g. antiaroside from *Antiaris*, allomethylose from *Gomphocarpus*). Again, we may conclude, therefore, that these substances are of more value at the species and variety levels than in classes above.

F. Saponins

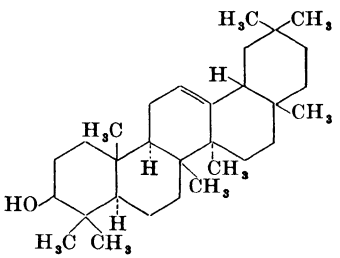
The saponins are found in about 70 families but their distribution is incompletely known, as in many cases they have only been detected by their ability to haemolyse blood. This is not surprising, as isolation in the pure state is rather difficult. They often contain 4–6 sugar residues per molecule (usually hexoses, pentoses, methyl pentoses or occasionally uronic acids), and the aglycones can be divided into two classes, steroids (e.g. XXV) or triterpenes (e.g. XXVI).

The steroidal saponins are less widespread, and apart from some families (Scrophulariaceae) are found in the monocotyledons (Liliaceae, Dioscoreaceae, Amaryllidaceae, Bromeliaceae). Triterpenoid saponins (e.g. α- or β-amyrin) are rare in monocotyledons, but are present in Loranthaceae, Phytolaccaceae, Chenopodiaceae, Amarantaceae, Ranunculaceae, Berberideae, Papaveraceae, Violaceae, Caryophyllaceae, Linaceae, Zygophyllaceae, Rutaceae, Sapindaceae, Polygalaceae, Magnoliaceae, Myrtaceae, Cucurbitaceae, Umbelliferae, Araliaceae, Primulaceae, Sapotaceae, Eberaecae, Oleaceae, Verbenaceae, Labiatae,

Campanulaceae, Rubiaceae, Compositae. They are thus compounds of extremely widespread occurrence, which lessens their taxonomic value. Some families, however, are particularly rich in saponins of the triterpenoid type (Caryophyllaceae, Sapindaceae, Polygalaceae, Sapotaceae), and although all species have not been examined, the majority of plants in them appear to contain these substances. It is not easy, however, to determine whether or not the individual glycosides are specific to certain species, or common to a large number, because of the difficulty of isolating them in a pure state.



(XXV)
Digitogenin



(XXVI)
β-Amyrin

Among the best known compounds of the triterpenoid class are aescine (glycoside of aecigenin) which has only been isolated from *Aesculus hippocastanum*, on the other hand the saponins of *Agrostemma githago*, which are derived from gypsogenin, are similar to those in other Caryophyllaceae. Aralin (glucoside of aralidin) has only been isolated from *Aralia japonica*, whereas cyclamin (*Cyclamin europaeum*) is probably present in other Primulaceae, although its structure is not completely known. It is the same with gypsophilo-saponin (*Gypsophila*), which appears to be present in a number of Caryophyllaceae. The glycosides of hederagenin (hederins) were first isolated from *Hedera helix*, but have also been detected in other genera of Araliaceae and Sapindaceae. One of the species of the Rosaceae, the Chilean soap-tree (*Quillaja saponaria*), is particularly rich in saponins, one of which gives glucuronic acid and quillajic acid (hydroxygypsogenin) on hydrolysis.

Glycyrrhizin (IIIa), the principle sweeting agent in liquorice (*Glycyrrhiza glabra*, Leguminosae) is a uronide derivative of (IIIb) and has been found also in the Sapotaceae (*Pradosia*) and ferns (*Polypodium*) although this requires confirmation.

The most specific compound, perhaps, of the steroidal saponins (Stoll and Jucker, 1955) is digitonin (two moles of galactose, two of glucose, xylose and XXV), which is only found in *Digitalis*.

The steroidal saponins of the Liliaceae have, however, been most

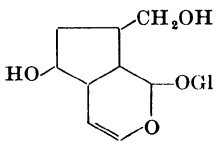
examined, because of their potential interest as precursors for cortisone synthesis. Marker and Lopez (1947) isolated numerous saponins from *Chlorogalum*, *Dioscorea*, *Yucca*, *Trillium*, *Agave*, *Hechtia*, *Manfreda* and so on. Often, one species was found to contain several saponins, and some saponins were found in neighbouring species and genera, so that there appears to be no close connection between the presence of these compounds and botanical classification.

In the last few years other families have been extensively examined (Amaryllidaceae, Dioscoreaceae) by Wall and his collaborators (1961). They found that although more than half of the species examined contained haemolytic substances, only a quarter of these contained steroidal saponins. In the different genera, the proportion with saponins varied widely, being 47% in *Yucca*, 24% in *Agave* and only 10% in *Dioscorea*. Of fourteen sapogenins isolated, six (diosgenin, gitogenin, hecogenin, sarsasapogenin, smilagenin and yammogenin) were found to be relatively specific; hecogenin in *Agave*, diosgenin in *Dioscorea*, and sarsasapogenin in *Yucca*. More interestingly a relationship was found between the configuration of the steroid ring and certain groups of plants. For example, some species of *Agave* yielded *trans* derivatives (tigogenin, hecogenin, gitogenin), while others gave *cis* compounds (smilagenin). The same happens in *Yucca* and *Dioscorea*. This considerable investigation indicates the need to undertake systematic research to obtain results of real value in chemical plant taxonomy.

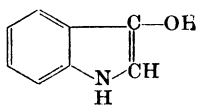
G. Other Glycosides

There are a number of glycosides which do not fit into the previous classes discussed. For example, the chromogenic glycosides which are in many cases responsible for dark coloration in plants occurring after frost or during drying. Aucubin (XXVII), which was first isolated from *Aucuba japonica* (Cornaceae) was also found in the neighbouring genera, *Garrya* and *Plantago*. By 1954 it had been demonstrated in twenty-odd species in five different families and, using paper chromatography, Paris and Chaslot (1959) have detected it in fifty new species. It is not found in lower plants, gymnosperms or monocotyledons. It is rarely present in Apetalae (Urticaceae) or Dialypetalae (Haloragaceae, Cornaceae), but mainly found in Gamopetalae (Loganiaceae, Scrophulariaceae, Utriculariaceae, Orobanchaceae, Globulariaceae, Plantaginaceae). In the Loganiaceae, it is found only in *Buddleia*; the sub-family of Buddleioidae is close to the Scrophulariaceae, which is rich in aucubin. Aucubin is not found in Umbelliferae or Araliaceae, both of which are neighbouring families of the Cornaceae, which contains the genus *Aucuba*.

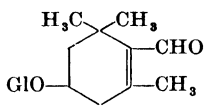
Another chromogenic glycoside, asperuloside, just isolated from *Asperula odorata* (Rubiaceae), is less well distributed than aucubin, although it is found in such diverse families as Euphorbiaceae (*Daphniphyllum*) and Saxifragaceae (*Escallonia*).



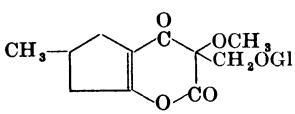
(XXVII)
Aucubin



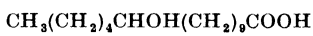
(XXVIII)
Indoxyl



(XXIX)
Picrocrocin



(XXX)
Verbenalin



(XXXI)
Jalapinolic acid

Indican (glucoside of indoxyl, XXVIII), which yields indigo, is also found in a number of well differentiated families; Leguminosae (*Indigofera tinctoria*), Cruciferae (*Isatis tinctoria*), Polygonaceae (*Polygonum tinctorium*) and Apocynaceae (*Wrightia tinctoria*). On the other hand, another chromogen, catalpin, has only been extracted from *Catalpa bignonoides*.

Certain specific glycosides are found in the class of bitter principles. Gentiopicrin (VI) is found in the Gentianaceae (with the exception of the tribe Menyanthoideae); picrocrocin (XXXIX) in *Crocus sativus*, and verbenalin (XXXc) in *Verbena officinalis*.

The Convolvulaceae contain certain specific glycosides (glucoresins) such as jalapin (2 mols glucose, rhamnose, rhodeose and jalapinolic acid, XXXI), convolvulin, and turpethein, not found in other families.

Other glycosides which are at present only known from one species are rhaponticin (3′-glucoside of 3,3′,5′-trihydroxy-4-methoxystilbene) from *Rheum rhaponticum*, arctiin (lignan glucoside) from *Arctium lappa*, and atractylic acid (a complex tribasic C_{30} acid) from *Atractylis gummifera*.

Thus the diverse glycosides of this group are distributed like those of other groups already discussed; certain being specific to a single plant, others being found in widely different families.

V. Discussion

What conclusions can be reached from this brief and very incomplete survey of the distribution of glycosides in the Plant Kingdom? First of all, it is obvious that the distribution of many classes has hardly been examined, and much work remains to be done. Again, certain glycosides have only been isolated in an impure state and their structure is not known with certainty. Furthermore, some compounds that had previously been thought to be specific to an individual species have, with the application of new methods, been found in other plants.

Bearing these difficulties in mind, some general conclusions can, however, be made. On the whole, the lower plants do not contain many glycosides apart from the complex nitrogen-containing compounds in the *Streptomyces*, lycoperdin, aminoglucoside from *Lycoperdon* and floridoside from red algae. Even in the Cryptogamae, glycosides, apart from a few flavonoids and saponins, are rare.

In the Gymnospermae, glucosides (coniferin, macrozamin) are not abundant, and only the Angiospermae contain the whole range of glycosides discussed above. Apart from certain special cases, analogous glycosides are found both in monocotyledons and dictyledons. Only a few families contain only one predominant type of glycoside (saponins in Caryophyllaceae, glucoresins in Convolvulaceae), and usually several categories are found, not only in the same family, but also in the same genus or species. It is thus difficult to establish any general rule, and one must examine particular cases. From the taxonomic point of view, glycosides appear to be interesting at the level of species or variety (Hegnauer, 1957) and one can distinguish "chemical" races as described previously (p. 348).

In such cases, or in those where a whole family has been well studied, the distribution of glycosides is of incontestible value in chemical taxonomy. But there are too many gaps in our knowledge, and if we wish to assess their importance to plant taxonomy further, we must continue to investigate their presence, structure and function in the Plant Kingdom.

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CHAPTER 13

Distribution of Anthocyanins in Higher Plants

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I. Introduction

In most surveys of the natural occurrence of the anthocyanins (e.g. Lawrence *et al.*, 1939; Taylor, 1940; Beale *et al.*, 1941; Hayashi and Abe, 1955; Forsyth and Simmonds, 1954), the main emphasis has been upon the type of aglycones (or anthocyanidins) present, rather than upon the glycosides. Even with these limitations, three points of general systematic interest have arisen from these studies. (1) Pelargonidin and delphinidin were found to occur with greater frequency in the more advanced plants, replacing cyanidin, which was considered to be the most "primitive" anthocyanidin. (2) It was discovered that normal anthocyanins were replaced by nitrogenous pigments in the flowers of eight families, all of which belong to the order Centrospermae. (3) Pigments which differed slightly from the usual pattern were found very rarely (e.g. hirsutin from *Primula* (Karrer and Widmer, 1927), gesnerin from *Gesneria* (Robinson *et al.*, 1934) and carajurin from *Bignonia* (Chapman *et al.*, 1927)).

It was not possible to draw any other systematic conclusions from the

data available because of the lack of detailed information about structures, which followed from the technical difficulties of isolation and identification. However, paper chromatography which was introduced into this field by Bate-Smith (1948), as in so many other biochemical endeavours, provided the technique necessary for speeding up anthocyanin identifications (Harborne, 1958b). As a result, the pigments present in many food crops and ornamental plants have been completely characterized for the first time. In this laboratory alone, over a hundred anthocyanins have been identified in connection with genetical studies. Much progress has been made in identifying the sugar moieties of anthocyanins and a remarkably wide range of glycosidic types has been observed (Harborne, 1962a). In addition four new anthocyanidins have been found, and the structures of an appreciable number of those pigments which had been studied by the older methods were found to require revision.

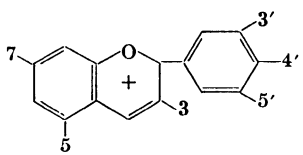
It is the purpose of this chapter to collect together all the data that has now accumulated about the detailed structures of anthocyanins and consider them in relationship to plant taxonomy.

II. Broad Distribution of Anthocyanins

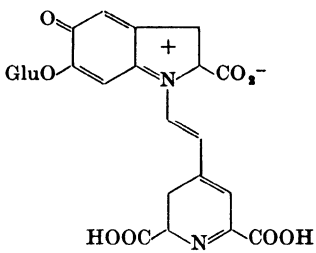
The anthocyanins, which are all based on the flavylium skeleton (I), are characteristic of, and almost confined to the higher plants. They have been recorded in Gymnospermae, but are present with much greater frequency in Angiospermae. There is only one reliable report of occurrence in lower plants; Bendz *et al.* (1962) isolated the 5-mono- and the 5-diglucoside of luteolinidin (see Table II) from the moss, *Bryum cryophilum*. It is not certain whether anthocyanins are present in ferns. Price *et al.* (1938) reported that the young fronds of several Pteridophyta, yielded anthocyanin-like solutions on extraction; the aglycones were said to resemble 6-hydroxycyanidin or 6-hydroxypelargonidin. However, spectral re-examination of one such pigment (from *Osmunda regalis*) showed that the compound was not an anthocyanin (Harborne, 1962b). Reports of the presence of anthocyanins in the algae have been discounted by Alston (1958). Very recently, a pigment with spectral properties (λ_{max} 280 and 500 mμ) similar to those of anthocyanins has been isolated from a fungus by Peterson *et al.* (1961); on the available evidence, however, it could equally well be an anthraquinone.

Anthocyanins are responsible for nearly all the red, pink, mauve and blue colours in higher plants. They are, however, replaced by another kind of pigment in eight of the nine families, which comprise the order Centrospermae. The characteristic pigments of this order are the beta-

cyanins (Reznik, 1957; Dreiding, 1961) formerly called "nitrogenous anthocyanins". Betanin, the pigment of *Beta vulgaris*, is now known (Mabry *et al.*, 1962) to have a structure identical with or tautomeric with (II). It is thus quite different from the flavylium pigments and is presumably derived biogenetically from two molecules of 3,4-dihydroxyphenylalanine.



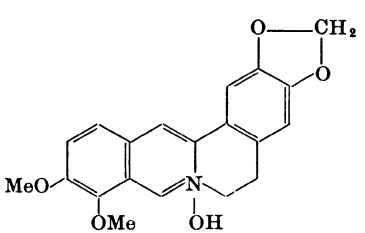
(I)
Flavylium ion



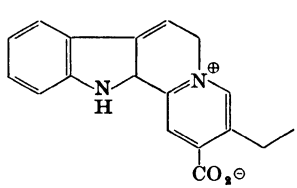
(II)
Betanin

From the systematic point of view, the position of the Caryophyllaceae, the only family of the Centrospermae that lacks betacyanins, is of great interest. Normal anthocyanins have been found in all the genera of the Caryophyllaceae which have been studied to date. The chemical data therefore suggests the removal of Caryophyllaceae from this order (cf. Mabry *et al.*, 1963). It is worth adding that the ability of members of the Cactaceae to synthesize betacyanins helped to secure them a place in the Centrospermae.

Betacyanins are accompanied in the Centrospermae by a related series of yellow pigments called betaxanthins (Dreiding, 1961), the structures of which have not yet been determined. It is not clear whether these pigments are confined to the Centrospermae. It should be noted in passing that yellow nitrogenous pigments have been found in other plants. The alkaloid, berberine (III) is present (mainly in the roots) of many plants of the Berberidaceae, Ranunculaceae, Anonaceae, Menispermaceae, Papaveraceae and Rutaceae (Henry, 1949). Flavocarpine (IV), which



(III)
Berberine



(IV)
Flavocarpine

occurs in stem bark of *Pleiocarpa mutica* (Apocynaceae), is reported to have a brilliant yellow colour (Buchi *et al.*, 1962). Another nitrogenous pigment (nudicaulin), which has only been partly characterized, is present in the petals of *Papaver nudicaule* (Price *et al.*, 1939).

Anthocyanins may occur in any part of the plant, but there are frequently differences in the type of pigment found in different organs. Thus the sepals, petals and pollen of the same plant may each have a different anthocyanin. Broadly speaking, leaves and fruits tend to have simpler pigments than flowers (Lawrence *et al.*, 1939). In systematic surveys, it has been customary to examine the pigments of only one organ, and since the flower pigments have been most commonly chosen, the results presented here refer mainly to the floral constituents. However, it is clear that, although the nature of the anthocyanidins may vary from tissue to tissue, the glycosidic pattern is often more consistent. For example, in the Solanaceae, the characteristic acylated petunidin 5-glucoside 3-rutinoside, petanin, occurs in tubers and seeds (potato), leaves (tomato), berries (*Solanum quitoense*) and flowers (*Petunia*) (Harborne, 1960a). Therefore, if data on flower pigments are not available, it seems reasonable to use results obtained from studies on leaves or fruits, remembering that the aglycones themselves may sometimes be of simpler structure.

A handicap in carrying out surveys of anthocyanin distribution is that a considerable number of plants do not show any visible pigmentation. Anthocyanin formation, can, however be induced, in the leaves of some plants (e.g. the tomato), by altering the environment. Indeed, transient anthocyanin coloration often occurs in the young or dying leaves of many species which are not normally cyanic, and surveys of such material have been carried out (e.g. Hayashi and Abe, 1955). In addition Reznik and Neuhäusel (1959) have shown that some aquatic plants contain anthocyanins in colourless form as the pseudo bases; extraction with cold acid produces normal pigments. To summarize, it is likely that many plants, which are apparently acyanic, will produce anthocyanin pigment given the right conditions. Use could perhaps be made of this fact when carrying out surveys of critical groups.

III. Systematic Distribution of Anthocyanina

The results of all reliable anthocyanin identifications that have been carried out to date are included in Table I. The data have been compiled from the following sources. (1) The work of the 1920–40 period, which has been collected together by Mayer and Cook (1943) and Karrer (1958). Of these results, only those which have been subsequently confirmed by

paper chromatographic studies are included. Although most of the earlier identifications of simple glycosides are reliable, many of the first reports of acylated anthocyanins have required subsequent revision. (2) The work of the Japanese school, which has been summarized by Hayashi (1962). (3) *Chemical Abstracts* entries, up to September 1962. (4) Work carried out at the John Innes Institute (Harborne, 1962a,c and unpublished).

The plant families listed in Table I are arranged according to the system of Engler and Prantl. This is a departure from the practice followed in nearly all the previous surveys (e.g. Lawrence *et al.*, 1939), in which Hutchinson's (1926) system has been adopted. The Hutchinson classification has not been used here partly because his revised system (Hutchinson, 1959) differs considerably from his earlier one, partly for the sake of uniformity with other workers in this field (e.g. Bate-Smith, 1962) and partly also because the Engler system is still the more widely accepted of the two.

The results in Table I refer to flower pigments unless otherwise stated (see remarks column), and no distinction is made between results obtained from cultivated plants as against wild species.

The emphasis in Table I is on the type of sugar present in the anthocyanins, since early surveys have dealt adequately with the distribution of the aglycones. Pigments in which the position and nature of the sugar residues have not been accurately determined are therefore excluded. However, all occurrences of unusual anthocyanidins are reported, irrespective of knowledge of their sugars.

IV. Hydroxylation Patterns of Anthocyanins

Ignoring methylation for the moment, there are seven different anthocyanidins known in nature (Table II); the hydroxylation pattern is thus more restricted here than in other flavonoids (e.g. flavonols, isoflavones etc.). 6-Hydroxylation is unknown, except for the rare carajurin; reports of the natural occurrence of 6-hydroxycyanidin (see p. 360) have not been substantiated. 8-Hydroxyanthocyanidins, corresponding to the flavonols gossypetin (V, R = OH) and herbacetin (V, R = H), have not so far been found. Leucoanthocyanidins which yield the flavylium salts (VI, R = H and OH) have, however, been found in nature; they are teracacidin and melacacidin, respectively (Clarke-Lewis, 1962).

Three anthocyanidins, pelargonidin, cyanidin and delphinidin (Table II), occur far more frequently than any other types. Of these three, cyanidin is the most common, being present in 80% of permanently pigmented leaves, 69% of fruits and 50% of flowers (Lawrence *et al.*

TABLE I

Natural distribution of anthocyanins

Order, family and genus	No. of species examined	Anthocyanin			Remarks and references
		Glycosidic type(s)*	Aglycones†	Acyl groups	
MONOCOTYLEDONEAE					
GLUMIFLORAE					
Gramineae					
Hordeum	1	3-Arabinoside	Dp, Cy, Pg	None	In aleurone (Metche and Urion, 1961)
Oryza	1	3-Glucoside 3,5-diglucoside 3-Rhamnosylglucoside	Mv, Cy	None	In leaves, etc. (Suzushino *et al.*, 1961)
Pennisetum	1	3-Glucoside	Cy	None	In spike hair (Shibata and Sakai, 1958)
Zea	1	3-Glucoside	Cy, Pg	None	In endosperm (Straus, 1959)
SPATHIFLORAE					
Lemnaceae					
Spirodela	2	3-Glucoside	Cy, Pt	None	Leaf pigments (Ng and Thimann, 1962; Harborne, 1962b)
FARINOSAE					
Commelinaceae					
Commelina	1	3,5-Diglucoside	Dp	*p*-Coumaric	Present as a metal complex (Mitsui *et al.*, 1959)
LILLIFLORAE					
Liliaceae					
Fritillaria	23	3-Rutinoside 3-Xylosylrhamnoside	Cy	None	3-Xylosylrhamnoside only in one species (Shibata, 1958; Harborne, 1962a)
Hyacinthus	1	3,5-Diglucoside	Pg, Cy, Dp	*p*-Coumaric	(Harborne, 1958b)
Lilium	1	3-Rutinoside	Cy	None	(Harborne, 1962b)
Scilla	1	3,5-Diglucoside	Cy, Dp	*p*-Coumaric	(Riding, 1961)

Tulipa	20	3-Glucoside 3-Rutinoside 3,5-Diglucoside 3-Rhamnosylglucoside (see Table III)	Pg, Cy, Dp	None	Many cultivars have also been examined (Shibata and Ishikura, 1960; Halevy, 1962)
Amaryllidaceae					
Lycoris	1	3-Glucoside 3-Sambubioside	Cy	None	(Hayashi, 1942)
Iridaceae					
Babiana	1	3,5-Diglucoside	Mv	None	(Harborne, 1962b)
Chasmanthe	1	3-Rhamnosylglucoside (see Table III)	Cy, Pn	None	(Harborne, 1962b)
Crocosma	1	3,5-Diglucoside 3-Rutinoside, 5-glucoside	Cy	None	(Harborne, 1962b)
Crocus	8	3,5-Diglucoside	Mv, Dp	None	(Hayashi, 1960)
Freesia	1	3-Glucoside	Mv	None	(Harborne, 1962b)
Iris	2	3,5-Diglucoside	Mv, Dp	*p*-Coumaric	(Hayashi, 1940)
Lapeyrousa	1	3-Rhamnosylglucoside (see Table III)	Cy	None	(Harborne, 1962b)
Tritonia	2	3-Gentiobioside	Pg	None	(Harborne, 1962a)
Watsonia	3	3-Sophoroside-7-glucoside 3-Sophoroside 3-Glucoside	Pg	None	(Harborne, 1962a)
SCITAMINEAE					
Cannaceae					
Canna	1	3-Rhamnosylglucoside	Cy	None	(Hayashi *et al.*, 1954)
MICROSPERMAE					
Orchidaceae					
Anacamptis	1	3,5-Diglucoside	Cy	None	(Harborne, 1962b)
		DICOTYLEDONEAE—Archichlamydeae			
FAGALES					
Fagaceae					
Fagus	1	3-Galactoside	Cy, Pg	None	In leaf of "copper beech" (Harborne and Sherratt, 1957)

* For structures of disaccharides see Table III.

† Ap = apigeninidin, Cy = cyanidin, Dp = delphinidin, Hs = hirsutidin, Lu = luteolinidin, Mv = malvidin, Pg = pelargonidin, Pn = peonidin, Pt = petunidin Rs = rosinidin (see Table II).

TABLE I—*continued*

Order, family and genus	No. of species examined	Anthocyanin			Remarks and references
		Glycosidic type(s)*	Aglycones†	Acyl groups	
URTICALES					
Urticaceae					
Morus	1	3-Glucoside	Cy	None	In mulberry (Harborne, 1962b)
POLYGONALES					
Polygonaceae					
Polygonum	1	3-Galactoside	Cy	None	(Sugano and Hayashi, 1960)
RANALES					
Calycanthaceae					
Calycanthus	1	3-Glucoside	Cy	None	(Hayashi and Noguchi, 1952)
Ranunculaceae					
Anemone	1	3-Glucoside	Pg, Cy, Mv	None	In pollen; flower pigments not yet identified (Tappi and Monzani, 1955)
Delphinium	1	3,5-Diglucoside	Dp	None	(Harborne, 1962c)
Paeonia	8	3,5-Diglucoside	Cy, Pn	None	(Beale *et al.*, 1941)
RHOEADALES					
Papaveraceae					
Papaver	6	3-Sophoroside-7-glucoside 3-Sophoroside 3-Glucoside	Pg, Cy	None	*P. nudicaule* contains unidentified yellow pigment (not anthocyanin) (Harborne, 1962a)
Cruciferae					
Matthiola	1	3-Sambubioside-5-glucoside 3-Glucoside	Pg, Cy	*p*-Coumaric Ferulic and sinapic	(Harborne, 1962a)
Raphanus	2	3-Sophoroside-5-glucoside	Pg, Cy, Mv	Ferulic, *p*-coumaric and caffeic	In pods and roots as well as flowers (Harborne, 1958a, 1962a; Ishikura and Hayashi, 1962)
Brassica	1	3-Sophoroside-5-glucoside	Cy	Sinapic (1 and 2)	In purple cabbage leaf (Harborne, 1962a; Stroh, 1959)

ROSALES					
Crassulaceae					
Kalanchoe	1	3,5-Diglucoside	Cy	None	(Harborne, 1962b)
Rosaceae					
Fragaria	2	3-Glucoside	Cy, Pg	None	In fruit (Sondheimer and Karash, 1956)
Malus	2	3-Galactoside	Cy	None	In apple skin (Sando, 1937)
Prunus	3	3-Glucoside 3-Rutinoside 3-Glucosylglucoside	Cy	None	In cherries and plums (Li and Wagenknecht, 1956, 1958; Parkinson, 1954)
Pyrus	1	3-Galactoside	Cy	None	In the bark (Harborne, 1962b)
Rubus	3	3-Glucoside 3-Sophoroside 3-Rutinoside 3-(2^G-glucosylrutinoside)	Cy, Pn, Pg	None	In fruits (Huang, 1956; Harborne, 1962b)
Rosa	21	3,5-Diglucoside 3-Glucoside	Pg, Cy, Pn	None	The same pigments are present in cultivar roses (Harborne, 1961)
Saxifragaceae					
Hydrangea	1	3-Glucoside	Dp	None	(Asen *et al.*, 1957)
Ribes	1	3-Glucoside 3-Rutinoside	Cy, Dp	None	In berries (Chandler and Harper, 1962)
Leguminosae					
Glycine	1	3-Glucoside	Cy	None	In seed coat (Kuroda and Wada, 1935)
Lathyrus	12	3-Rhamnoside-5-glucoside 3-Rhamnoside 3-Xylosylgalactoside 3-Galactoside	Pg, Cy, Pn Dp, Pt, Mv	None	Galactoside types occur only in garden Sweet Pea (Harborne, 1962a)
Lespedeza	1	3,5-Diglucoside	Mv	None	(Hayashi *et al.*, 1955)
Lupinus	1	3,5-Diglucoside	Pg, Cy, Dp	None	Anthocyanin as metal complex in blue lupin (Bayer, 1959)
Pisum	4	3-Rhamnoside-5-glucoside	Mv, Pt, Dp Pn, Cy	None	A third glycosidic type is present in the flower of this genus (Dodds and Harborne, 1962)

* For structures of disaccharides see Table III.

† Ap = apigeninidin, Cy = cyanidin, Dp = delphinidin, Hs = hirsutidin, Lu = luteolinidin, Mv = malvidin, Pg = pelargonidin, Pn = peonidin, Pt = petunidin, Rs = rosinidin (see Table II).

TABLE I—*continued*

Order, family and genus	No. of species examined	Anthocyanin			Remarks and references
		Glycosidic type(s)*	Agylcones(†)	Acyl groups	
Phaseolus	2	3-Sophoroside 3-Glucoside 3,5-Diglucoside	Pg, Cy, Pn Dp, Pt, Mv	None	Mainly seed coat pigments (Feenstra, 1960; Harborne, 1962a)
Tamarindus	1	3-Glucoside	Cy	None	(Lewis and Johar, 1956)
Wisteria	1	3,5-Diglucoside	Dp	None	(Harborne, 1962b)
GERANIALES					
Geraniaceae					
Pelargonium	1	3,5-Diglucoside	Pg, Pn, Mv	None	(Harborne, 1961)
Tropaeolaceae					
Tropaeolum	2	3-Sophoroside	Pg	None	(Harborne, 1962a)
Rutaceae					
Citrus	1	3-Glucoside	Cy, Dp	None	Pigments of blood orange (Chandler, 1958)
Euphorbiaceae					
Poinsettia	1	3-Glucoside 3-Rutinoside	Pg, Cy	None	Bract pigments (Asen, 1958)
SAPINDALES					
Empetraceae					
Empetrum	2	3-Galactoside	Dp, Mv	None	In berries (Hayashi *et al.*, 1951)
Aceraceae					
Acer	6	3-Glucoside	Cy	None	In leaf (Hattori and Hayashi, 1937)
Balsaminaceae					
Impatiens	1	3,5-Diglucoside	Pg, Pn	None	(Hayashi *et al.*, 1953)
Aquifoliaceae					
Ilex	1	3-Xylosylglucoside	Cy	None	(Hayashi, 1942)
RHAMNALES					
Vitaceae					
Vitis	2	3-Glucoside 3,5-Diglucoside	Cy, Pn, Dp Pt, Mv	*p*-Coumaric	In fruit (Riberau-Gayon, 1959)
Rhamnaceae					
Ceanothus	1	3-Rhamnoside	Mv	None	(Harborne, 1962b)

MALVALES					
Malvaceae					
Hibiscus	2	3-Glucosylglucoside	Cy	None	(Hayashi, 1944)
Malva	1	3,5-Diglucoside	Mv	None	(Willstäter and Meig, 1915)
Sterculiaceae					
Theobroma	1	3-Arabinoside 3-Galactoside	Cy	None	In cocoa pod (Forsyth and Quesnel, 1957)
Chiranthodendron	1	Glucoside	Ap	Gallic acid	In the calyx of "Maxpalxochitl" (Pallares and Garza, 1949)
PARIETALES					
Theaceae					
Camellia	2	3-Glucoside	Cy	None	Tricetinidin formed in processed leaf (Roberts and Williams, 1958; Chandra, 1960)
Violaceae					
Viola	1	3-Rhamnosylglucoside-5-glucoside	Cy, Dp	*p*-Coumaric	(Endo, 1959)
Begoniaceae					
Begonia	56	3-Sambubioside 3-Sophoroside 3-(2^G-xylosylrutinoside) 3-(2^G-glucosylrutinoside)	Cy, Pg	None	(Bopp, 1957; Harborne, 1962b)
Passifloraceae					
Passiflora	1	3-Glucosylglucoside	Pg	None	In fruit rind (Pruthi *et al.*, 1961)
MYRTIFLORAE					
Punicaceae					
Punica	1	3,5-Diglucoside	Pg, Dp	None	The Dp, 3,5-diglucoside is in the pomegranate juice (Harborne, 1962a, b)
Lythraceae					
Cuphea	1	3,5-Diglucoside	Pg, Mv	None	(Harborne, 1962b)
Lythrum	1	3,5-Diglucoside	Mv	None	(Harborne, 1962b)

* For structures of disaccharides see Table III.

† Ap = apigeninidin, Cy = cyanidin, Dp = delphinidin, Hs = hirsutidin, Lu = luteolinidin, Mv = malvidin, Pg = pelargonidin, Pn = peonidin, Pt = petunidin, Rs = rosinidin (see Table II).

TABLE I—*continued*

Order, family and genus	No. of species examined	Anthocyanin			Remarks and references
		Glycosidic type(s)*	Aglycones(†)	Acyl groups	
Myrtaceae					
Eucalyptus	1	3-Glucoside	Cy, Dp	None	Leaves (Hillis, 1956)
Metrosideros	1	3-Glucoside	Dp, Mv	None	(Cambie and Seelye, 1961)
Melastomaceae					
Tibouchina	1	3,5-Diglucoside	Mv	*p*-Coumaric (2)	(Harborne, 1962b)
Onagraceae					
Clarkia	1	3,5-Diglucoside	Mv	None	(Harborne, 1962b)
Fuchsia	1	3,5-Diglucoside	Pn, Mv	None	(Harborne, 1962b)
Oenothera	1	3-Glucoside	Cy	None	(Wada, 1950)
		DICOTYLEDONEAE—Sympetalae			
ERICALES					
Ericaceae					
Rhododendron	83	3-Galactoside 3-Arabinoside 3,5-Diglucoside	Cy, Mv	None	Azalein occurrs in 44 species but not in Cy-containing spp. (Harborne, 1962d)
Vaccinium	3	3-Galactoside	Cy, Pn, Mv	None	In berries (Sakamura and Francis, 1961; Suomalainen and Keranen, 1961; Hayashi, 1949)
PRIMULALES					
Myrsinaceae					
Ardisia	1	3-Galactoside	Cy, Pn	None	In berries (Harborne, 1962a)
Bladhia	1	3-Galactoside	Dp, Mv	None	(Yeh and Huang, 1961; see also Harborne, 1962a)
Primulaceae					
Cyclamen	1	3-Glucoside	Mv	None	Other unidentified glycosides in cultivars (Karrer and Widmer, 1927; Harborne, 1962b)

Primula	15	3-Glucoside 3,5-Diglucoside 3-Gentiobioside 3-Glucosylgentiobioside	Pg, Cy, Pn Rs, Dp, Pt Mv, Hs	None	In leaves and stems as well as flowers. *Note*: 7-methylated pigments (Rs, Hs); kampferol-3-glucosylgentibioside present in most species (Harborne and Sherratt, 1961)
PLUMBAGINALES					
Plumbaginaceae					
Ceratostigma	2	Not known	Dp, Pt, Mv	None	Azalein present, cf. *Plumbago* (Harborne, 1962b)
Limonium	3	Not known	Dp	None	Azalein absent (Harborne, 1962b)
Plumbago	2	3-Rhamnoside	Pg, Cy, Dp, Cp	None	Azalein and Cp present (Harborne, 1962d)
CONTORTAE					
Oleaceae					
Ligustrum	1	3-Glucoside	Mv	None	In fruit (Hayashi, 1943)
Apocynaceae					
Lochnera	1	Not known	Hs	None	Only other source of Hs is in Primulaceae, see above (Forsyth and Simmonds, 1957)
TUBIFLORAE					
Convolvulaceae					
Ipomoea	1	3,5-Diglucoside	Pg	None	(Kataoka, 1936)
Polemoniaceae					
Gilia	1	3,5-Diglucoside	Pg	*p*-Coumaric	(Harborne, 1962a)
Phlox	1	3,5-Diglucoside	Pg	None	(Harborne, 1962b)
Boraginaceae					
Anchusa	1	3,5-Diglucoside	Pt	None	(Harborne, 1962b)
Verbenaceae					
Clerodendron	1	3-Glucoside	Pg	None	(Harborne, 1962b)
Lantana	1	3-Glucoside	Cy	None	(Harborne, 1962b)

* For structures of disaccharides see Table III.

† Ap = apigeninidin, Cy = cyanidin, Dp = delphinidin, Hs = hirsutidin, Lu = luteolinidin, Mv = malvidin, Pg = pelargonidin, Pn = peonidin, Pt = petunidin, Rs = rosinidin (see Table II).

TABLE I—*continued*

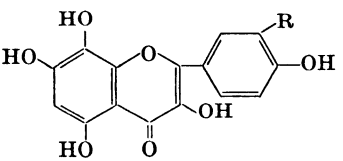
Order, family and genus	No. of species examined	Anthocyanin			Remarks and references
		Glycosidic type(s)*	Aglycones(†)	Acyl groups	
Verbena	6	3-Glucoside 3,5-Diglucoside	Pg, Cy, Dp	None	(Scott-Moncrieff and Sturgess, 1940; Harborne, 1962b)
Labiatae					
Monarda	3	3,5-Diglucoside	Pg	*p*-Coumaric	Acylated pigments also present in seven other genera in this family (Harborne, 1958a; Asen, 1961; Kuroda and Wada, 1935)
Perilla	1	3,5-Diglucoside	Cy	*p*-Coumaric	
Salvia	7	3,5-Diglucoside	Pg, Cy, Dp	*p*-Coumaric, caffeic	
Solanaceae					
Atropa	1	5-Glucoside-3-rutinoside	Pt	*p*-Coumaric	Also in berry (Harborne, 1960a)
Browallia	1	3,5-Diglucoside	Dp	*p*-Coumaric (2)	(Harborne, 1962b)
Brunfelsia	1	5-Glucoside-3-rutinoside	Mv	*p*-Coumaric	(Harborne, 1962b)
Cestrum	3	3-Rutinoside	Pg, Cy	None	(Harborne, 1962b)
Iochroma	2	3-Glucoside	Pg, Mv	None	(Harborne, 1962b)
Nicotiana	2	3-Rutinoside	Cy	None	(Harborne, 1962b)
Lycopersicon	1	5-Glucoside-3-rutinoside	Pt	*p*-Coumaric	In leaf and stem (Harborne, 1960a)
Petunia	2	5-Glucoside-3-rutinoside	Dp, Pt, Mv	*p*-Coumaric	(Harborne, 1960a)
Solanum	66	5-Glucoside-3-rutinoside	Pg, Cy, Pn	*p*-Coumaric	In all parts of plant (Harborne, 1960a; Dodds and Long, 1955)
Scrophulariaceae					
Antirrhinum	8	3-Rutinoside 3,5-Diglucoside	Pg, Cy, Dp	None	(Scott-Moncrieff, 1930; Harborne, 1962b)
Collinsia	12	3-Glucosylglucoside	Pg, Cy, Dp	None	(Garber, 1958)
Maurandia	1	3,5-Diglucoside	Dp	None	(Harborne, 1962b)
Pentstemon	2	3,5-Diglucoside	Dp	None	(Harborne, 1962b)
Phygelius	1	3-Rutinoside	Pg	None	(Harborne, 1962b)
Bignoniaceae					
Arrabidaea	1	None	Carajurin	None	Occurs without sugar attachment in leaves (Chapman *et al.*, 1927)
Gesneriaceae					
Achimenes	1	5-Glucoside-3-rutinoside	Pg, Mv	None	(Harborne, 1962a)

Columnea	2	Probably none	Columnidin	None	All contain pigments lacking 3-OH (Harborne, 1960b, 1962a)
Gesneria	1	5-Glucoside	Ap, Lu	None	
Kohleria	1	5-Glucoside	Ap, Lu	None	
Kohleria hybrid	1	5-Glucoside-3-rutinoside	Mv	None	Leaves contain pigment lacking 3-OH (Harborne, 1962b)
Streptocarpus	10	5-Glucoside-3-rutinoside 3,5-Diglucoside 3-Sambubioside 3-Glucoside	Pg, Cy, Pn Dp, Pt, Mv	None	(Harborne, 1962a)
Saintpaulia	1	5-Glucoside-3-rutinoside	Mv	None	(Harborne, 1962a)
RUBIALES					
Caprifoliaceae					
Sambucus	1	3-Sambubioside 3-Glucoside and 3-sambubioside-5-glucoside	Cy	None	In berry (Harborne, 1962a; Reichel and Reichwald, 1960)
Rubiaceae					
Rubia	1	3-Glucosylglucoside	Cy	None	In berry (Hayashi, 1944)
CAMPANULATAE					
Campanulaceae					
Campanula	1	3,5-Diglucoside	Dp	None	(Harborne, 1962b)
Compositae					
Callistephus	1	3-Glucoside	Pg, Cy	None	(Willstäter and Burdick, 1916)
Cosmos	1	3-Rutinoside	Cy	None	(Hayashi, 1941)
Coreopsis	1	3-Glucoside	Cy	None	(Shimokoriyama, 1957)
Centaurea	3	3,5-Diglucoside	Cy	None	As a metal complex (Bayer, 1958)
Chrysanthemum	1	3-Glucoside	Cy	None	Willstäter and Bolton, 1916)
Dahlia	5	3,5-Diglucoside	Pg, Cy	None	(Hayashi, 1933; Harborne and Sherratt, 1957)
Helenium	1	3,5-Diglucoside	Cy	None	(Willstäter and Bolton, 1916)
Solidago	1	3-Glucosylglucoside	Cy	None	(Bjorkman and Holmgren, 1958)
Zinnia	1	3,5-Diglucoside	Cy	None	(Willstäter and Bolton, 1916)

* For structures of disaccharides see Table III.

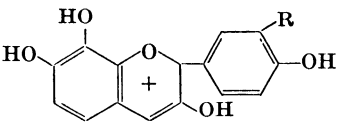
† Ap = apigeninidin, Cy = cyanidin, Dp = delphinidin, Hs = hirsutidin, Lu = luteolinidin, Mv = malvidin, Pg = pelargonidin, Pn = peonidin, Pt = petunidin, Rs = rosinidin (see Table II).

1939). Delphinidin is next in order of frequency, followed by pelargonidin. Delphinidin is noticeably absent from certain families, e.g. the Rosaceae, the Papaveraceae and the Orchidaceae. By contrast, it is particularly abundant in other families, notably in the related group: Boraginaceae, Campanulaceae, Polemoniaceae and Hydrophyllaceae. There is here, of course, a strong correlation between the presence of delphinidin and blue flower colour. Only 10% of the blue-flowered species examined by Gascoigne *et al.* (1948) did not have delphinidin.



(V)

Gossypetin (R = OH);
Herbacetin (R = H)



(VI)

Teracacidin (R = H);
Melacacidin (R = OH)

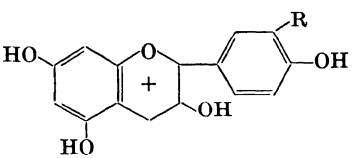
Pelargonidin is found much more frequently in cultivated than in wild plants, probably because there is a strong human selection for brilliant scarlet and orange-red colours among ornamental plants. There is, however, no reason for believing that pelargonidin occurs more frequently in tropical than in temperate plants, as suggested by Beale *et al.* (1941). Thus, Forsyth and Simmonds (1954) found pelargonidin in only 17% of the tropical genera they studied (a comparable figure for temperate plants is 23%, cf. Beale *et al.*, 1941). It is true that Gascoigne *et al.* (1948) found pelargonidin to be scarce in Australian plants; this was possibly because they were sampling a totally different flora and also because they examined only wild species.

Anthocyanins lacking a 3-hydroxyl group are very rare; they have been found in only four families: Bignoniaceae, Gesneriaceae, Sterculiaceae and Theaceae. One has also been recorded in a moss (see p. 360). The first pigment of this type to be isolated was carajurin (Chapman *et al.*, 1927) which is present (apparently in the free state) in *Arrabidaea chica* (formerly *Bignonia chica*). It is interesting that Forsyth and Simmonds (1954) have indicated that a related gesnerin-like pigment occurs in *Tecomaria capensis* (also Bignoniaceae). Gesnerin (apigeninidin 5-glucoside) itself was first found in *Gesneria fulgens* (Robinson *et al.*, 1934) and has since been identified in *Kohleria eriantha*. It is accompanied, in both these plants, by luteolinidin 5-glucoside (Harborne, 1960b). Two incompletely characterized pigments have been found in other members of the Gesneriaceae; both are almost certainly new

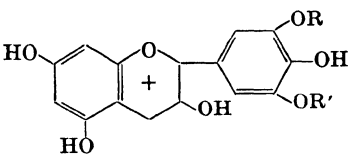
anthocyanins lacking a 3-hydroxyl group. One is present in the leaves of *Achimenes* and derived hybrids; the other is present in the orange-red flowers of *Columnea banksii* and *C. stavengeri*. Work on their identification is in progress (Harborne, unpublished).

TABLE II

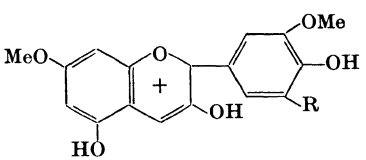
The structural variation of anthocyanins



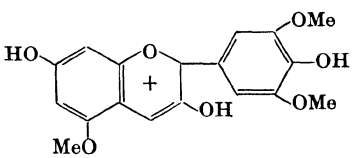
R = H, Pelargonidin (Pg)
R = OH, Cyanidin (Cy)
R = OMe, Peonidin (Pn)



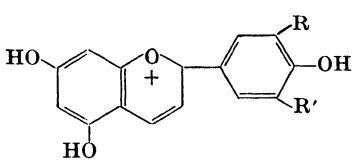
R = R′ = H, Delphinidin (Dp)
R = Me, R′ = H, Petunidin (Pt)
R = R′ = Me, Malvidin (Mv)



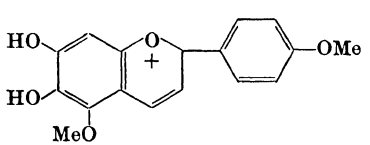
R = H, Rosinidin (Rs)
R = OMe, Hirsutidin (Hs)



Capensinidin (Cp)



R = R′ = H, Apigeninidin (Ap)
R = OH, R′ = H, Luteolinidin (Lu)
R = R′ = OH, Tricetinidin (Tc)



Carajurin (Cj)

Apigeninidin has also been found, in association with glucose and gallic acid, in a plant unrelated to the Gesneriaceae, that is, in *Chiranthodendron pentadactylon* (Sterculiaceae), a tree commonly known as "maxpalxochitl" (Pallares and Garza, 1949). The delphinidin analogue in this series, tricetinidin, has been found recently in processed tea leaves, *Camellia sinensis*, by Roberts and Williams (1958), who claim that it is

formed during processing by autoxidation of *l*-epigallocatechin gallate. Vuatez *et al.* (1959) however report that fresh tea leaves contain two anthocyanins, one of which appears from its R_F values and spectral properties, to be a derivative of tricetinidin.

Since anthocyanins lacking a 3-hydroxyl group occur in several unrelated plants, they do not appear to be of outstanding value as taxonomic markers. It is, nevertheless, interesting that two of the families containing them, Bignoniaceae and Gesneriaceae, are certainly very closely allied. Furthermore, the Verbenaceae, another family of the Tubiflorae, appears also to contain gesnerin in one species *Holmskioldia sanguinea* (Forsyth and Simmonds, 1954; Harborne, 1962b) but this result requires confirmation. Further work on the pigments of these three families may well yield more results of systematic interest.

V. Methylation Patterns of Anthocyanins

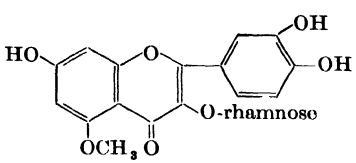
Two methyl ethers of cyanidin (peonidin and rosinidin) and four of delphinidin (petunidin, malvidin, hirsutidin and capensinidin) are known (Table II). Carajurin is the 7,4′-dimethyl ether of the as yet unknown pigment 5,6,7,4′-tetrahydroxyflavylium ion. Of these seven compounds, only malvidin, petunidin and peonidin are at all common.

Malvidin is found particularly frequently as a floral pigment, being especially abundant in such families as the Geraniaceae, Malvaceae and Primulaceae. By contrast, petunidin, the pigment intermediate between the more fully methylated malvidin and the unmethylated delphinidin, occurs relatively infrequently as a major pigment. It was not, for example, found at all by Forsyth and Simmonds (1954) in their survey of 246 tropical plants. It is, however, present characteristically and abundantly in plants of the Solanaceae (see Table I). Indeed, it is no coincidence that it was first isolated from a solanaceous plant, the garden *Petunia* (Willstäter and Burdick, 1917). Furthermore, in the tuber-bearing Solanums, petunidin is present in almost every species; malvidin, by contrast, is rare. Petunidin does occur quite widely in other sympetalous families, but it is normally present in trace amounts accompanying the more abundant malvidin. The reason that it was not detected frequently in earlier surveys is that the Robinson distribution tests did not distinguish between it and cyanidin-delphinidin mixtures (cf. Lawrence *et al.*, 1939). Even in later studies employing paper chromatography, similarities in the R_F values of petunidin and cyanidin have led to some misidentifications.

Peonidin is uncommon in the flowers of wild plants mainly because the systems for methylation and for adding the third hydroxyl group to the

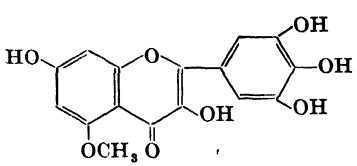
anthocyanidin B-ring appear to have evolved together; the result is that malvidin predominates. Peonidin was first isolated from *Paeonia* blooms (Willstäter and Nolan, 1915) and this remains the best known source. It is also fairly common among colour mutants of many garden plants, e.g. *Primula*, *Streptocarpus*, *Cyclamen* and *Rosa*.

Very few plants have the ability to methylate the hydroxyl groups in the 5- or 7-position of anthocyanidins. 5- or 7-Methylated anthocyanidins have been isolated from plants of the Primulaceae, Plumbaginaceae and Apocynaceae and it is significant that these three families are in adjacent orders (Table I). In *Primula*, hirsutidin (7-methylmalvidin) and rosinidin (7-methylpeonidin) are known. Hirsutidin is common (present in 9 out of 15 species examined), whereas rosinidin is rare (only in *P. rosea* and *P. polyanthus*) (cf. malvidin-peonidin relationship described above). Hirsutidin is also present in *Lochnera rosea* (fam. Apocynaceae) (Forsyth and Simmonds, 1957). The only 5-methylated anthocyanidin known is capensinidin; this is present in *Plumbago capensis*. This pigment is almost certainly 5-methylmalvidin, but its structure has still to be confirmed by synthesis (Harborne, 1962d).



(VII)

Azalein



(VIII)

5-*O*-Methylmyricetin

In most plants that have been studied, it is clear that separate systems exist for methylating anthocyanidins and for methylating flavonols (Harborne, 1962e). *Plumbago capensis* is exceptional in that the system for methylating the 5-hydroxyl group of flavonols (which gives rise to azalein (VII)) appears to have lost some of its specificity, thus allowing formation of 5-*O*-methylmalvidin. This appears to be the only reasonable explanation for the exceptional occurrence of a 5-methylated anthocyanin and is supported by the following facts. (1) Azalein and 5-methylmyricetin (VIII) are widely distributed in the genus *Rhododendron* (azalein is present in 44 out of 83 species examined). None of the anthocyanins, however, contains 5-*O*-methyl groups (Harborne, 1962d; Egger, 1962). (2) A search of other Plumbaginaceae has shown that azalein is also present in two species of *Ceratostigma* but the accompanying anthocyanidin is delphinidin.

TABLE III

List of anthocyanidin glycosides

Glycosidic types	Occurrence¶
	3-Monosides
3-Glucoside	The most common type
3-Galactoside	Fairly common: e.g. *Vaccinium, Fagus sylvatica, Malus, Empetrum*
3-Rhamnoside*	Rare; *Lathyrus, Plumbago* and *Ceanothus* spp.
3-Arabinoside (α-linkage)	Rare; *Theobroma cacao, Hordeum vulgare* and *Rhododendron* spp.
	3-Biosides
3-Rhamnosylglucoside† (rutinoside, α 1→6)	Common; e.g. *Antirrhinum* sp.
3-Xylosylglucoside (sambubioside, β 1→2)	Fairly common; e.g. *Begonia, Sambucus, Streptocarpus, Ilex*
3-Xylosylgalactoside‡	Rare; *Lathyrus odoratus* cultivars
3-Glucosylglucoside (gentiobioside, β 1→6)	Rare; *Primula sinensis* and *Tritonia* spp.
3-Glucosylglycoside (sophoroside, β 1→2)	Common; e.g. *Papaver, Phaseolus*
3,5-Diglucoside	Very common
3-Rhamnoside-5-glucoside	Rare; *Lathyrus* and *Pisum* spp.
	3-Triosides
3-Glucosylglucosylglucoside (links probably β 1→6)	Rare; *Primula sinensis*
3-Rutinoside-5-glucoside	Fairly common; especially in Solanaceae (e.g. *Solanum*) and Gesneriaceae (e.g. *Streptocarpus*)
3-Sambubioside-5-glucoside	Rare; *Matthiola incana* and *Sambucus nigra*
3-Sophoroside-5-glucoside	Rare; *Raphanus sativus* and *Brassica oleraceae*
3-Sophoroside-7-glucoside	Rare; *Papaver* and *Watsonia* spp.
3-(2^G-glucosylrutinoside)	Rare; *Begonia* and *Rubus*
3-(2^G-xylosylrutinoside)	Rare; *Begonia*

* May be α-linked, as it is relatively resistant to hydrolysis by anthocyanase.

† A pelaronidin 3-rhamnosylglucoside isomeric with the 3-rutinoside is reported to be present in the tulip variety "President Eisenhower" (Halevy, 1962). In this laboratory, we have noted that the cyanidin and peonidin 3-rhamnosylglucosides isolated from *Chasmanthe* and *Lapeyrousa* do not correspond exactly in R_F values with the corresponding 3-rutinosides. Since the nature of the disaccharide linkage has not yet been determined for the 3-biosides of a number of plants, it should not be assumed that the 3-rhamnosylglucosides isolated from all the plants mentioned in Table I are 3-rutinosides.

‡ Linkage β1→2; the name lathyrose is proposed for this new disaccharide.

¶ Percentage occurrences by genera of monosides, biosides and triosides are 45, 61 and 16 respectively (data in Table I). 3-Glycosides (63%) are more frequent than 3,5-diglycosides (50%). For references. see Table I.

VI. Glycosidic Patterns of Anthocyanins

Twenty classes of anthocyanidin glycoside are now known (Harborne, 1962a,c). These are listed in Table III, together with some indication of their taxonomic distribution. Detailed surveys of the glycosidic pattern in individual genera have also been carried out. The results obtained so far (Table IV) indicate that each genus has a characteristic glycosidic pattern

TABLE IV

Distribution of anthocyanins according to their sugar components

Genus	No. of species and cultivars*	Glycosidic types
Begoniaceae		
Begonia	55	3-Sambubioside, 3-(2^G-xylosylrutinoside) 3-sophoroside and 3-(2^G-glucosylrutinoside)
Ericaceae		
Rhododendron	46	3-α-Arabinoside and 3-β-galactoside or 3,5-diglucoside
Vaccinium	4	3-β-Galactoside
Gesneriaceae		
Streptocarpus	22	3-Rutinoside-5-glucoside or 3-sambubioside
Leguminosae		
Lathyrus	40	3-Rhamnoside-5-glucoside, 3-rhamnoside, 3-xylosylgalactoside and 3-galactoside
Pisum	10	3-Rhamnoside-5-glucoside and 3-rhamnoside
Liliaceae		
Fritillaria	23	3-Rutinoside or 3-xylorhamnoside
Tulipa	120	3-Rutinoside, 3-glucoside and 3,5-diglucoside
Papaveraceae		
Papaver	8	3-Sophoroside-7-glucoside, 3-sophoroside and 3-glucoside
Primulaceae		
Primula	12	3-Glucoside, 3,5-diglucoside, 3-gentiobioside and 3-glucosylgentiobioside
Ranunculaceae		
Peonia	9	3,5-Diglucoside
Rosaceae		
Rosa	115	3,5-Diglucoside and 3-glucoside
Scrophulariaceae		
Antirrhinum	7	3-Rutinoside or 3,5-Diglucoside
Solanaceae		
Solanum	55	3-Rutinoside-5-glucoside

* This number is an approximate one; in some instances, it is difficult to differentiate wild and cultivated forms: in others, it is difficult to eliminate errors due to nomenclatural synonymy.

and species which do not conform to the generic pattern are exceptional in other respects. Most genera that have been examined have only one glycosidic type present (e.g. *Solanum*) and it is only the genera in which more than one class of glycoside occur that require further discussion.

Lathyrus

The only exceptional wild species is *L. sativus*, which has malvidin 3-rhamnoside, instead of the usual malvidin 3-rhamnoside-5-glucoside in the flowers. Since the 3-rhamnoside is presumably an intermediate in the synthesis of the 3-rhamnoside-5-glucoside, this is simply a plant in which glycosylation has not been carried to completion. The only other known source of 3-rhamnoside-5-glucosides is *Pisum*, a genus which is closely allied botanically to *Lathyrus*. Cultivated forms of both *Pisum* and *Lathyrus* do contain other types of anthocyanidin glycoside: 3-galactosides and 3-xylosylgalactosides of peonidin, cyanidin and pelargonidin have been found, for example, in crimson and orange mutant forms of the sweet pea, *Lathyrus odoratus*. A related flavonol glycoside, kampferol 3-xylosylgalactoside-7-rhamnoside, is present in the flowers of wild and mutant forms of *L. odoratus* and it is likely that the enzyme system controlling flavonol glycosylation loses some of its specificity in the mutant forms, thus permitting the synthesis of related anthocyanins. The same may hold for *Pisum*, although the "unusual" anthocyanins here have not yet been fully identified.

Rosa

Most cultivars and wild species contain 3,5-diglucosides. A few, however, have mainly 3-glucosides, presumably also because glycosylation is incomplete (as in *Lathyrus*). The flavonol glycosides in rose flowers are the 3- and 4′-glucosides and 3-sophorosides of kampferol and quercetin.

Rhododendron

This genus is unusual in having two apparently mutually exclusive glycosidic patterns: (1) the 3-arabinoside and the 3-galactoside of cyanidin in red flowered species, and (2) the 3,5-diglucoside of malvidin in mauve and blue forms. Thus diglucosylation appears to be associated with a higher level of methylation of the aglycone. It is not clear yet whether this chemical division of the genus bears any relationship to known taxonomic groupings.

Antirrhinum

This is another genus with two separate glycosidic patterns (3-rutinoside and 3,5-diglucoside) but, in this instance, there is a correlation with

taxonomy. Of the seven species examined, six have cyanidin 3-rutinoside and are placed together in the section Antirrhinum by Rothmaler (1956). The seventh, *A. cornutum*, a New World species in the section Saerorhinum of the genus, has delphinidin 3,5-diglucoside. It is also of interest that chemical affinities exist between *Antirrhinum* and closely related genera, since both the *Antirrhinum* glycoside types are present in some species which were once included under *Antirrhinum*. Thus, *Maurandia speciosa* (formerly *A. maurandiodes*) contains delphinidin 3,5-diglucoside and *Asarina procumbens* (formerly *A. asarina*) has cyanidin 3-rutinoside.

Begonia

Although he studied the anthocyanins of this genus in some detail, Bopp (1957) was not able to identify the sugars of the anthocyanins. Studies which are still in progress (Harborne, 1962) indicate that the situation is complex; two patterns can be again discerned (cf. *Rhododendron* and *Antirrhinum*). Some species have cyanidin 3-sambubioside with or without cyanidin 3-(2^G-xylosylrutinoside); others have cyanidin (or pelargonidin) 3-sophoroside accompanied sometimes by a cyanidin 3-(2^G-glucosylrutinoside). Only two plants have been found so far to have all four glycosidic types but both are of known hybrid origin. The two trisaccharides present in the *Begonia* anthocyanins are unusual in being branched; the rhamnose is attached to glucose in both oligosaccharides (as in rutinose) by means of an α 1$\rightarrow$6 link.

Fritillaria

Of 24 species examined, 23 have cyanidin 3-rutinoside. *F. kamchatchensis* is exceptional in having a cyanidin 3-(xylorhamnoside) or 3-(rhamnoxyloside) present (Shibata, 1958). Significantly, the taxonomic status of this species is still in dispute.

Primula

The usual patterns are 3-glucoside or 3,5-diglucoside. *Primula sinensis* is unusual in producing in both flowers and stems complex mixtures of anthocyanidin 3-glucosides, 3-gentiobiosides and 3-(glucosylgentiobiosides). However, the related flavonol glucosides, especially the 3-(glucosylgentiobioside) occur widely throughout the genus. Thus, the enzymic mechanism required for adding two and three glucose residues to the 3-hydroxyl group of flavonoids is present in most *Primulas*. *P. sinensis* is exceptional only in that both flavonols and anthocyanidins are used as substrates for these systems (cf. *Lathyrus* above).

Streptocarpus

S. dunnii is the exceptional species; its flowers are brick red in colour and contain cyanidin 3-glucoside and 3-sambubioside. All other species examined have blue flowers, in which the pigment is malvidin 3-rutinoside-5-glucoside. There is, however, no lack of uniformity in glycosidic pattern, since all the other species have the *dunnii* flower pigments in their stems and leaves. *S. dunnii* is thus a "relict" species, from the pigment point of view (Lawrence and Sturgess, 1957). It is very distinct morphologically, being the only species with green filament colour and sticky pollen (Lawrence, 1957). It is also unusual in having α-naphthoquinone pigment, dunnione, on the underside of its leaves; only one other species also has this pigment. The unique floral pigmentation of *S. dunnii* has provided the basis of most of the colour variation present in the garden *Streptocarpus*, which is a complex hybrid derived from *S. dunnii*, *S. rexii* and *S. parviflorus*.

VII. Acylated Anthocyanins

It has long been recognized that many anthocyanins occur in nature acylated with organic acids but progress in the study of these pigments has been slow because of the lability of the acyl–anthocyanin linkage. In

TABLE V

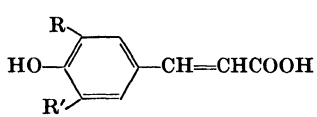
Distribution of acylated anthocyanins

Family	Genera	Acyl groups
Monocotyledons		
Commelinaceae	*Commelina*	*p*-Coumaric
Liliaceae	*Hyacinthus*, *Scilla*	*p*-Coumaric
Iridaceae	*Iris*	*p*-Coumaric
Dicotyledons—Archichlamydeae		
Cruciferae	*Brassica*,* *Matthiola*,* *Raphanus*	*p*-Coumaric, caffeic, ferulic and sinapic
Vitaceae	*Vitis*	*p*-Coumaric
Violaceae	*Viola*	*p*-Coumaric
Melastomaceae	*Tibouchina**	*p*-Coumaric
Dicotyledons—Sympetalae		
Polemoniaceae	*Gilia*	*p*-Coumaric
Labiatae	*Monarda*, *Perilla*, *Salvia*	*p*-Coumaric and caffeic
Solanaceae	*Atropa*, *Browallia*,* *Brunfelsia*, *Lycopersicon*, *Petunia*, *Solanum*	*p*-Coumaric

* Anthocyanins with two acyl groups attached have been detected in these genera.

the earlier reports, anthocyanins with malic, malonic, *p*-hydroxybenzoic, protocatechuic or gallic acid as acyl groups were described (see, e.g., Karrer, 1958) and Robinson and Robinson (1932) suggested that in some anthocyanins acyl groups are directly attached to the 3-hydroxyl position. More recent studies indicate, however, that the only acyl groups attached to anthocyanins are hydroxycinnamic acids (and usually *p*-hydroxycinnamic acid) and these groups are attached to the anthocyanins through the sugar hydroxyl groups. Acylated anthocyanins can now be recognized readily by spectral and chromatographic means (Harborne, 1958a, b) and they have been thus identified with certainty in ten families (Table V). When present in a particular genus they are present with a certain regularity; they are especially abundant in genera of the Cruciferae and the Solanaceae. The only three sympetalous families having acylated pigments are all included in the order Tubiflorae by Engler; acylation is thus a feature of some taxonomic interest.

The Cruciferae is the only family to have anthocyanins acylated with sinapic (IX, R = R′ = OMe) and ferulic acids (IX, R = H, R′ = OMe), as well as with *p*-coumaric acid (IX, R = R′ = H). The system controlling acylation in the Crucifers is thus different from that present in the more highly evolved families, such as the Labiates (acyl groups: *p*-coumaric and caffeic acid (IX, R = OH, R′ = H)) and the Solanaceae (acyl group: *p*-coumaric acid).



(IX)

p-Coumaric acid (R = R′ = H)
Caffeic acid (R = OH, R′ = H)
Ferulic acid (R = OCH_3, R′ = H)
Sinapic acid (R = R′ = OCH_3)

It is possible that acylated anthocyanins occur in families other than these listed in Table V. Indeed, they are reported as occurring in *Delphinium* (Ranunculaceae), *Impatiens* (Balsaminaceae), *Gentiana* (Gentianaceae) and *Dianthus* (Caryophyllaceae). The pigments of all these plants require re-examination before the presence of an acylated pigment can be definitely recorded because the presence of impurities readily make normal anthocyanins behave like acylated anthocyanins in preliminary tests. The pigment of *Delphinium consolida*, which is reported to be acylated with *p*-hydroxybenzoic acid, has, in fact, been re-examined and the purified anthocyanin is clearly not acylated (Harborne, 1962a).

Until recently, it was thought that anthocyanins were the only class of flavonoid to occur in acylated form. Hörhammer *et al.* (1959) found a *p*-coumaroylkampferol 3-glucoside in flowers of *Tilia argentea* (Tiliaceae); since then, several other acylated flavonol glycosides have been described. Birkoffer and Kaiser (1962) have isolated a kampferol 3-(feruloylsophoroside) from flowers of *Petunia* (Solanaceae); a quercetin-3-(caffeoylsophoroside)-7-glucoside occurs in flowers of *Helleborus foetidus* (Ranunculaceae) (Harborne, 1962b). *Pisum* species and cultivars (Leguminosae) contain a range of *p*-coumaroyl and feruloyl flavonol-3-glucosylsophorosides (Furuya *et al.*, 1962; Harborne, 1962b). Thus acylated flavonols may occur as widely as acylated anthocyanins but there does not seem to be any obvious relationship between the two groups of pigments. *Petunia* is the only plant in which both anthocyanins and flavonols are acylated; however, the acyl group of the anthocyanins (*p*-coumaric acid) differs from that present in the flavonol (ferulic acid).

VIII. Anthocyanins and Flower Colour

Anthocyanins are the most important group of plant pigments concerned with flower colour and any study of their distribution must take this aspect into account. As flower colour has evolved, there has been a tendency for plants to produce more complex pigments; in structural terms, the trend has been away from the simple cyanidin-3-glucoside towards a complex acylated co-pigmented malvidin triglycoside (Lawrence and Sturgess, 1957). The evolution towards blue colours is primarily related to the need to provide the colour most attractive to insect pollinators (Fritsch, 1915). A secondary advantage of this evolutionary trend is in terms of pigment stability; a complex pigment without *o*-dihydroxy groups and having several sugars attached is much more stable to light and to enzymic attack than a simple cyanidin derivative.

Blueness is produced in flowers by the interaction between anthocyanin and one or more of a number of modifying factors. In rare instances, cyanidin derivatives may be the basis of blue colours; more usually the pigment is malvidin, petunidin or delphinidin. Of the various modifying factors, two appear to be of prime importance. They are (a) chelation of anthocyanins by metals such as iron, magnesium and molybdenum, and (b) co-pigmentation of anthocyanins by flavones or by similar phenolic substances. It is conceivable that both these systems might operate together in some flowers to produce blueness, but it is much more probable that either one or the other system is present. In plants, containing hirsutidin, malvidin, peonidin or pelargonidin derivatives, metal complexing cannot be involved since none of the anthocyanidins

have the *o*-dihydroxy grouping required for metal chelation. Thus in blue forms of *Primula sinesis* and *P. obconica*, there is very good evidence that co-pigmentation by kampferol glycosides is the mechanism for blueing. Pecket and Selim (1962) has recently shown, from studies of interspecific hybrids, that the same is true for blueness in *Lathyrus* flowers. Cyanidin and delphinidin metal complexes have been isolated from blue flowers of *Lupinus*, *Commelina* and *Centaurea* (Bayer, 1958, 1959; Mitsui *et al.*, 1959) but it is not known, at present, how widely such complexes are distributed (see also Hayashi, 1962).

Another factor in flower coloration is that of the interaction between anthocyanins and pigments, other than flavones. For example, a number of iridaceous plants (e.g. *Chasmanthe*, *Crocosmia Lapeyrousia*) have yellow-red flowers and contain cyanidin with a supplementary water-soluble yellow pigment (Harborne, 1962b). Much further study of the factors modifying flower colour *in vivo* is required before the results can be related to plant systematics.

Finally, some mention of studies of anthocyanin patterning is appropriate, since at least two systematic studies of this have been made. In *Streptocarpus*, Lawrence (1957) surveyed the occurrence of six flower colour patterning genes. He found that the distribution of the flower pattern genes in the wild was not random, but that individual species and taxonomic groups were characterized by different combinations of these genes. A study of the distribution of anthocyanin patterning genes has been made in cultivated diploid potatoes. The purpose here was to show that six so-called species were, in fact, all part of one interbreeding cultivar complex. In accordance with expectation, Dodds and Paxman (1962) found that the same patterning genes were common to all the cultivated diploids examined. Using other evidence as well, they were thus able to revise the nomenclature of the group.

IX. Conclusions

Only about a thousand plant species have been examined for their anthocyanins; and in only a fifth of this number have the sugars of the anthocyanins been identified. The sample of higher plants so far studied in this respect is thus an extremely small one; there must be a considerable number of new anthocyanin structures yet to be discovered. The results so far do indicate that glycosidic pattern of anthocyanins is related to plant systematics. The glycosidic type is a more useful character than that of the anthocyanidin type, because it is much less variable genetically. The glycosidic pattern of anthocyanins is related to that of the flavonol glycosides and future studies of distribution in nature of

plant pigments should include the identification of both groups of glycoside.

From the systematic point of view, it is the unusual chemical structures which provide the best kind of "taxonomic marker", and further exploration of the pigments present in those families, which are already noted for their chemical versatility (e.g. Leguminosae, Compositae, Verbenaceae, Gesneriaceae, Apocynaceae, Bignoniaceae, Iridaceae) is bound to be rewarding.

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CHAPTER 14

The Taxonomic Significance of Alkaloids

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I. Introduction

About 15–20% of all vascular plants contain alkaloids. These compounds often have considerable physiological effects on both man and animals, and so have been known for centuries as medicines, poisons and magical potions. The beginnings of alkaloid chemistry, however, only go back 140 years when Sertürner (1817) recognized that morphine was the principle responsible for the effect of opium, and that it was a basic substance which was capable of forming salts; later it was shown to contain nitrogen (Bussy, 1822; c.f. Delépine, 1953). Today, more than a thousand individual alkaloids are known, and the literature on their chemistry, biochemistry and pharmacology is now vast.

This chapter, therefore, only covers, albeit briefly, those aspects of alkaloids that are of direct interest to plant taxonomy. This task

has been rendered easier by the recent publication of two mongraphs on alkaloid chemistry (Boit, 1961; Manske and Holmes, 1950–60). The importance of alkaloids in chemical plant taxonomy was reviewed by the author five years ago (Hegnauer, 1958), but the rapid advances made since that time justify a new treatment of the subject.

The taxonomic system used throughout this chapter is based on that of Wettstein (1935) and unless otherwise stated the names and limits of all families, orders and other taxa are those used and defined by him.

II. Criteria for Using Alkaloids in Taxonomy

Several surveys, some of which were systematic (e.g. for medicinal or poisonous plants), have been carried out for alkaloid-containing plants in various parts of the world (e.g. Arthur, 1954; Arthur and Cheung, 1960; Bisset, 1957; Blinova and Stuckey, 1960; Douglas and Kiang, 1957; Ismailov, 1958; Kiang *et al.*, 1961; Kuvayev and Blinova, 1960; Lazur'evskiĭ and Sadykov, 1939; Massagetov, 1946; Meyer-Pernet, 1957; Orechoff, 1934; Sokolow, 1952, 1956; Swanholm *et al.*, 1959, 1960; Webb, 1949, 1952, 1953, 1955; Willaman and Schubert, 1955, 1961). The results of these investigations have shown that alkaloids are unexpectedly widely distributed in the Cormophytes (flowering plants and ferns), and also occur in numerous families which were previously regarded as being free from them. Manske (1950) has suggested that 38- 39 families can be regarded as alkaloid-containing families. It must be assumed, therefore, that alkaloids are present in about one-sixth of the vascular plants, and that none of the larger families will be free from them. Since alkaloids can easily be detected, they are obviously of great interest to taxonomists. To make the best use of these compounds it is necessary to consider the meaning of the terms alkaloid and alkaloidal plant, and to consider the biogenetic interrelationships of these plant constituents.

A. Definition of an Alkaloid

It is not at all easy to define what is meant by the term alkaloid. The first definition corresponding to present-day conceptions was given by Winterstein and Trier (1910). These authors defined alkaloids in the broadest sense as basic, nitrogen-containing compounds that are distributed in both the vegetable and animal kingdoms. Within this broad group they defined the "alkaloids proper" as compounds containing heterocylic nitrogen, having a more or less distinctly basic character, and a complex molecular structure. Such compounds are restricted to the

plant kingdom and are distributed sporadically in certain genera and families, rarely being universally present in larger groups of plants. These compounds all have recognizable pharmacological activity.

This definition of the "alkaloids proper" is in accordance with present day views and shows that chemical, botanical and pharmacological properties must all be taken into account when defining an alkaloid.

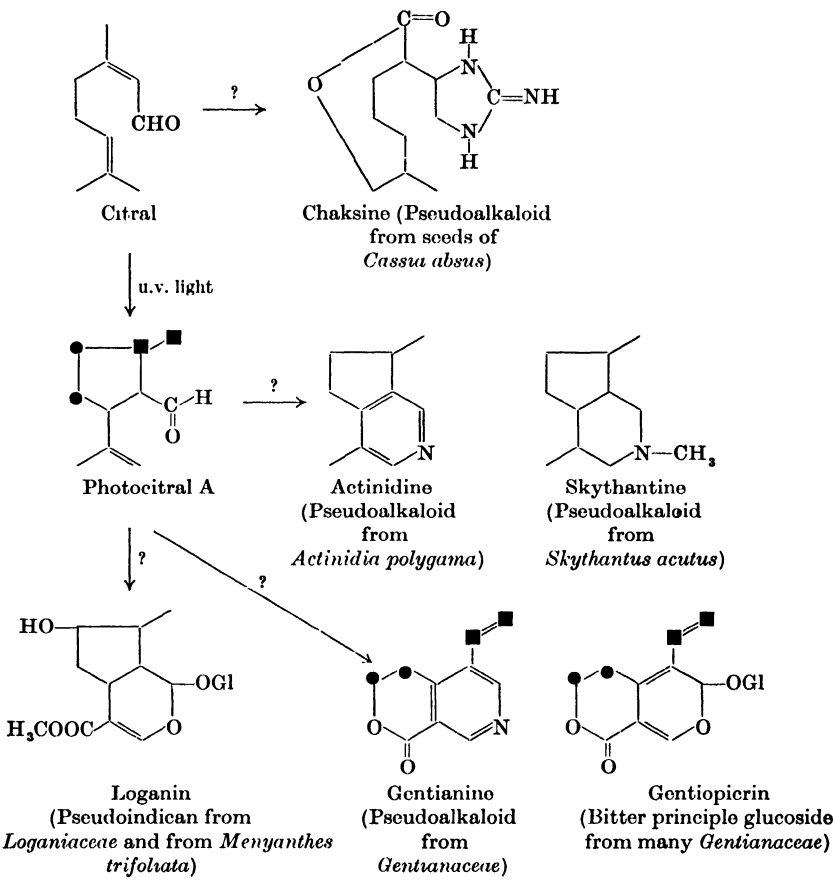
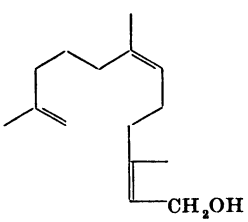


FIG. 1. Some monoterpene pseudoalkaloids and their presumed relation to non-alkaloid compounds in plants (cf. Thomas, 1961; Cookson, 1962).

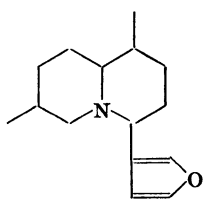
However, on this definition there is no sharp distinction made between alkaloids and many other nitrogenous compounds in plants. For example, colchicine contains no heterocyclic nitrogen and is not basic, and yet is counted as an alkaloid because of its pharmacological activity and

restricted distribution. Thiamine, on the other hand, in spite of being a heterocyclic nitrogenous base, is not counted as an alkaloid as it is universally distributed in living matter.

Winterstein and Trier (1910) also developed the suggestions made by Pictet (1906) about the biogenesis of alkaloids in plants and postulated a *biological unit* (1910, pages 265, 309) to which the compounds could be referred. Thus the alkaloids can be thought of as by-products of protein metabolism, which are methylated on either nitrogen or, when present, on hydroxyl groups (detoxification) and so removed from general metabolism. They further suggested that the main precursors were proline, lysine, ornithine, phenylalanine and tryptophan, and their proposed scheme for the biosynthesis of isoquinoline alkaloids (1910, p. 307) corresponds exactly with that accepted today.



Farnesol



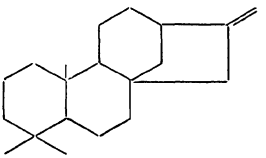
Desoxynupharidine
(Sesquiterpene pseudo-alkaloid from *Nuphar japonicum*)

FIG. 2. Sesquiterpenoid pseudoalkaloids of the genus *Nuphar*.

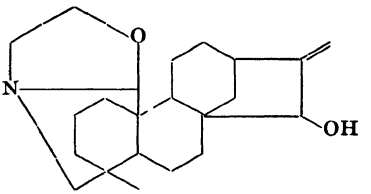
This concept of Winterstein and Trier, of characterizing the alkaloids by their mode of biogenesis is extremely useful for chemotaxonomic purposes. Thus, different alkaloids which, however, are known to be related biogenetically (i.e. are of the same alkaloid family) can be used to support a phylogenetic relationship between the plants from which they were isolated. For the purpose of chemical plant taxonomy, therefore, alkaloids are best defined as follows.

> Alkaloids are more or less toxic substances which act primarily on the central nervous system. They have a basic character, contain heterocyclic nitrogen, and are synthesized in plants from amino acids or their immediate derivatives. In most cases they are of limited distribution in the plant kingdom.

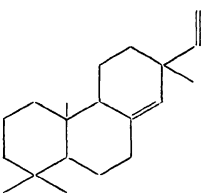
Such a definition, of course, does exclude a number of nitrogen-containing plant constituents which are normally thought of as alkaloids.



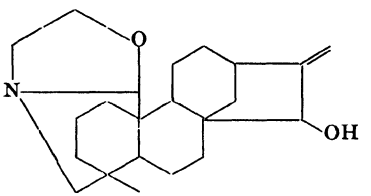
Phyllocladene
(Diterpene hydrocarbon
of conifers)



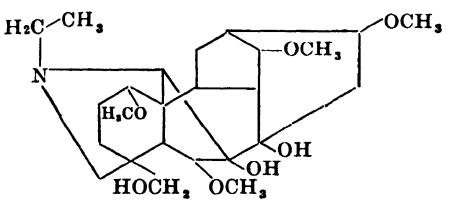
Veatchine
(Diterpene alkaloid from *Garrya* species,
e.g. see Mosettig *et al.*, 1961)



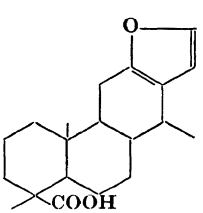
Pimaradiene (Hypothetical
precursor of the phyllocladene
and atisine skeleton; see
Wiesner and Valenta, 1958)



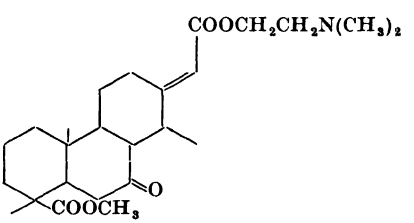
Atisine
(Alkamine from *Aconitum*
species)



Lycoctonine (= Delsine = Royline;
alkamine from *Aconitum*- and *Delphinum*-
species and from *Inula royleana*)



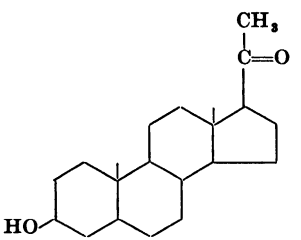
Vouacapenic acid and
vinhaticoic acid
(Epimeric diterpene acids
from the wood of
Vouacapoua and
Platymenia species)



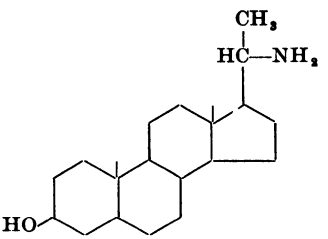
Cassamine
(*Erythrophloeum*base)

FIG. 3. Some diterpene pseudoalkaloids and their relation to non-alkaloid compounds in plants.

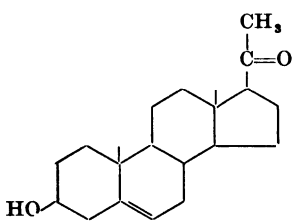
One such group comprises the large number of compounds which have no heterocyclic ring such as the biological amines (e.g. ephedrine, hordenine, mescaline, narceine and galegine), betaines such as betaine itself (glycine betaine), and aliphatic quaternary bases such as choline, acetylcholine, muscarine, and sinapin. Stachydrine (proline betaine) and tryptamine are also excluded, although in this case they are clearly derived from amino acids and contain heterocyclic nitrogen.



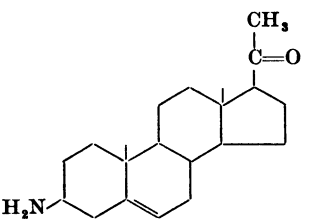
5α-Pregnan-3β-ol-20-one
(From *Xysmalobium undulatum*)



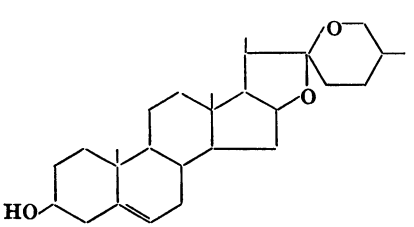
Funtuphyllamine A
(Pseudoalkaloid from the leaves of *Funtumia africana*)



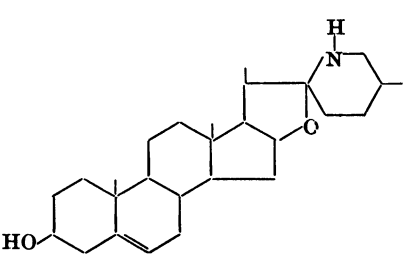
Δ^5-Pregnen-3β-ol-20-one
(From *Xysmalobium undulatum*)



Holaphyllamine
(Pseudoalkaloid from the leaves of *Holarrhena floribunda*)



Diosgenin
(Widespread in Liliaceae and Dioscoreaceae; also in the genus *Solanum*)



Solasodine
(Alkamine of the glycoalkaloids of the genus *Solanum*)

FIG. 4. Some pseudoalkaloids of the C_{21}- and C_{27}-steroid-type (cf. Tschesche, 1961).

All the compounds mentioned above are usually referred to as biological amines (Guggenheim, 1951) or "protoalkaloids" (Ackermann, 1956). When such protoalkaloids occur in the same genus or family as

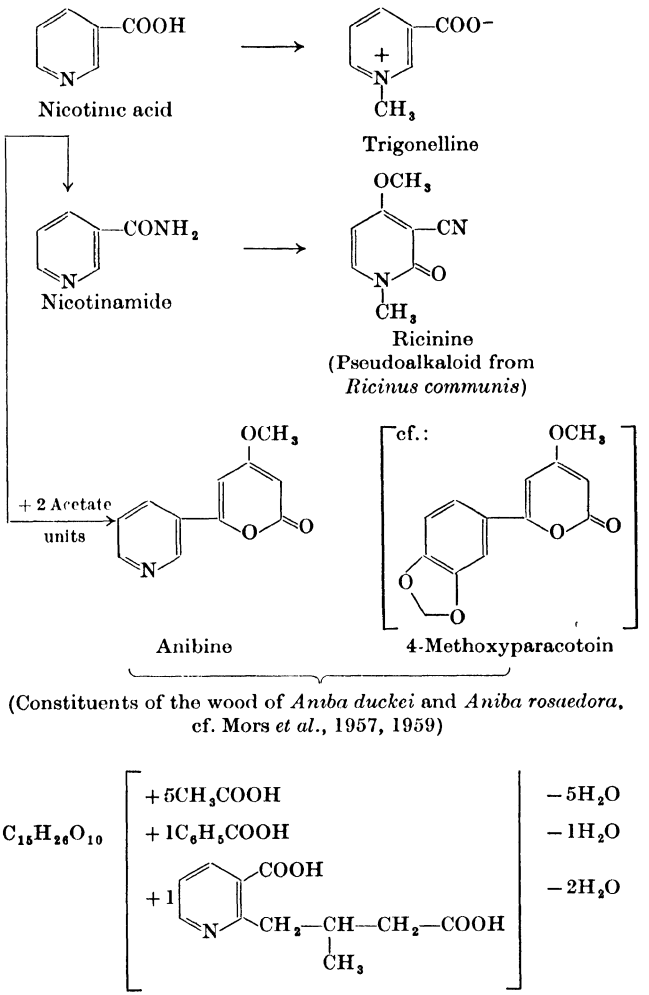
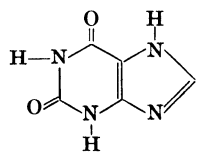


FIG. 5. Nicotinic acid and some pseudoalkaloids presumably directly derived from it.

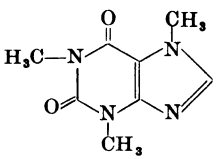
true alkaloids to which they are biogenetically related (e.g. hordenine, candicine and mescaline in the Cactaceae; narceine in *Papaver somniferum*) then it is usual to classify them as alkaloids also. On the other

hand, if they occur in a taxon that contains no true alkaloids, then such taxa are not classified as alkaloid-containing (e.g. the Loranthaceae, which contain only phenylethylamine and tyramine).

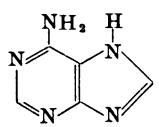
Another extensive group of nitrogenous compounds that falls outside our definition of alkaloids, comprises those which are unrelated biosynthetically to the amino acids. These are mainly based on mono-,



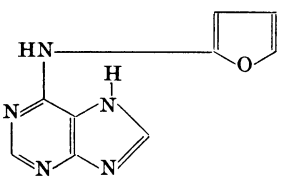
Xanthine (Widespread in the plant kingdom)



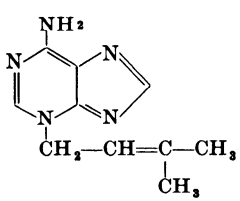
Caffeine (=Trimethylxanthine; sporadically distributed, e.g. in tea and Mate leaves, coffee beans, Cola nuts, etc.)



Adenine (= 6-Aminopurine, generally distributed)



Kinetin (Growth hormone; probably generally distributed in trace amounts)



Triacanthine (=Togholamine=Chidlovine; present in young leaves of *Gleditschia triacanthos*, *Chidlovia sanguinea* and *Holarrhena* species; disappears during leaf enlargement and probably has growth hormone activity, Cavé *et al.*, 1962)

FIG. 6. Some pseudoalkaloids derived from purines.

sesqui- and di-terpenes, C_{21}- and C_{27}-steroids, nicotinic acid, and purines. Although, from a chemical point of view, these are usually regarded as true alkaloids, from a chemotaxonomic point of view they are best kept separate. They can be classified according to the precursor on which they are based but, on account of their general alkaloid character (they are, for example, toxic), they can be collected together in a group called the "pseudoalkaloids" or "alcaloida imperfecta" (Hegnauer, 1956, 1958).

From the taxonomic point of view protoalkaloids, pseudoalkaloids and true alkaloids should each be considered separately. The main groups of the pseudoalkaloids are shown in Figs. 1–7; discussion of the true alkaloids (on our definition) is given in Section I, C (p. 399).

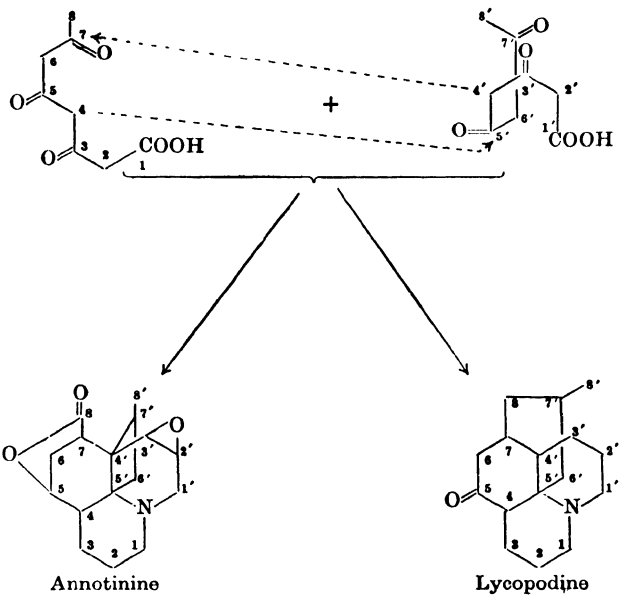


FIG. 7. *Lycopodium* alkaloids possibly pseudoalkaloids related to triketoöctanoic acid (4-acetate units) (Conroy, 1960; cf. Hegnauer, 1962).

B. DEFINITION OF AN ALKALOIDAL PLANT

During feeding-experiments with animals in attempts to investigate the chemical nature of antiscorbutic substances (vitamin C) in plants, Rygh and Laland (1932) and Laland (1932) unexpectedly found that narcotine was present in a number of common fruits and vegetables. They isolated this alkaloid (which had been long known to occur in *Papaver somniferum*) from unripe oranges (600 mg from 10 l. of press juice), unripe tomatoes (20 mg from 20 kg), cabbage (40 mg from 100 kg) and potatoes (12 mg from 20 kg) but not from cranberries (150 kg).

Nicotine has been shown to be widely distributed in plants; so far it has been detected in the following genera, *Equisetum*, *Lycopodium*, *Sedum*, *Mucuna*, *Erythroxylon* (Fikenscher, 1958), *Asclepias*, *Duboisia*, *Nicotiana*, *Salpiglossis* (Schröter, 1958), *Atropa*, *Datura* and *Lycopersicon* (Wahl, 1952), *Eclipta*, *Zinnia*, and probably *Acacia* and *Lupinus* (Hegnauer, 1959). (For further references, see Hegnauer, 1956.)

These two examples show that there is no sharp dividing line between alkaloid-containing and alkaloid-free plants. When large quantities of plant material are extracted (as for narcotine), traces of alkaloids can often be detected in species which are usually regarded as being free from such compounds. On the other hand, when an alkaloid is relatively easy to both isolate and detect (as is the case for nicotine which is steam volatile, and gives the very sensitive test for pyridine-containing compounds with cyanogen bromide and aniline), it obviously may be shown to be present in numerous species even when only small quantities of material are examined.

None of the plants examined by Rygh and Laland can be regarded as accumulating narcotine and, of the species containing nicotine, only those in the genera *Duboisia*, *Nicotiana*, *Salpiglossis* (nornicotine), *Eclipta* and *Zinnia* are regarded as true nicotine-containing plants.

In the other cases mentioned, the alkaloids are really trace-substances. This is often true for many other naturally occurring compounds (e.g. cinnamic acid derivatives; flavonoids; coumarins; sedoheptulose; monoterpenes of the linalool, citral, carvone, limonene, and pinene type; triterpenes of the amyrine and lupeol type) which are commonly found in the plant kingdom, but are only formed in traces in some species, and hence only detectable when relatively large quantities of material are used. Such trace occurrences are in most cases unimportant at present for taxonomic purposes, and it would appear from the taxonomists' point of view that the ability to accumulate a given compound is more important than the ability just to synthesize it.

We are justified, therefore, in regarding alkaloid-containing plants as those which accumulate relatively large amounts of these substances, and to exclude such species as *Asclepias syriaca* where only traces of alkaloid have been found. From the practical point of view (e.g. the possibility of detecting small amounts of alkaloids in herbarium material) we can define the lower limit as 0·01% of the dry weight.

Another unmistakable characteristic of a true alkaloid plant is that it always contains more than one alkaloid, the main component (or components) being accompanied by smaller quantities of a number of biogenetically related congeners. This is so invariably the case that it has led in many instances to a greater understanding of biosynthetic pathways (cf. the hygrine and tropine bases in the Solanaceae, and *Erythroxylum* species; indole and quinoline bases in *Cinchona*). The structures of the accompanying alkaloids are often important when deciding whether a compound has been synthesized by one route or another (homologous and analogous compounds, see Section II, D).

The facts that ricinine (Fig. 5) is the only alkaloid in *Ricinus communis*,

and that the majority of the Gentianaceae contain only gentianine, (Fig. 1), are sufficient to exclude these two bases from the class of true alkaloids.

C. Types of Alkaloid that can be Distinguished

Numerous hypotheses of alkaloid biosynthesis in plants, based on either theoretical considerations (e.g. Winterstein and Trier, 1910; Schöpf, 1949; Goutarel *et al.*, 1950; Robinson, 1955; Wenkert, 1954, 1959; Barton and Cohen, 1957; van Tamelen, 1961) or experimental observations (e.g. Marion, 1958; Mothes, 1959a,b; Boit, 1961; Battersby, 1961) point to the fact that the amino acids phenylalanine, tryptophan, ornithine, lysine, histidine, and anthranilic acid (*o*-aminobenzoic acid) are the primary precursors of alkaloids in plants. Each of these amino acids can be regarded as the starting point for the synthesis of one or more types of alkaloid. The hypothesis put forward by Wenkert (1959) that the products of carbohydrate metabolism, rather than the amino acids, are the main precursors of alkaloids does not appear to agree with the experimental results obtained so far. We may, therefore, put the known types of alkaloid into one of six families corresponding to the six amino acids mentioned above. It is possible to further sub-divide these families according to the types of reaction involved in their synthesis. There are also a number of alkaloids which can be regarded as hybrids as far as their origin is concerned (cf. evodiamine, Fig. 11).

There is no doubt that all these sub-divisions are important taxonomically, since it is the alkaloid family, rather than the individual compound, which gives the most useful systematic characters.

The most important biogenetically related groups of alkaloids so far recognized are shown in Figs. 8–17. It should be emphasized that many of the schemes are hypothetical. For example, two different routes of biosynthesis are shown for the indole alkaloids of the Loganiaceae, Apocynaceae and Rubiaceae. From the botanical point of view, it would seem more likely that the non-indole moiety of these alkaloids comes from cyclopentanoid monoterpenes as suggested by Thomas (1961).

If one also assumes that the orders Tubiflorae, Contortae and Rubiales can convert monoterpenes to secondary plant products in this way, a remarkable number of biochemical relationships are revealed (cf. Fig. 1).

The scheme shown for alkaloids of the sparteine group (lupin alkaloids, Fig. 16) is based on the suggestions of Schütte (1960, 1961) and his co-workers (Schütte *et al.*, 1959, 1961, 1962), which were developed from experimental observation. The anthranilic acid family of alkaloids is

based on the results of the Australian workers (Price, 1956) and on the taxonomical and botanical considerations put forward by Hegnauer (1958).

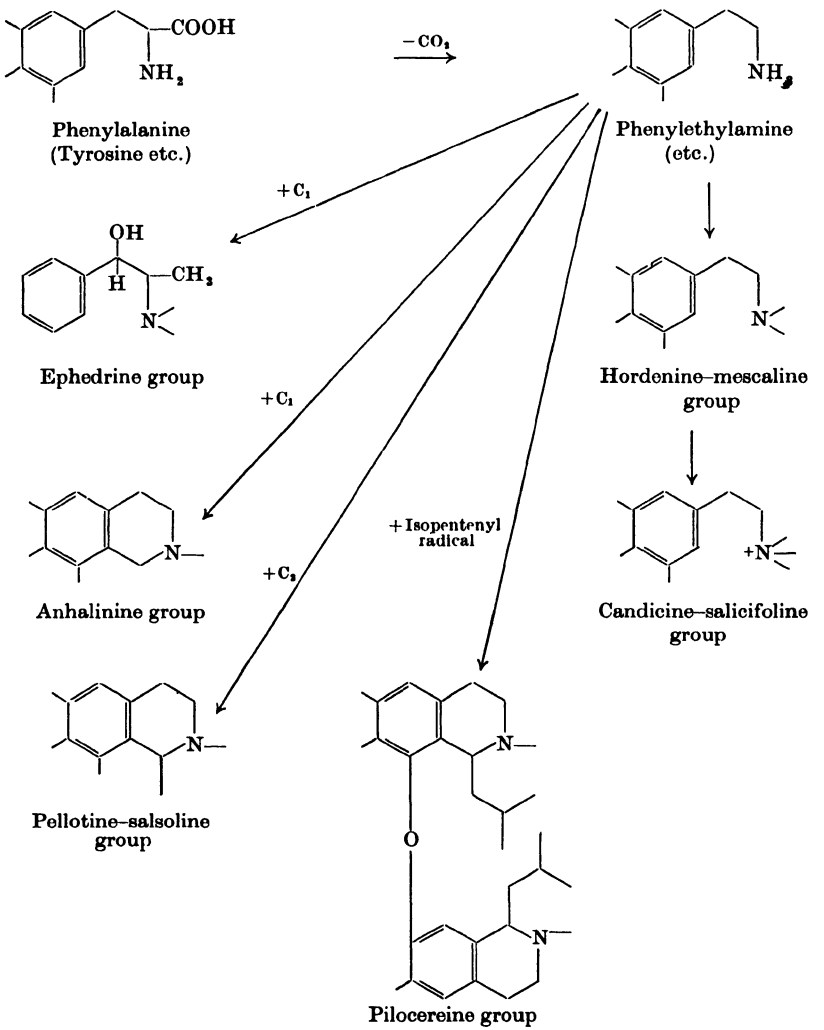


FIG. 8. Phenylalanine family. (a) Alkaloids or protoalkaloids formed from the corresponding amine only.

D. HOMOLOGOUS AND ANALOGOUS CHEMICAL CHARACTERS

The fact that the same substance, or two similar substances, may be isolated from two different plants is only of value taxonomically if the

compound(s) can be regarded as the result of exactly equivalent metabolic processes. Generally, it might be assumed that the presence of similar chemical products is circumstantial proof of such processes, but caution is required since there are cases where the possibility of an analogous, rather than a homologous metabolic pathway might exist. In the case of the alkaloids there are a number of instances that indicate the need to proceed with caution.

For example, anabasine (I) can be formed in two different ways (see Hegnauer, 1958; Mothes, 1959; Schütte, 1960). In the genus *Nicotiana* (Solanaceae), it is built up from one molecule of lysine (via Δ^1-piperideine) and one of nicotinic acid (Fig. 15; cf. nicotine from one molecule of ornithine and one of nicotinic acid, Fig. 14). In the Leguminosae, on the other hand, two molecules of lysine are involved (as Δ^1-piperideine, see Fig. 16). Since this family also contains derivatives of tetrahydroanabasine (e.g. ammodendrine, orensine, iso-orensine etc., Fig. 16), and in the Chenopodiaceae anabasine is accompanied by alkaloids of the sparteine and matrine type which are probably also derived from lysine (Fig. 16), it seems likely that the anabasine in the Solanaceae is not equivalent (homologous) to that in the other two families.

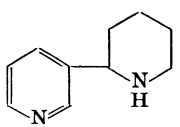
The distribution of the quinoline alkaloids of the quinine type is also noteworthy, and must be regarded as an indication of possible different pathways leading to the same group of compounds. Until recently quinine was regarded as occurring uniquely in *Cinchona*, but in the last few years it has been isolated from several unrelated genera (Table I).

TABLE I

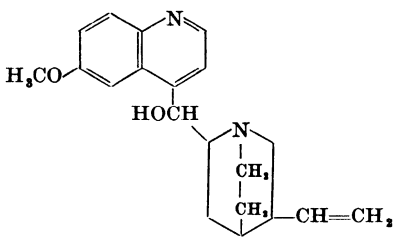
Distribution of quinine and related quinoline alkaloids

Genus	Family	Organ	Alkaloid	Author
Cinchona (all species)	Rubiaceae	Bark	Quinine Quinidine Cinchonine Cinchonidine, etc.	Distribution, cf. Boit (1961)
Enantia polycarpa Engler and Diels	Annonaceae	Bark	Palmatine Quinidine Hydroquinidine	Buzas *et al.* (1959)
Picrolemma pseudocoffea Ducke	Simarubaceae	Twigs	Quinine	Altman (1956)
Strychnos pseudoquina St. Hil.	Loganiaceae	Bark	Cinchonidine Quinidine Quinine Cupreine (?) (curare-like bases)	Altman (1956)

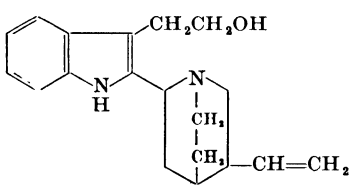
The genus *Cinchona* contains, besides quinine (II) and related quinoline bases, indole alkaloids such as cinchonamine (III) and quinamine. Such observations formed the basis of the hypothesis that the quinoline alkaloids in *Cinchona* were formed from indoles (Goutarel, 1950). Later this hypothesis was substantiated by Relijk (1958), who showed that indole alkaloids were first formed in the leaves of *Cinchona*, and subsequently translocated to the bark and converted into quinolines.



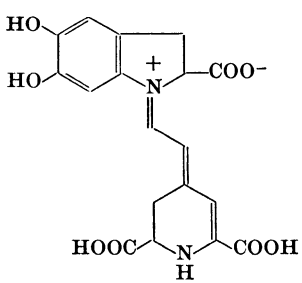
(I)
Anabasine



(II)
Quinine



(III)
Cinchonamine



(IV)
Betanidin

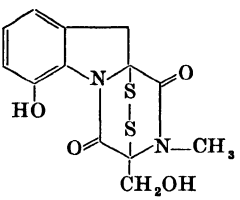
In the genus *Enanitia* (Annonaceae), on the other hand, quinine occurs together with palmatine (the main alkaloid) which is a member of the berberine group (see Fig. 9). Unfortunately no details of other alkaloids occurring in *Picrolemma pseudocoffea* have been recorded. However, although it is impossible to speculate on the origin of the quinine alkaloids in the Annonaceae and Simarubaceae without experimental facts, it appears highly probable that they are formed in quite a different way from that in *Cinchona*.

The possibility of analogous modes of formation is increased when one compares groups of alkaloids rather than individual substances.

The indole ring structure is a common feature of many alkaloids and in most cases arises from tryptophan (cf. Figs. 11–13). However, in some instances, especially where hydrogenated indole rings are found, it

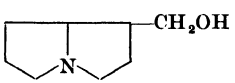
originates from phenylalanine (cf. Fig. 10, *Mesembryanthemum* and *Erythrina* alkaloids). The betanins of the Centrospermae similarly are derivatives of 3,4-dihydroxyphenylalanine. Betanidin itself has the structure (IV) (Wyler and Drieding, 1962; Mabry *et al.*, 1962).

Suhadolnik and Chenoweth (1958) established that the mould alkaloid gliotoxin (V), which has a hydrogenated indole ring, is also formed from phenylalanine.

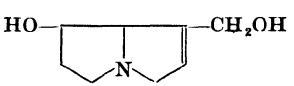


(V)
Gliotoxin

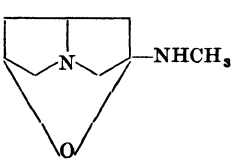
The pyrrolizidine skeleton (necine) can also be synthesized in different ways. Alkaloids of this type (VI–IX) have been isolated from five families and are distributed as follows: Gramineae (VIII, IX); Santalaceae (VI; lindelofidine and its esters with cinnamic acids); Papilionaceae (VI, VII), Boraginaceae (VI, VII) and Compositae (VIII), esterified with necic acids in these last three families.



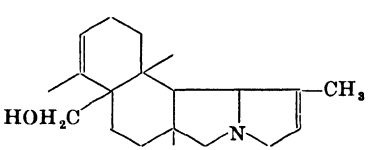
(VI)
Lindelofidine,
Trachelanthamidine,
Laburnine



(VII)
Retronecine,
Heliotridine

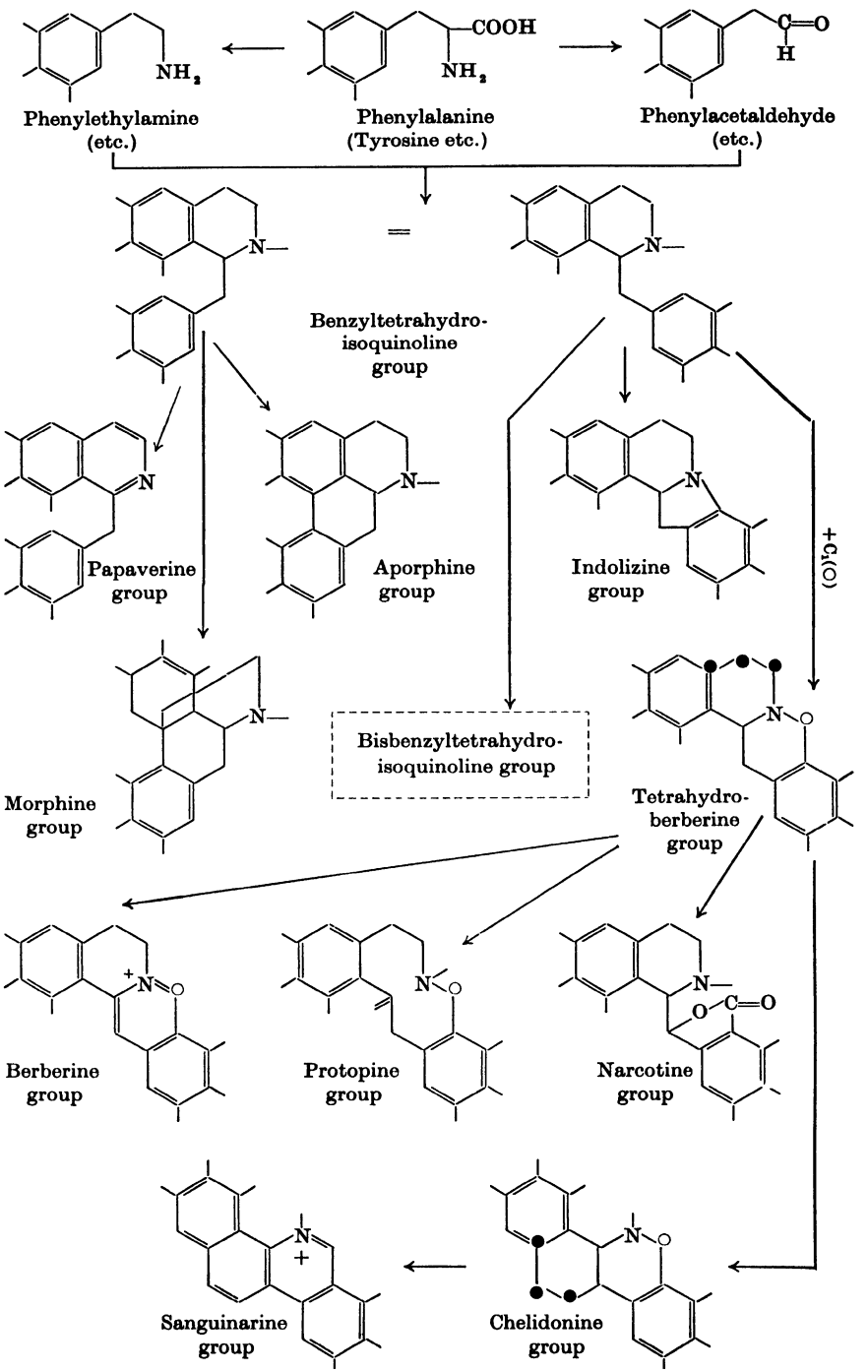


(VIII)
Loline

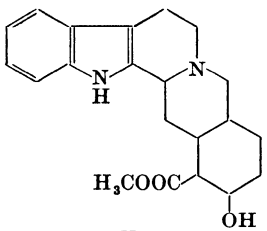


(IX)
Thelepogine $C_{20}H_{33}NO$
(Fridrichsons and Mathieson, 1960)

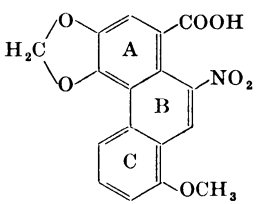
The pyrrolizidine alkaloids of the last three families are similar in the structures of both bases and esterifying acids, but those of the Santalaceae and Gramineae are quite different. In *Thesium* (Santalaceae) both *p*-coumaric and thesinic acids occur, and the alkaloids of the grasses are


NH₂
Phenylethylamine
(etc.)
COOH
NH₂
Phenylalanine
(Tyrosine etc.)
C=O
H
Phenylacetaldehyde
(etc.)
=
Benzyltetrahydro-
isoquinoline
group
Papaverine
group
Aporphine
group
Indolizine
group
+C₁(O)
Morphine
group
Bisbenzyltetrahydro-
isoquinoline group
Tetrahydro-
berberine
group
Berberine
group
Protopine
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Narcotine
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Sanguinarine
group
Chelidonine
group

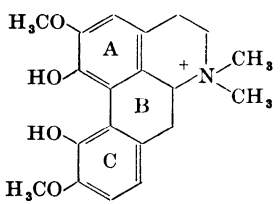
presumably of different origin from that in the dicotyledons. For example, thelepogine (IX) can be derived from a diterpene of the manoöl type and, if this hypothesis is correct, is not a true alkaloid according to our definition.



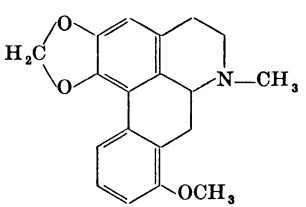
(X)
Yohimbine



(XI)
Aristolochic acid



(XII)
Magnoflorine



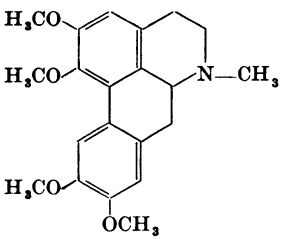
(XIII)
Stephanine

Besides such cases of analogous biosynthesis there are many instances of homologous formation of alkaloids. The example of quinine in the Rubiaceae has already been mentioned. The quinoline alkaloids of cinchona bark (II, III) are obviously quite different structurally from the yohimbine alkaloids (e.g. X) from yohimbe bark, but they are closely related biosynthetically (see Fig. 12) and this points to a close relation between the plants in which they occur.

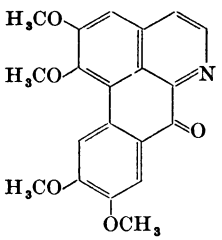
Hegnauer (1960) has recently drawn attention to a similar case of homologous origin of chemically unlike substances. Aristolochic acid (XI) has long been known in Aristolochiaceae, but recent work has shown that

it is accompanied by quaternary aporphine bases (e.g. magnoflorine-corytuberine methiodide, XII, see Fig. 9). Conversion of such bases into nitrophenanthrene structures of the aristolochic acid seems highly probable.

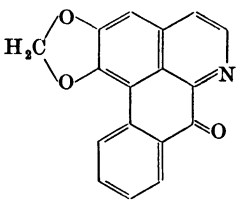
The position of the methoxyl group in the C ring of (XI) is of course unfavourable, but the alkaloid stephanine (XIII), for which a biogenetically plausible mode of formation was suggested by Barton and Cohen (1957), has an equivalent substitution pattern to aristolochic acid.



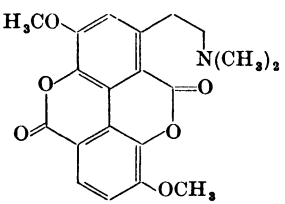
(XIV)
Glaucine



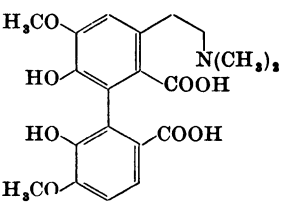
(XV)
Base II



(XVI)
Liriodenine



Taspine



(XVII)
Taspinic acid

If we are correct in our assumption that the aporphine bases and aristolochic acid are homologous (that is to say, biogenetically closely related) then the occurrence of the latter compound in the Aristolochiaceae, provides an additional argument for placing this family in the Polycarpicae.

Another type of alkaloid which is obviously derived from the aporphines has recently been found in the heartwood of *Liriodendron tulpiferum* (Taylor, 1961). Besides glaucine (XIV), Taylor isolated the corresponding base (XV) and the yellow alkaloid liriodenine (XVI). Finally, taspine (XVII), which has been isolated from *Leontice ewersmanii* and *Caulophyllum robustum* (Berberidaceae), can be regarded as an oxidation product of an aporphine base.

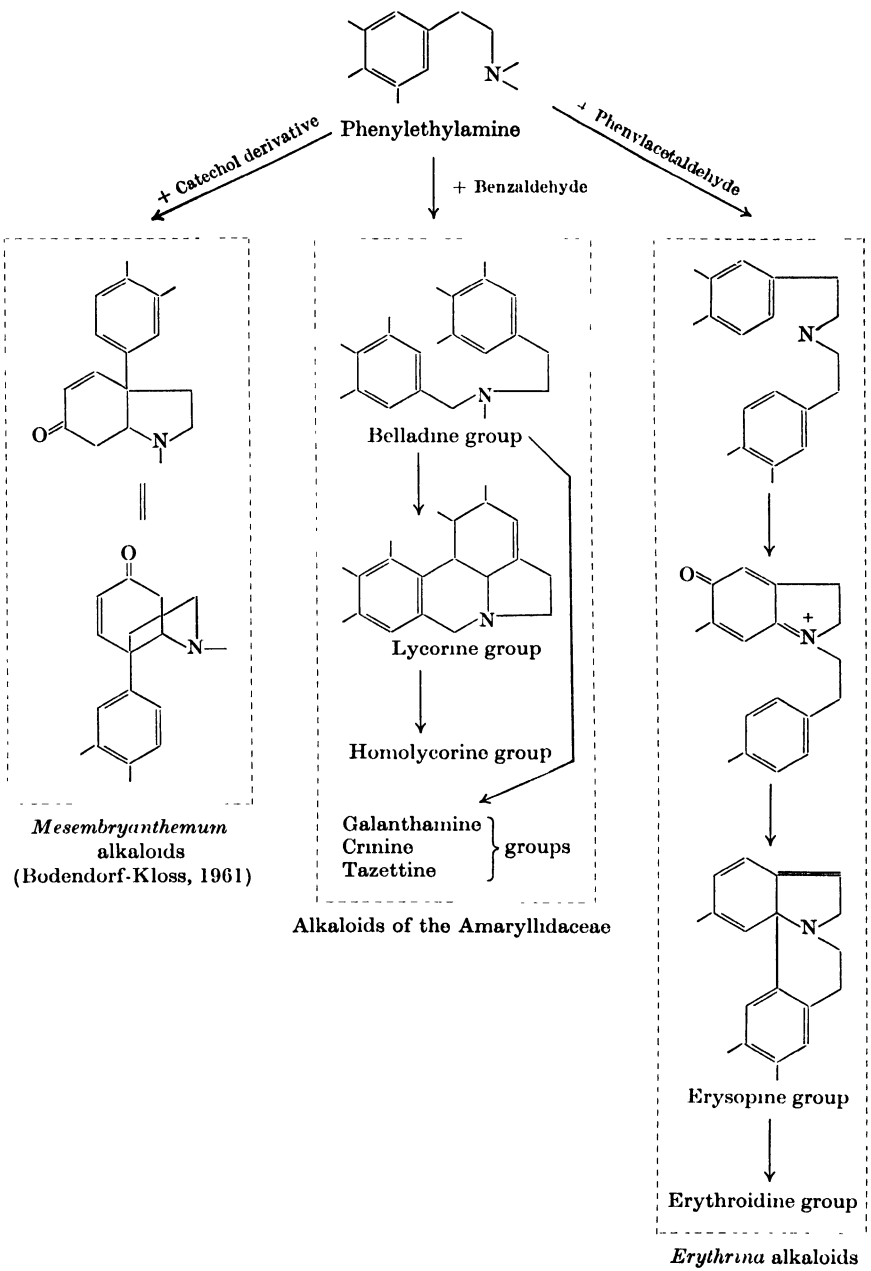


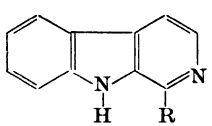
FIG. 10. Phenylalanine family. (c) Special modifications.

III. The Use of Alkaloids in Taxonomy

A. Introduction

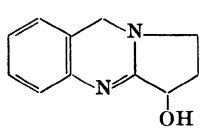
The distribution of individual alkaloids in the plant kingdom has recently been reviewed by Boit (1961), and Hegnauer (1958) has discussed the occurrence of the different groups of alkaloids in the Cormophytes. In this section, therefore, only selected examples will be considered to show the way in which the alkaloids can be usefully employed as taxonomic guides.

It is obvious that one cannot employ the occurrence of alkaloids as a whole for taxonomic purposes since, as discussed in the preceding section, the term covers a whole range of complex widely different substances. Differences in the chemistry of the individual compounds must therefore be critically evaluated before one can attempt to use their occurrence to compare one group of plants with another. Furthermore, alkaloids which either occur infrequently or are present in trace amounts must be distinguished from those which are found in all members of a given group. The widespread occurrence of traces of certain simple alkaloids suggests that they may often be by-products of common metabolism or in some cases be produced as artifacts. For example,



(XVIII, R = CH_3) Harmane

(XIX, R = H) Norharmane



(XX)

Vasicine

Poindexter and Carpenter (1962) found traces of harmane and norharmane (XVIII, XIX) in tobacco smoke and suggested that these compounds were produced by destructive distillation of tryptophan via tryptamine, and of the corresponding aldehydes (cf. Fig. 11). On this basis, in those cases where traces of nicotine have been obtained from plants by steam distillation, one should strictly speaking, show that the compound was actually present as such in the fresh plant.

Even when such simple bases are present in reasonably large amounts, their distribution may be quite fortuitous, and the taxonomic importance of alkaloids such as nicotine, the simple harmane derivatives, vasicine (= peganine, XX) and similar compounds cannot be judged at present. It would appear that such compounds are in general widely distributed in the plant kingdom and in exceptional cases, for reasons unknown, they are produced in readily detectable amounts. Once all the species which accumulate these compounds are known then it is probable that this ability to synthesize detectable quantities will be seen to have a definite, albeit limited, taxonomic value. For example it already appears that the Elaeagnaceae might be regarded as accumulators of alkaloids of the tryptophan–tetrahydroharmane type.

Leaving aside the simple bases, it is obvious, as has already been stressed, that one must distinguish between the true alkaloids and the proto- and pseudoalkaloids, otherwise one is comparing totally different characters. Attempts to classify plants phylogenetically on the basis of their content of alkaloids of increasing molecular complexity (McNair, 1935), therefore inevitably led to fallacious results, especially since he included alkaloid esters and glyco-alkaloids based on diterpenes or steroidal sapogenins (Figs. 3 and 4), apart from the question as to whether an increase in the size or complexity of a molecule can be regarded as a step in a phylogenetic progression.

Predictions about phylogeny can only be made if one restricts one's attention to a limited group of defined chemical substances, otherwise all sorts of interfering factors must be taken into account. For example, a reduction, rather than an increase, in the size of a molecule is often encountered such as in the case of the bitter principles (C_{26} skeleton) of the Rutaceae and Meliaceae which are today believed to be derived from triterpenes (C_{30}-compounds).

Although many characters can be used (cf. Harmann (1961) in his noteworthy work on the Farinosae) in the case of the alkaloids one should only use groups or families as discussed previously. Thus, although the leucoanthocyanins as a group could be used directly the work of Sporne (1960), one could certainly not use "alkaloids" (i.e. all alkaloids) in the same way.

Another example of the importance of considering the biogenetic and physiological aspects in the chemotaxonomy of alkaloids, is the surprising fact that different populations of *Duboisia myoporoides*, contain different combinations and proportions of hyocyamine (and related tropine bases), nicotine, isopelletierine, and anabasine (Figs. 14 and 15). It is probable that the plants contain the following combination of properties; (a) diamino acid decarboxylase (acting on ornithine and lysine; Figs.

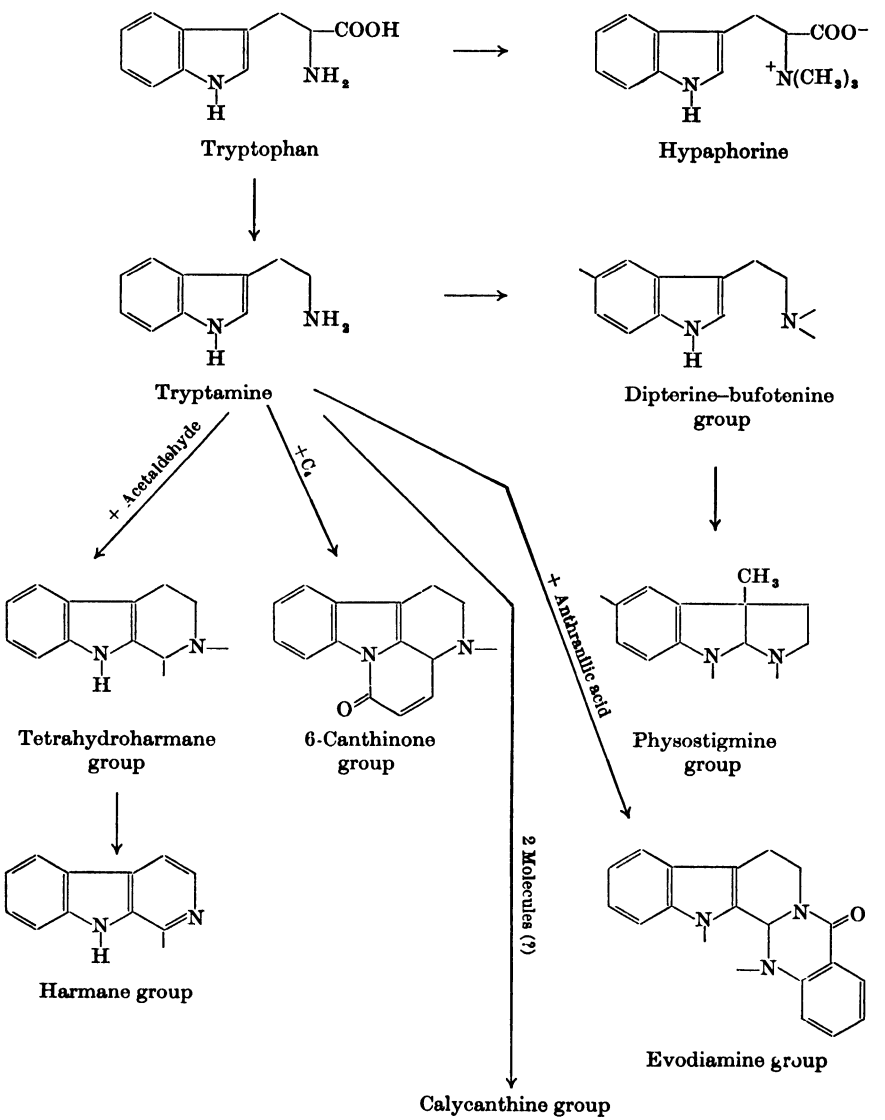


FIG. 11. Tryptophan family. (c) Alkaloids produced mainly from tryptamine.

14 and 15); (b) diamino oxidase (acting on putrescine and cadaverine), (c) linking of amino aldehydes (perhaps a spontaneous reaction in the vacuole with reactive metabolites to give the alkaloids found. Slight changes in the equilibrium or in the concentration or activity of enzymes

in this combination of biosynthetic steps could obviously lead to the accumulation of different bases. Generally, the ornithine and lysine families of alkaloids (Figs. 14 and 15) are closely related, and rarely appear separately. They should, therefore, perhaps be treated as one "unit" from the chemotaxonomic point of view.

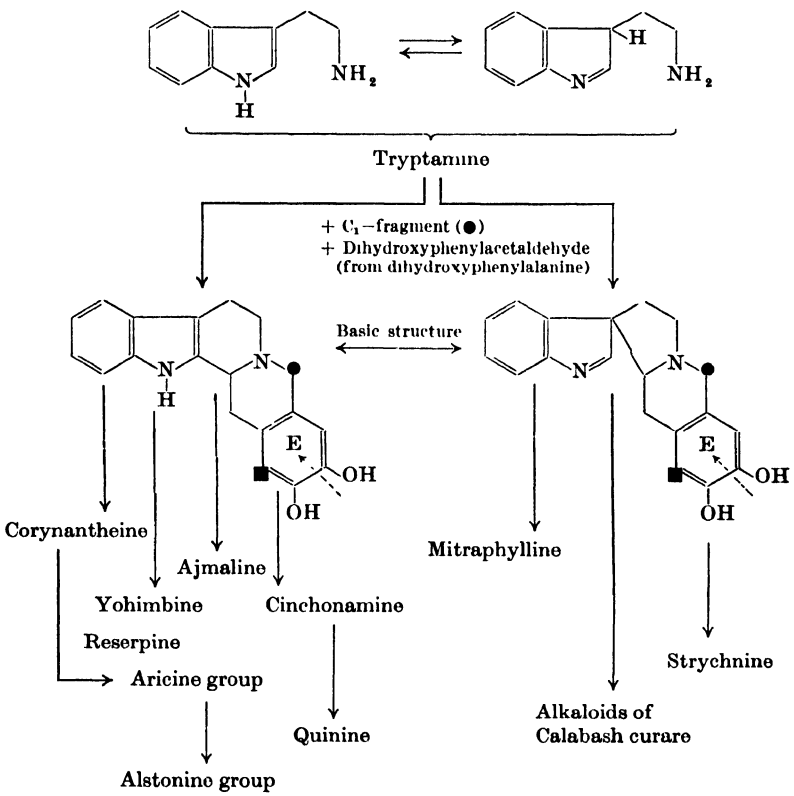


FIG. 12. Tryptophan family. (b) Complex indole bases: the "berberine hypothesis" includes methylation of the C-atoms marked ■, ring opening (↑) and hydrogenation of the E ring.

In spite of the limitations mentioned above, alkaloids can be very useful for taxonomic purposes as is illustrated in the succeeding sections.

B. Colchicine Group

Šantavý (1956) demonstrated that many of the results on the distribution of colchicine given by Klein and Pollauf (1929) were incorrect, and

so many chemotaxonomic speculations based on this latter paper had to be revised. In a long series of investigations using specific methods to detect tropolone-ring alkaloids, Šantavý and his co-workers (for literature see Boit, 1961; Moza *et al.*, 1962; Hegnauer, 1963) could only detect colchicine and related alkaloids in the *Gloriosa*, *Littonia*, *Ornithoglossum*, *Iphigenia*, *Colchicum*, *Androcymbium*, *Dipidax* and *Anguillaria*.

TABLE II

The colchicine-containing genera of the Liliaceae arranged according to the systems of Krause, Buxbaum and Hutchinson

	Krause	Buxbaum	Hutchinson
Sub-family	MELANTHIOIDEAE	WURMBAEOIDEAE (includes only the following 6 tribes)	———
Tribe	6. Uvularieae *Gloriosa* *Littonia*	Glorioseae *Gloriosa* *Littonia*	17. Uvularieae *Gloriosa* *Littonia*
	8. Anguillarieae *Androcymbium* *Dipidax* *Ornithoglossum* *Anguillaria* *Iphigenia*	Iphigenieae *Ornithoglossum* *Iphigenia* Baeometraea (not yet investigated)	21. Anguillarieae *Anguillaria* *Dipidax*
	9. Colchiceae *Colchicum* (incl. *Merendera* and *Bulbocodium*)	Colchiceae *Colchicum* (incl. *Merendera* and *Bulbocodium*) *Androcymbium* Neodregeae *Dipidax* Wurmbaceae *Anguillaria*	26. Colchiceae *Colchicum* *Merendera* *Bulbocodium* 27. Iphigenieae *Ornithoglossum* *Iphigenia* *Androcymbium*

Colchicine and related compounds occur also in *Sandersonia aurantiaca* (Glorioseae) and *Camptorrhiza strumosa* (Iphigenieae) (Šantavý, personal communication).

If one compares this distribution of the colchicines in the Liliaceae with the systems proposed by Krause (1930), Hutchinson (1959) and Buxbaum (1925, 1927, 1937), it is apparent that the last mentioned shows the best correlation (Table II).

It is also obvious that Hutchinson's reorganization of the Liliaceae does not appear to give any advantage, presumably because he ignored the detailed investigations made by Buxbaum. The assumption that the accumulation of colchicine and related compounds is a characteristic feature of the Wurmbaeoideae was already made by Buxbaum, and the

Czech investigators mentioned previously have now shown that he alone appeared to have appreciated the natural correlations of this group of the Liliaceae correctly. The lack of raphide-containing cells in the aerial parts of the Wurmbaeoideae is a further argument for the homogeneity of this sub-family.

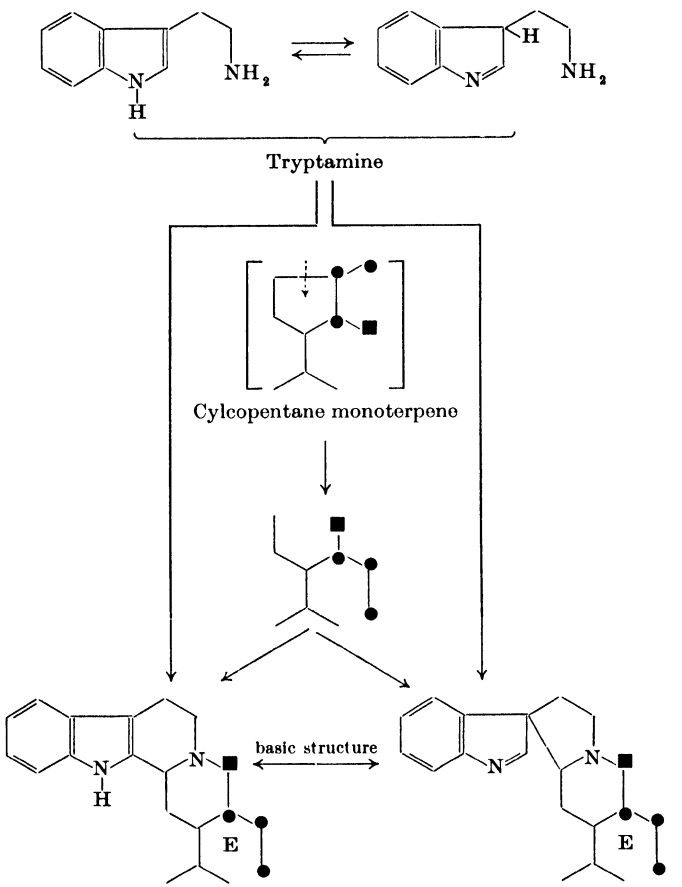


FIG. 13. Tryptophan family. (c) Complex indole bases: the "monoterpene hypothesis" includes secondary formation of the E-ring.

Finally, it should be noted that colchicine and related tropolone bases are probably not true alkaloids at all since Leete and Nemeth (1960, 1961) have suggested that the biosynthesis of this class of compound probably represents a variant of flavonoid biosynthesis. If this is the case the colchicines should really be regarded as pseudoalkaloids.

C. Amaryllidaceae

The alkaloids of the Amaryllidaceae (Fig. 10) have only been detected in the sub-family Amaryllidoideae of this family (in the sense of Pax and Hoffmann, 1930; Wettstein, 1935). Hutchinson has re-arranged this family, in particular removing the Agavoideae, Hypoxidoideae and

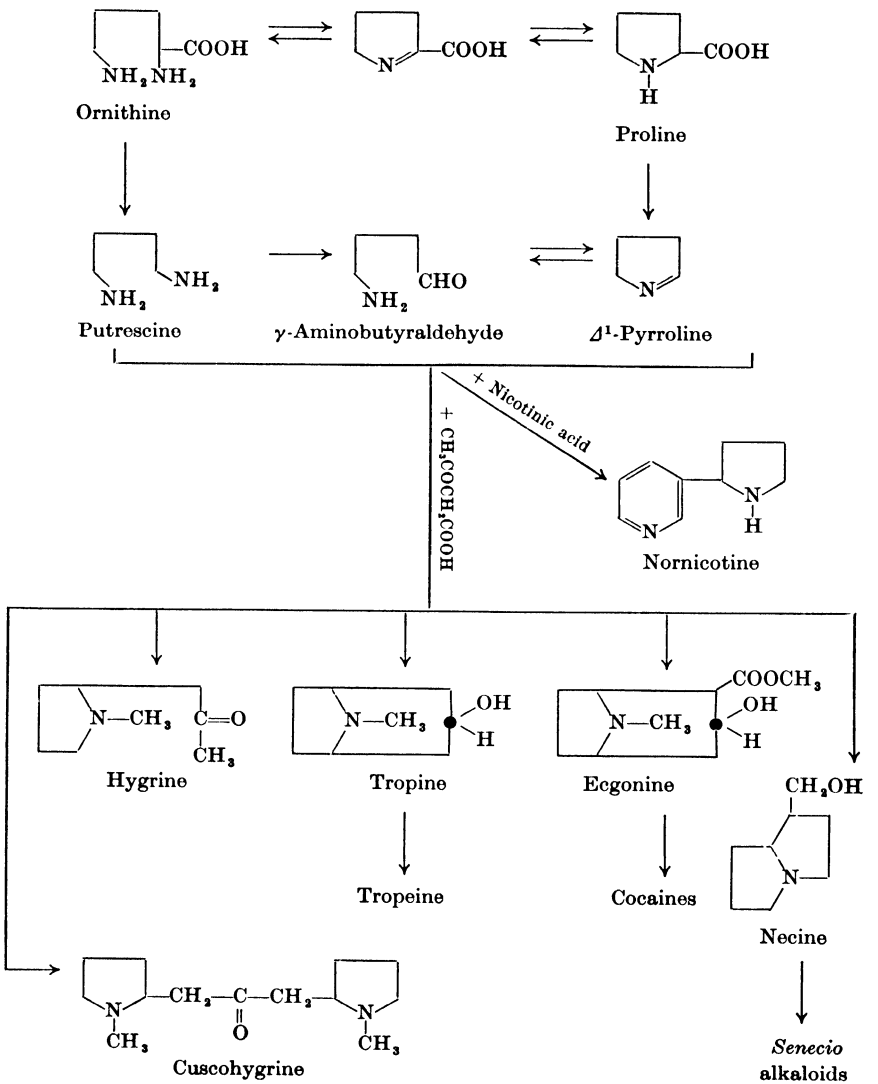


Fig. 14. Ornithine family.

Campynematoideae, retaining only the Amaryllidoideae. He has also placed the majority of genera of the Alloiideae (put in the Liliaceae by Krause and Wettstein) in the Amaryllidaceae because they also possess

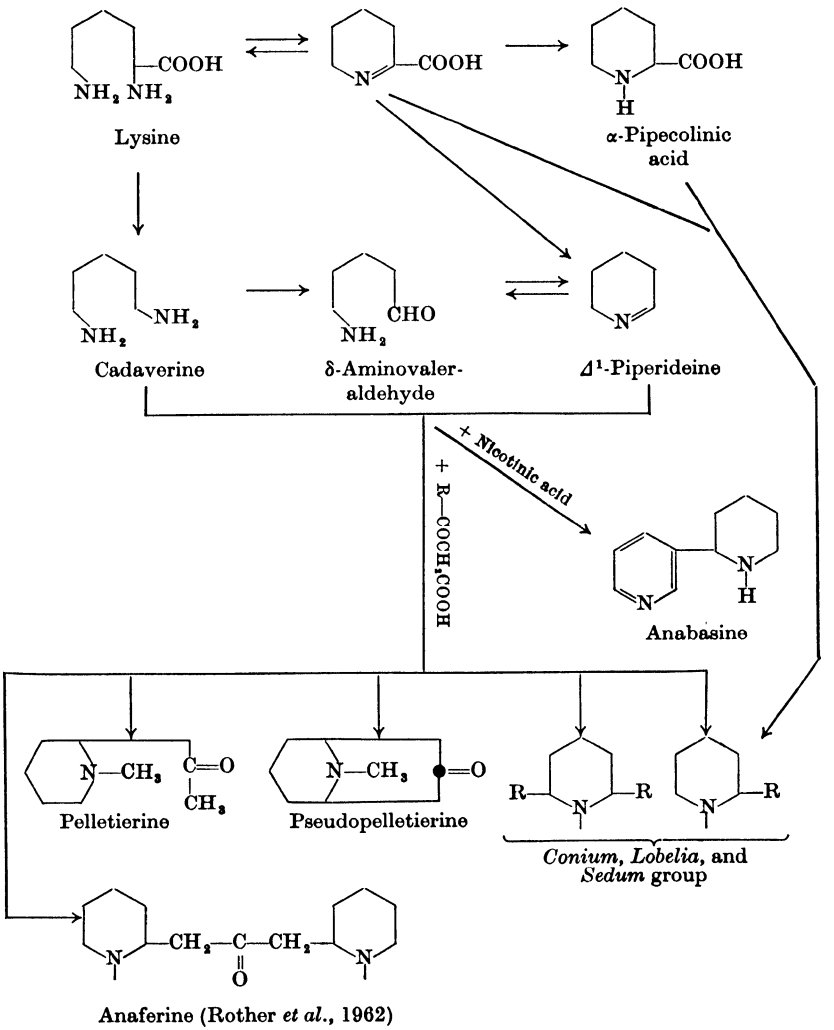


FIG. 15. Lysine family. (a) Alkaloids corresponding to hygrine and tropine.

umbellate inflorescences. It is interesting to examine if this new arrangement is more natural than the previous one. No chemical investigations have been carried out on Hypoxidoideae or Campynematoideae so these

can be excluded from the discussion. The Agavoideae undoubtedly fit better with the Dracaenoideae (put in the Liliaceae by Krause and Wettsteinf, both sub-families having many species which contain saponins and frequently raphides and other forms of calcium oxalate are present. The Amaryllidoideae, on the other hand, invariably contain alkaloids, but steroidal saponins have not been detected with certainty. Furthermore, raphides in idioblasts and in mucilage containing tubes are common. This general uniformity of the Amaryllidoideae is completely destroyed if one includes the Allioideae, since members of this group have neither alkaloids nor raphide-containing cells, but do contain steroidal saponins. Many members of the Allioideae also contain unusal S-containing amino acids (e.g. alliin) and so their re-arrangement in the Amaryllidaceae as suggested by Hutchinson is not at all satisfactory. It would appear better to leave the Allioideae in the Liliaceae, or if this is not desirable on other grounds, to elevate them to family rank, placed between the Liliace and Amaryllidaceae as already defined (cf. Hegnauer, 1963).

D. Polycarpicae

Thirteen of the twenty-five families placed by Wettstein in the Polycarpicae are considered to be free from alkaloids. These are mainly monotypic or oligotypic families of uncertain position. Alkaloids are found frequently in the other twelve families of this order. The majority of these alkaloids are benzyltetrahydroisoquinolines (Fig. 9), of which the two quaternary bases magnoflorine (XII) and berberine (XXI) can be taken as typical. These two compounds (or their congeners) have been isolated from various species of the Annonaceae, Aristolochiaceae, Berberidaceae, Magnoliaceae, Menispermaceae and Ranunculaceae, where they occur along with the corresponding tertiary base. In the Hernandiaceae, Lauraceae, Monimiaceae and Nymphacaceae, on the other hand, only the latter compounds are found. When all the results are taken into consideration it is apparent that the ability to synthesize and accumulate benzyltetrahydroisoquinoline alkaloids is a remarkably constant feature of the Polycarpicae. There are, however, some points that should be noted in certain families.

Aristolochiaceae: the typical secondary products of this family, aristolochic acid (XI) and related compounds, have been discussed earlier (Section II, D). More important from the chemotaxonomic point of view, however, is the fact that magnoflorine (XII) and related bases of the benzylisoquinoline type have been isolated from some species. The occurrence of these latter compounds is of great systematic

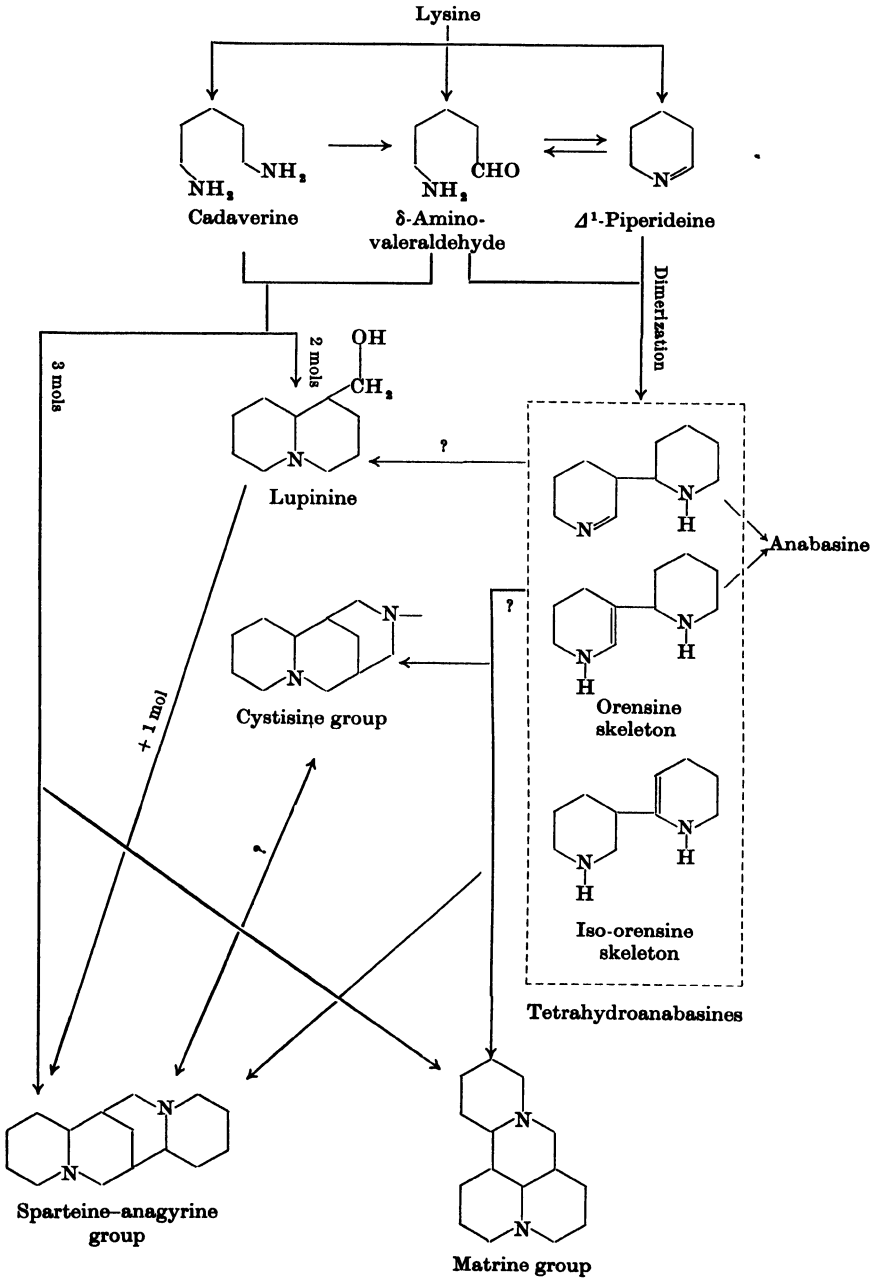


FIG. 16. Lysine family. (b) Lupin alkaloids.

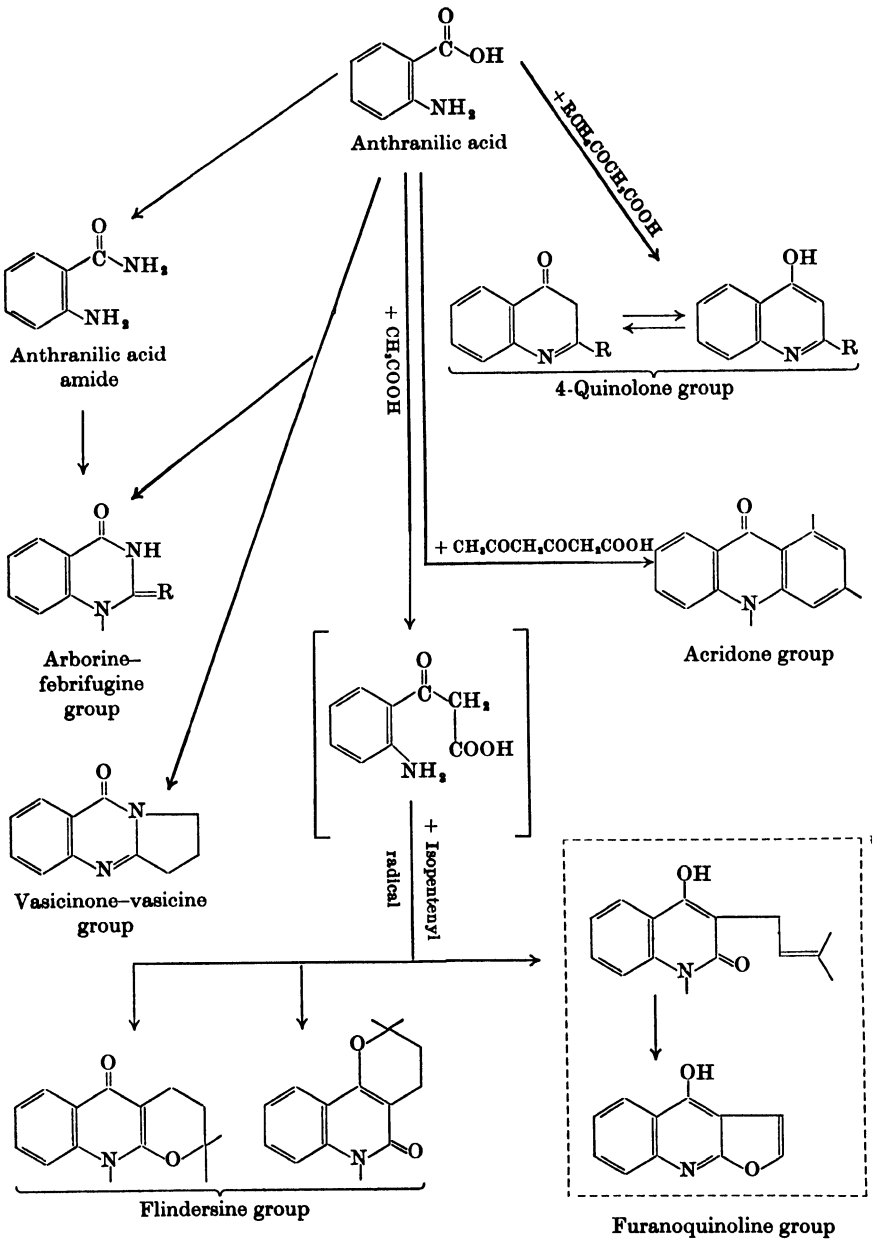
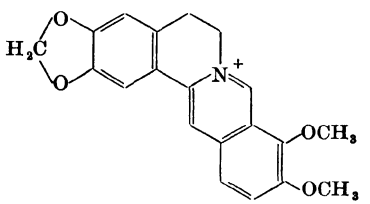


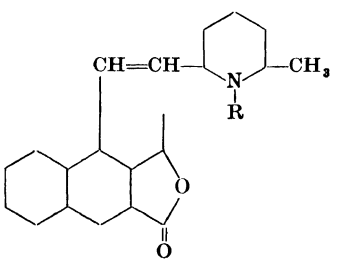
FIG. 17. Anthranilic acid family.

importance and supports the inclusion of this family in the order (see Hegnauer, 1960).

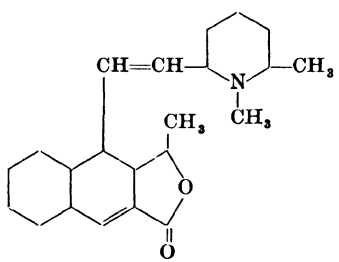
Berberidaceae: besides the widely distributed benzylisoquinolines like (XXI), bases of the lupin type have been obtained from the genera *Caulophyllum* and *Leontice*. Sparteine, lupanine, methylcytisine and leontine (optical antipode of allomatrine) (Fig. 16) have all been isolated, sometimes alone, but more often together with benzylisoquinoline bases.



(XXI)
Berberine



(XXII)
Himbacine (R = CH_3)
Himbeline and Himandravine (R = H)

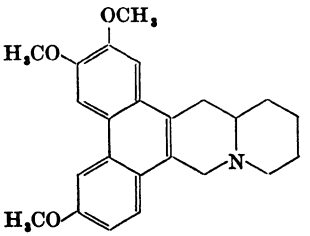


(XXIII)
Himgravine

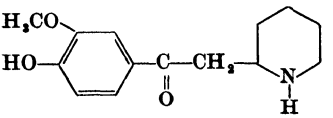
Calycanthaceae: no benzylisoquinoline alkaloids have been isolated from this family; the bases calycanthine, calycanthidine and folicanthine are believed to be tryptamine derivatives.

Himantandraceae: this family also contains no benzyltetrahydroisoquinoline alkaloids. A series of alkaloids (XXII, XXIII) of quite different structure has been isolated from the bark of *Himantandra* (*Galbulimia*) *baccata* and *H.* (*G.*) *belgraveana* (Piney *et al.*, 1961; Abraham and Bernstein, 1961). It is not clear how these bases are to be classified biogenetically.

Lauraceae: besides many typical benzylisoquinolines, two alkaloids of quite a different structure, cryptopleurine (XXIV) and pleurospermine (XXV) have been isolated from the bark and leaves respectively of the Australian species, *Cryptocarya pleurosperma*.

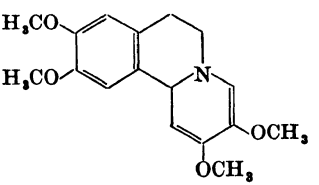


(XXIV)
Cryptopleurine

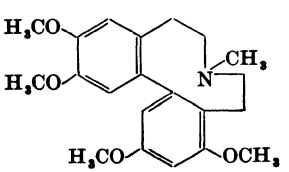


(XXV)
Pleurospermine

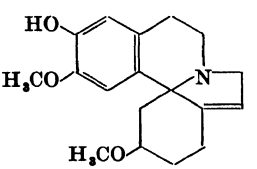
Menispermaceae: this family contains mainly bisbenzylisoquinoline bases and members of the berberine group. In addition there are also some alkaloids related to aporphine alkaloids, such as rotundine (XXVI) (*Stephania rotunda*). Protostephanine (XXVII) (*Stephania japonica*) and dihydroerysodine (XXVIII) (*Cocculus laurifolia*) on the other hand seem to belong to the *Erythrina* group (Fig. 10).



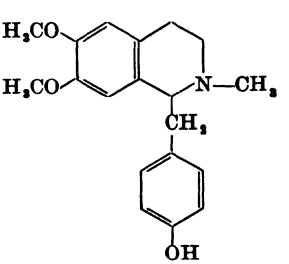
(XXVI)
Rotundine



(XXVII)
Protostephanine



(XXVIII)
Dihydroerysodine



(XXIX)
Armepavine

Nymphaeaceae: the occurrence of the pseudoalkaloids nupharidine and deoxynupharidine (Fig. 2) in this family has been known for some time. The recent identification of the aporphine derivatives, roemerine, nuciferine, nornuciferine and armepavine (XXIX) in *Nelumbo nucifera* is taxonomically more important since it shows the relationship between the Nymphaeaceae and the Polycarpicae.

The unexpected occurrence of many different types of alkaloid in the Polycarpicae can be interpreted if one classifies the families of this order in four groups. (a) Those containing only benzyltetrahydroisoquinolines. (b) Those containing both isoquinolines and other types of alkaloid. (c) Those containing only other alkaloids. (d) Those lacking alkaloids altogether.

One can assume that the evolution of the Polycarpicae might have followed one of the two possibilities shown in Fig. 18.

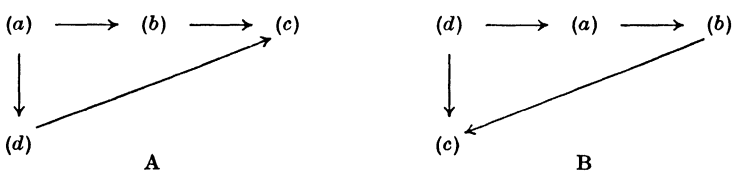


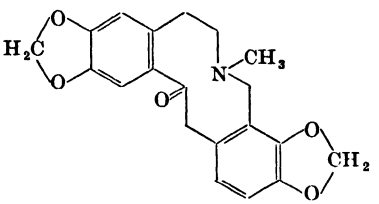
FIG. 18.

Since alkaloids are absent from the Winteraceae, which are considered to be very primitive, it would appear that the scheme B (Fig. 18) is more likely to be correct. Such schemes are of course purely hypothetical but are of value in so far as they point to the need for a more strict chemical and taxonomic examination of the members of the groups (c) and (d). Only when we can allot a family to these two groups with absolute certainty will it be possible to use the alkaloids fully for help in tracing affinities of the families within the Polycarpicae.

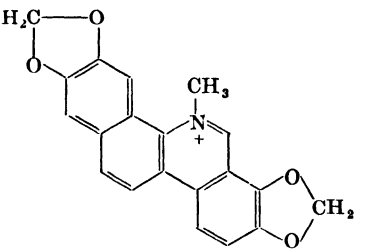
E. RHOEADALES

According to Wettstein the Rhoeadales contain the Papaveraceae, Capparidaceae, Cruciferae, Resedaceae, and the two minor families Tovariaceae and Moringaceae. True alkaloids have only been found in the Papaveraceae, all species of which contain compounds of the benzyltetrahydroisoquinoline type. For a long time it was thought that protopine (XXX) was the most widespread, but recently Hakim *et al.* (1961) have shown that coptisine and sanguinarine (XXXI) are equally well distributed. From the phytochemical point of view therefore the family belongs to the Polycarpicae, with the protopine, chelidonine and

sanguinarine as the alkaloids most characteristic for the family as a whole. Since the Papaveraceae differ from the Rhoeadales by other characters as well, it would seem better to incorporate them into the Polycarpicae where they would find their closest relative in Nymphaeaceae and Berberidaceae.



(XXX)
Protopine



(XXXI)
Sanguinarine

F. Rutaceae

In the system of Wettstein, the Rutaceae are the main family of the Terebinthales, while Hutchinson places them, along with the Simarubaceae in the Rutales. In neither system is any nearer relationship to the Polycarpicae suggested. From the point of view of alkaloid chemistry, the Rutaceae are characterized by having a large number of bases of the anthranilic acid family (Fig. 17) and it is to be expected that representatives of such compounds are going to be found in the Simarubaceae. Besides the furanoquinoline and acridone alkaloids, derivatives of histidine (pilocarpine, XXXII) and tryptophan (6-canthinone, Fig. 11) occur more rarely. However, the interesting feature from the chemotaxonomic point of view is that representatives of the benzyltetrahydroisoquinolines have also been isolated. It is important to note that no completely new compounds of this type have been found in the Rutaceae, but that those which have been isolated are also present in the Polycarpicae and Papaveraceae (Table III).

The most widespread alkaloids of the Polycarpicae (magnoflorine (XII) and berberine (XXI)) occur in the Rutaceae along with alkaloids of the Papaveraceae (allocryptopine and chelerythrine). If, then, one merely considers the benzylisoquinoline alkaloids the Rutaceae are related to the Polycarpicae as discussed for the Papaveraceae in the previous section. When, however, all the different types of alkaloid are considered it is obvious that this family is much more distinct from the Polycarpicae than are the Papaveraceae.

Of course it could be argued that the Rutaceae belong to type (b) of the Polycarpicae in the scheme put forward earlier (Fig. 18), but the alkaloids

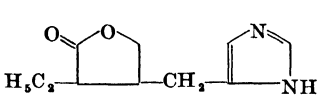
of the anthranilic family are much more numerous than those of the benzylisoquinoline group. This could perhaps be accounted for by assuming that the order in which the Rutaceae occur was originally derived from the Polycarpicae. One proposal of this kind has actually been put forward by Hallier (1912); in his system the Berberidaceae are suggested to give rise to the Terebinthinae.

TABLE III

Benzyltetrahydroisoquinoline alkaloids of the *Rutaceae*

Alkaloid	Main distribution	Genera of the Rutaceae in which the alkaloids have been detected
Magnoflorine (XII) (Corytuberine methiodide)	Widespread in the Polycarpicae	*Phellodendron, Zanthoxylum*
Menisperine ((+)-Isocorydine methiodide)	Menispermaceae, Lauraceae	*Fagara, Zanthoxylum*
Berberine (XXI)	Berberidaceae, Menispermaceae, Ranunculaceae, Papaveraceae	*Evodia, Fagara, Phellodendron, Toddalia, Zanthoxylum*
Allocryptopine (Homochelidonine, Fagarine I, α-Fagarine)	Papaveraceae	*Fagara*
Chelerythrine (Toddaline)	Papaveraceae	*Fagara, Toddalia*

If the importance of individual characters is overstressed in phylogenetic speculations it can lead to error, and further arguments are therefore required before the above postulates can be accepted. This can readily be done in the case of the Rutaceae by considering the general distribution in the Polycarpicae of the compounds isolated from one member of the family, *Phellodendron amurense* (Table IV). If this species did not contain the Rutaceae bitter principles, limonin and obacunone, it would be phytochemically a typical member of the Polycarpicae.



(XXXII)
Pilocarpine

TABLE IV

Compounds from *Phellodendron amurense* compared with those found in members of the Polycarpicae

Substance	Polycarpicae	*Phellodendron amurense*
SiO_2 in membranes	Widespread in woody members	+ (also in other Rutaceae)
Alkaloids: Berberine	Widespread	+
Palmatine		+
Jatrorrhizine		+
Magnoflorine		+
Essential oil	Common in woody members (oil cells)	+ (Common in the family; lysigenic cavities)
Ferulic acid	Common	+ (lumecaerulic acid)
Amurensin	Des-*O*-methylicariin from *Epimedium* has the same aglycone	+
Isoprenoid bitter principles	Columbin (C_{20}) in the Menispermaceae	Limonin (C_{26}) Obacunone (C_{26})

G. Complex Indole Alkaloids

The complex indole alkaloids (Figs. 12 and 13) are known to occur in the Loganiaceae, Apocynaceae and Rubiaceae. This indicates that these families may be related as suggested in the systems of both Hallier (1912) and Hutchinson (1929) in which the Rubiaceae are derived from the Loganiaceae. The Contortae (Loganiaceae, Apocynaceae, Asclepiadaceae and Gentianaceae) and the Rubiaceae are also similar in their ability to synthesize "pseudoindicans" and related compounds of the type of monoterpenoid semi-acetal glucosides (asperuloside, loganin, genipin, gentiopicrin etc. (Fig. 1)). There are also a number of similarities to the Tubiflorae. Generally speaking the families of the Sympetalae are interrelated chemically (the families of the orders Contortae and Tubiflorae and perhaps the Oleaceae and Dipsacaceae) but an exact analysis must be reserved for future consideration.

IV. Conclusions

Biochemical characters for the evaluation of natural relationships in the plant kingdom will certainly be taken into greater consideration in the future. Among such characters, the alkaloids, in many cases, play

an important role. However, much greater efforts need to be made until we are sufficiently well informed about their chemistry, biogenesis and distribution to profit fully from the knowledge we already possess.

It must be stressed once again that individual characters are in most cases of limited importance, and if possible, the whole range of plant products should be taken into account when one group of plants is compared with another.

Finally, one should always distinguish between groups of compounds produced by analogous and homologous pathways of plant metabolism. This means, of course, that we need to be more certain about the biosynthetic pathways in the different families. It can be seen, therefore, that at present chemical plant taxonomy is only at the beginning of a promising future.

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CHAPTER 15

The Distribution of Alkaloids in the Rutaceae

J. R. PRICE

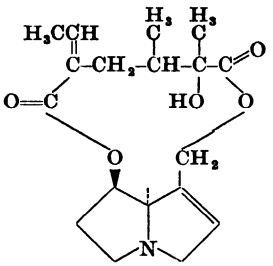
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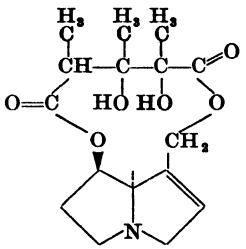
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I. Introduction

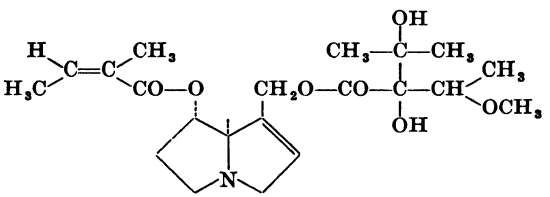
The conversion of carbon dioxide and water, through the mediation of small amounts of nitrogen, phosphorus, potassium and other elements, to the building materials which lead to the development of an adult plant from a germinated seed, is clearly an essentially chemical or physico-chemical process. The end-product of this complex process is, equally clearly, a combination of substances each capable of definition in physico-chemical terms. However difficult it may be at present to define these chemical systems adequately, there are certain constituents of them, such as the low molecular weight components, which can be identified precisely. The structures of these low molecular weight substances are characteristic of the overall metabolic patterns of the organisms producing them, in just the same way as are such morphological features as the shape of the leaves, the number of petals, the type of fruit and so on. Each is the end-product of an integrated series of gene-controlled processes and, in the absence of detailed biogenetic data, each should be accorded the same weight as a taxonomic "character". But we can go further: it is not even the nature of the end-product, i.e. its molecular structure, that is of primary importance. It is the biogenetic pathway that is phylogenetically the more significant; not the structure itself but the series of gene-controlled reactions which give rise to it. This,



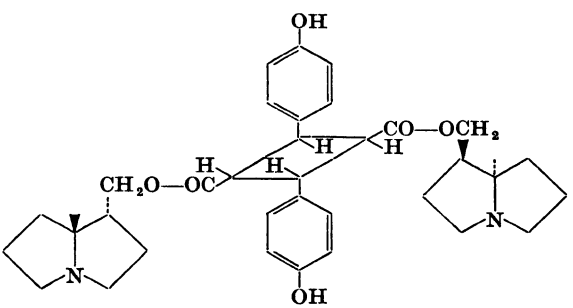
(I)
Senecionine (*Senecio*)



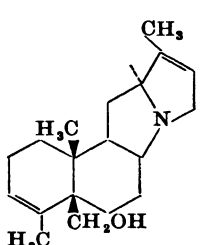
(II)
Monocrotaline (*Crotalaria*)



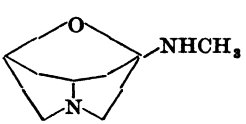
(III)
Lasiocarpine (*Heliotropium*)



(IV)
Thesine (*Thesium*)



(V)
Thelepogine (*Thelepogon*)

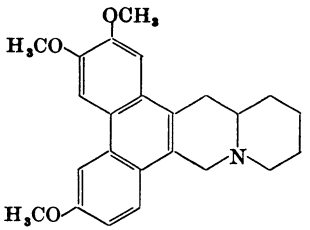


(VI)
Loline (*Lolium*)

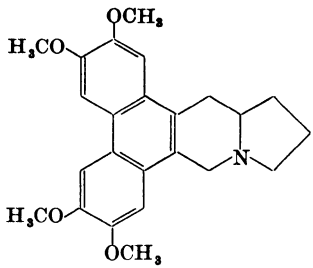
of course, is because the sequence of biogenetic steps must to some extent at least reflect the evolutionary history of the organism. For many classes of plant products in the molecular weight range 250–500, several biosynthetic routes can be postulated so that the occurrence of a particular substance in two groups of plants does not necessarily mean it is produced by the same route in the two groups. Even if it should be, there is the ever-present possibility that evolutionary pressures have led to the development of similar biogenetic routes to similar end-products in otherwise unrelated groups of organisms. To illustrate, one could not argue that the occurrence of pyrrolizidine ester alkaloids (I–IV) in the genera *Senecio*, *Crotolaria*, *Heliotropium* and *Thesium* necessarily implies a close phylogenetic relationship between the Compositae, the Leguminosae, the Boraginaceae and the Santalaceae. Still less of course, could one contend that these families are related to the Gramineae because of the occurrence of alkaloids containing the pyrrolizidine ring system (V) and (VI) in *Thelepogon* and *Lolium*.

A further example is provided by the occurrence of phenanthroindolizidine and -quinolizidine alkaloids which are clearly biogenetically related. In *Cryptocarya pleurosperma*, family Lauraceae, is found the vesicant phenanthroquinolizidine base cryptopleurine (VII), while the phenanthroindolizidine alkaloids tylophorine (VIII) (Boit, 1961) and tylocrebrine (IX) (Gellert *et al.*, 1962) have been found in *Tylophora* species, family Asclepiadaceae. Recently Russel (personal communication) has isolated these two phenanthroindolizidines (VIII and IX) from two *Ficus* species, family Moraceae. But no taxonomist, I think, would agree that this establishes that the Lauraceae, Asclepiadaceae and Moraceae have evolved from a common stock, and it is obvious that one must be wary of attaching too much weight to the support which such occurrences might provide for a postulated relationship between two groups of plants. In practice, one is confronted not only with diverse occurrences of a particular substance but more often, as in the cited examples of pyrrolizidine alkaloids, with occurrences of substances which seem closely related because of certain common structural chemical features rather than actual identity. There are, of course, many metabolites which are ubiquitous and therefore have no value for differentiating one organism, and its phylogeny, from another. Unfortunately, as yet our knowledge of the distribution of extractives is very limited and, though the grosser aspects of the morphology of every named species have been described, the chemical identification of substances produced by these species lags very, very far behind.

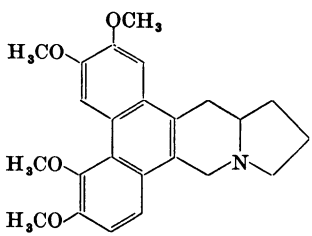
There are a number of reasons why this is so. Firstly, of course, the great majority of named species have never been subjected to a chemical



(VII)
Cryptopleurine



(VIII)
Tylophorine



(IX)
Tylocrebrine

examination of any sort. Secondly, apart from a few commercially important species, what has been done is (of necessity) superficial, even when it has led to complete chemical identification of one or more metabolites. Such factors as seasonal variation, limitation of occurrences to particular organs and so on, enhance the difficulties. Thirdly, of those species that have been examined, many were subjected—necessarily—to tests of limited significance, which leave the results open to such doubt that they are valueless for taxonomic purposes. The so-called "spot tests" for alkaloids are in this category, and there are others. While we may be led to further work by the results of these superficial tests, I believe that the only chemical data, the taxonomic implications of which can be seriously considered, are those in which an unequivocal structural identification has been achieved. Finally, however self-evident it may be, the point must be made that postulation of a phylogenetic relationship between two groups of plants on the basis of chemical data can only be valid when there is available positive evidence in respect of each group. In other words, no conclusion can be drawn from the absence of a particular substance or class of substances. The difference between presence and absence of a substance might be the reflection of profound biochemical and genetical differences or of nothing more than a single gene difference occasioning inability to synthesize or accumulate detectable amounts of the substance.

These introductory remarks may be summed up by stating that, although a detailed knowledge of the chemistry of a wide range of plant species will undoubtedly be of great value in the study of phylogeny, we have not yet reached a stage where we can often argue with any confidence on chemical grounds with the inferences drawn by the orthodox taxonomist from morphological evidence; our data are too limited and too unreliable. The subsequent sections of this Chapter are subject to this overriding reservation.

II. Alkaloids in the Rutaceae

Among those groups of plant extractives which have received considerable attention from the organic chemist are the alkaloids, a structurally variable but widespread group of substances, characterized by the presence in the molecule of more or less basic nitrogen atoms, and not classifiable into any other clear-cut group of natural substances. Present evidence suggests that they occur in detectable amounts in 5–10% of plant species. This, of course, limits their usefulness for taxonomic purposes considerably since, as already pointed out, no conclusion can be drawn from negative data. Among those families known to be relatively rich in species containing alkaloids is the Rutaceae, a family consisting of approximately one hundred and fifty genera comprising more than a thousand species. The alkaloids whose structures have been determined and the species from which they have been isolated are listed in Table I.

The members of the Rutaceae are mostly trees and shrubs, widely distributed in tropical and subtropical habitats, and particularly abundant in Australia and South Africa. The best known genus is *Citrus* and there are also a number of useful commercial timbers. In most species the leaves are gland-dotted and a large proportion of the family is relatively rich in essential oils. Geissman (1962) has directed attention to the wide occurrence of oxygen-alkylated flavonoid compounds in the Rutaceae, describing this as "a notable example of the capacity for a closely allied group of plants to perform a single kind of synthetic reaction". I have drawn attention elsewhere (Price, 1961) to the circumstance that the capacity to add an isopentane unit to a wide variety of molecular types appears to be a biochemical characteristic of the family. In addition to alkaloids and essential oils the Rutaceae is particularly rich in coumarins; further reference to these will be made subsequently.

Engler and Prantl (1931) divide the Rutaceae into seven subfamilies —the Rutoideae, Toddaliodeae, Aurantioideae, Flindersioideae, Dictyolomatoideae, Spathelioideae and Rhabdodendroideae. Of these, the

TABLE I
Occurrence of alkaloids in the Rutaceae

Plant	Alkaloid(s)*	Category†	Reference
Acronychia acidula	Melicopine	A	Boit (1961)
A. baueri	Acronycine (XII)	A	Boit (1964)
	1,3-Dimethoxy-10-methylacridone	A	
	Melicopicine (XI)	A	
	Melicopidine	A	
	Melicopine	A	
	Acronidine	B	
	Acronycidine	B	
	Kokusaginine	B	
	Skimmianine (XIV)	B	
A. haplophylla	1-Hydroxy-2,3-dimethoxy-10-methylacridone	A	Lahey (pers. comm.)
Aegle marmelos	Dictamnine	B	Boit (1961)
	γ-Fagarine	B	
	7-Hydroxy-1-phenyldihydroquinazol-4-one	D	
	Aegeline (XXXIX)	K	
Balfourodendron riedelianium	Evoxanthine	A	Boit (1961); Rapoport and Hiem (1960); Rapoport and Holden (1960)
	Flindersiamine	B	
	Isobalfourodine (XXI)	B	
	Maculosidine	B	
	O-Methyl balfourodinium$^+$ (XIX)	B	
	1-Methyl-2-phenyl-4-quinolone	C	
Boenninghausenia albiflora	Dictamnine	B	Boit (1961)
Boronia ternata	Skimmianine (XIV)	B	Jefferies (pers. comm.)
	1-Acetoxymethyl-2-Propyl-4-quinolone	C	
Casimiroa edulis	Dictamnine	B	Boit (1961); Mechoulam *et al.* (1961); Raman *et al.* (1962)
	γ-Fagarine	B	
	Skimmianine (XIV)	B	
	Casimiroine (XXVI)	C	
	Eduleine (XXIV)	C	
	Casimiroedine (XXXIII)	G	
	NN-Dimethylhistamine	G	
	Zapotidine (XXXII)	G	
	N-Benzoyltyramine	K	
Chloroxylon swietenia	Skimmianine (XIV)	B	Boit (1961)
Choisya ternata	Evoxine	B	Frolova *et al.* (1958)
	Skimmianine (XIV)	B	
Citrus aurantium	Quinoline	C	Boit (1961); Willaman and Schubert (1961)
	Narcotine (XXXV)	H	
	Noradrenaline	K	
	Tryptamine	K	
	Tyramine	K	

TABLE I—*continued*

Plant	Alkaloid(s)*	Category†	Reference
Citrus sinensis	Narcotine (XXXV)	H	Willaman and Schubert (1961)
Cusparia macrocarpa	Evolitrine	B	Rapoport and Hiem (1960); Rapoport and Holden (1960)
Dictamnus albus	Dictamnine	B	Boit (1961)
Dictyoloma incanescens	*NN*-Dimethyl-5-methoxy-tryptamine	K	Boit (1961)
Eriostemon difformis	Maculosidine	B	Jefferies (pers. comm.)
	Skimmianine (XIV)	B	
E. coccineus	Skimmianine (XIV)	B	Jefferies (pers. comm.)
E. thryptomenioides	Maculosidine	B	Jefferies (pers. comm.)
	Skimmianine (XIV)	B	
E. brucei	Maculosidine	B	Jefferies (pers. comm.)
E. tomentellus	Maculosidine	B	Jefferies (pers. comm.)
	Skimmianine (XIV)	B	
Evodia alata	Evoxanthine	A	Boit (1961)
	1-Hydroxy-2,3-dimethoxy-10-methylacridone	A	
	Melicopidine	A	
	1,2,3-Trimethoxy-10-methylacridone	A	
	Evolatine (XV)	B	
	Kokusaginine	B	
E. littoralis	Dictamnine	B	Boit (1961)
	Evolitrine	B	
	Kokusaginine	B	
E. meliaefolia	Berberine	H	Boit (1961)
E. rutaecarpa	Evodiamine	E	Boit (1961)
	Rhetsinine	E	
	Rutaecarpine	E	
E. xanthoxyloides	Evoxanthidine (X)	A	Boit (1961); Ritchie (pers. comm.)
	Evoxanthine	A	
	1-Hydroxy-2,3-dimethoxy-10-methylacridone	A	
	Melicopidine	A	
	Xanthevodine	A	
	Xanthoxoline	A	
	Evodine	B	
	Evoxine	B	
	Evoxoidine	B	
	Kokusaginine	B	
Fagara angolensis	Skimmianine (XIV)	B	Boit (1961)

TABLE I—*continued*

Plant	Alkaloid(s)*	Category†	Reference
Fagara coco	γ-Fagarine	B	Boit (1961); Comin and Deulofeu (1959)
	Skimmianine (XIV)	B	
	Allocryptopine	H	
	Fagarine II (XXXVII)	H	
	N-Methylisocorydine	H	
F. macrophylla (= *Z. macrophyllum*)	Fagaramide (XLI)	K	Willaman and Schubert (1961)
F. mantchurica (= *Z. schinifolium*)	Skimmianine (XIV)	B	Boit (1961)
F. semiarticulata	Chelerythrine (XXXVIII)	H	Scheuer *et al.* (1962)
	Dihydrochelerythrine	H	
F. tingoassuiba (= *Z. tingoassuiba*)	6-Hydroxy-2,3,5-trimethoxy-*NN*-dimethylaporphine+	H	Riggs *et al.* (1961)
F. viridis	Skimmianine (XIV)	B	Boit (1961)
F. xanthoxyloides (= *Z. senegalense*)	Skimmianine (XIV)	B	Boit (1961)
	Fagaramide (XLI)	K	
Flindersia acuminata	Dictamnine	B	Boit (1961); Ritchie (pers. comm.)
	Maculine	B	
F. australis	Flindersine (XX)	B	Boit (1961); Ritchie (pers. comm.)
F. bennettiana	Flindersiamine	B	Boit (1961); Ritchie (pers. comm.)
	Maculine	B	
	Skimmianine (XIV)	B	
F. bourjotiana	Flindersiamine	B	Boit (1961); Ritchie (pers. comm.)
	Skimmianine (XIV)	B	
F. collina	Flindersiamine	B	Boit (1961); Ritchie (pers. comm.)
	Kokusaginine	B	
F. dissosperma	Dictamnine	B	Boit (1961); Ritchie (pers. comm.)
	Flindersiamine	B	
	Maculine	B	
	Skimmianine (XIV)	B	
F. ifflaiana	Ifflaiamine	B	Ritchie (pers. comm.)
F. laevicarpa	Skimmianine (XIV)	B	Ritchie (pers. comm.)
F. maculosa	Dictamnine	B	Boit (1961); Ritchie (pers. comm.)
	Flindersiamine	B	
	Kokusaginine	B	
	Maculine	B	
	Maculosidine	B	
	Maculosine	B	
F. pimenteliana	Dictamnine	B	Ritchie (pers. comm.)

TABLE I—*continued*

Plant	Alkaloid(s)*	Category†	Reference
Flindersia pubescens	Dictamnine	B	Ritchie
	Flindersiamine	B	(pers. comm.)
	Kokusaginine	B	
	Maculosidine	B	
	Skimmianine (XIV)	B	
F. schottiana	Kokusaginine	B	Ritchie
	Maculine	B	(pers. comm.)
F. xanthoxyla	Flindersiamine	B	Boit (1961); Ritchie
	Maculine	B	(pers. comm.)
Galipea officinalis	Cuspareine	C	Boit (1961)
	Cusparine (XXV)	C	
	Galipine	C	
	Galipoline	C	
	4-Methoxy-2-pentylquinoline	C	
	1-Methyl-2-quinolone	C	
	2-Pentylquinoline (XXVII)	C	
	Quinaldine	C	
	Quinoline	C	
Gleznowia verrucosa	Skimmianine (XIV)	B	Jefferies (pers. comm.)
Glycosmis arborea	Arborinine	A	Boit (1961);
	Kokusaginine	B	Pakrashi *et al.*
	Skimmianine (XIV)	B	(1961); Banerjee
	Arborine (XXVIII)	D	*et al.* (1961)
Haplophyllum bucharicum	Skimmianine (XIV)	B	Boit (1961)
H. dubium	Dubamine	B	Boit (1961);
	Dubinidine	B	Men'shikov (1961)
H. foliosum	Skimmianine (XIV)	B	Boit (1961)
H. pedicellatum	γ-Fagarine	B	Boit (1961)
	Skimmianine (XIV)	B	
H. perforatum	Skimmianine (XIV)	B	Boit (1961)
Hortia arborea	Dictamnine	B	Boit (1961);
	γ-Fagarine	B	Pachter *et al.*
	Skimmianine (XIV)	B	(1960, 1961)
	Rutaecarpine	E	
H. brazileana	Hortiacine (XXIX)	E	Boit (1961)
	Hortiamine	E	
Lunasia amara	Hydroxylunacridine	B	Boit (1961)
	Hydroxylunacrine	B	
	Hydroxylunidine	B	
	Hydroxylunine	B	
	Kokusaginine	B	
	Lunacridine (XXIII)	B	
	Lunacrine	B	
	Lunine (XVI)	B	

TABLE I—*continued*

Plant	Alkaloid(s)*	Category†	Reference
Lunasia amara	Skimmianine (XIV)	B	
	Lunamarine	C	
	4-Methoxy-2-(3′,4′-methylene-dioxyphenyl)-quinoline	C	
	4-Methoxy-2-phenylquinoline	C	
	Eduleine (XXIV)	C	
L. quercifolia	Lunacrine	B	Boit (1961)
	Lunasine (XVIII)	B	
	Lunine (XVI)	B	
	Eduleine (XXIV)	C	
Medicosma cunninghamii	Medicosmine	B	Boit (1961)
Melicope fareana	Melicopicine (XI)	A	Boit (1961)
	Melicopidine	A	
	Melicopine	A	
	Acronycidine	B	
	Skimmianine	B	
Orixa japonica	Evolitrine	B	Boit (1961)
	Kokusagine	B	
	Kokusaginine	B	
	Orixine (XXII)	B	
	Skimmianine (XIV)	B	
Pentaceras australis	Canthinone	F	Boit (1961)
	5-Methoxycanthinone (XXX)	F	
	4-Methylthiocanthinone	F	
Phebalium nudum	Dictamnine	B	Boit (1961)
	Evolitrine	B	
	γ-Fagarine	B	
	Kokusaginine	B	
Phellodendron amurense	Berberine	H	Boit (1961); Kunitomo (1962)‡
	Jatrorrhizine	H	
	Magnoflorine+	H	
	Menisperine+	H	
	Palmatine	H	
	Phellodendrine+	H	
	Candicine (XL)	K	
P. lavallei	Berberine	H	Boit (1961)
P. wilsonii	Berberine	H	Boit (1961)
Pilocarpus heterophyllus	Pilocarpine (XXXI)	G	Boit (1961)
P. jaborandi	Isopilocarpine	G	Boit (1961)
	Pilocarpidine	G	
	Pilocarpine (XXXI)	G	
P. microphyllus	Isopilocarpine	G	Boit (1961)
	Pilocarpine (XXXI)	G	
	Pilosine	G	
P. pennatifolius	Pilocarpine (XXXI)	G	Boit (1961)
P. racemosus	Pilocarpine (XXXI)	G	Boit (1961)

TABLE I—*continued*

Plant	Alkaloid(s)*	Category†	Reference
Platydesma campanulata	Evolitrine	B	Scheuer (pers. comm.)
	Kokusagenine	B	
	6-Methoxydictamnine	B	
	Platydesmine	B	
	Pilokeanine	B	
	1,2-Dimethyl-4-quinolone	C	
Ruta graveolens	Kokusaginine	B	Boit (1961); Arthur and Cheung (1960)
	Skimmianine (XIV)	B	
	Graveoline	C	
Skimmia japonica	Skimmianine (XIV)	B	Boit (1961)
S. laureola	Skimmianine (XIV)	B	Boit (1961)
S. repens	Dictamnine	B	Boit (1961)
Teclea grandifolia	Evoxanthine	A	Boit (1961)
T. sudanica	Flindersiamine	B	Boit (1961)
Toddalia aculeata (= *T. asiatica*)	Berberine	H	Boit (1961)
	Chelerythrine (XXXVIII)	H	
	Dihydrochelerythrine	H	
Vepris bilocularis	Flindersiamine	B	Govindachari and Sundararajan (1961)
	Kokusaginine	B	
	Skimmianine (XIV)	B	
Zanthoxylum ailanthoides	Dictamnine	B	Boit (1961)
	Skimmianine (XIV)	B	
	Laurifoline+ (XXXIV)	H	
	Magnoflorine+	H	
Z. alatum	Dictamnine	B	Boit (1961); Ishii (1961); Ishii and Harada (1961)
	γ-Fagarine	B	
	Skimmianine (XIV)	B	
	NN-Dimethyllaurotetanine+	H	
	Magnoflorine+	H	
Z. avicennae	Avicine	H	Boit (1961)
Z. brachyacanthum	Allocryptopine	H	Boit (1961)
	Chelerythrine (XXXVIII)	H	
	N-Methyl-α-canadine+ (XXXVI)	H	
	N-Methylisocorydine+	H	
Z. clava-herculis (= *Z. americanum*)	Berberine	H	Boit (1961)
	Herclavin	K	
Z. nitidum	Nitidine	H	Boit (1961)
	Oxynitidine	H	
Z. piperitum	Magnoflorine+	H	Boit (1961)
Z. rhetsa	Skimmianine (XIV)	B	Boit (1961)
	Rhetsine	E	
	Rhetsinine	E	
	Chelerythrine (XXXVIII)	H	
Z. suberosum	Canthinone	F	Boit (1961)

* Only the formulae of the illustrative examples given in the text are indicated.

† The structural categories to which the alkaloids belong are indicated as follows. A, acridines; B, furoquinolines; C, quinolines; D, quinazolines; E, indoloquinazolines; F, canthinones; G, imidazoles; H, benzylisoquinolines; K, amines or amides.

the three last are monogeneric; no information is available concerning the occurrence of alkaloids in Spathelioideae or Rhabdodendroideae. The bulk of the genera and species in the family are found in the Rutoideae, Toddalioideae and Aurantioideae; Flindersioideae consists of two genera only. According to Willis (1960), the groups of which the family is made up differ considerably among themselves and several of them were formerly regarded as independent families. They are considered to be closely allied to the Meliaceae, Burseraceae, Simarubaceae, Zygophyllaceae and Cneoraceae. These relationships are set out in Fig. 1. Before considering them further let us examine the alkaloid pattern in the family.

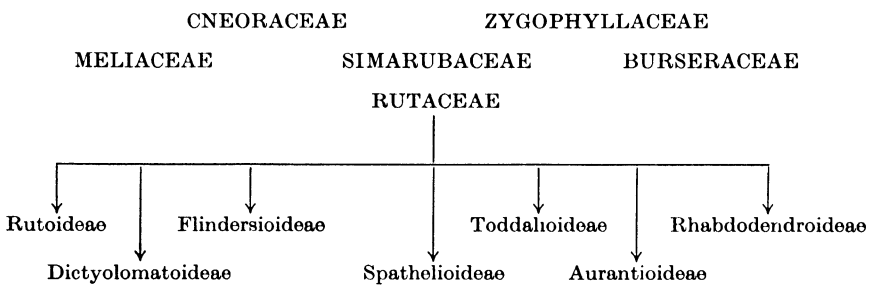
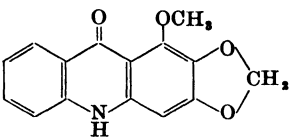


FIG. 1. Rutaceae and related families.

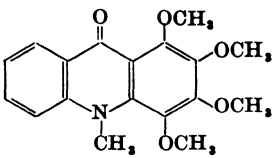
From a purely chemical point of view the Rutaceae is a fascinating group and in respect of the alkaloids it produces is probably the most versatile of all the families of higher plants. For our purposes the alkaloids may be considered as representing nine structural classes—acridines (X–XIII), furoquinolines (XIV–XXIII), quinolines (XXIV–XXVII), quinazolines (XXVIII), indoloquinazolines (XXIX), canthinones (XXX), imidazoles (XXXI–XXXIII), benzylisoquinolines (XXXIV–XXXVIII) and aromatic amines or amides (XXXIX–XLI).* Since on biogenetic grounds the furoquinolines may reasonably be regarded as derivatives of 4-hydroxy-3-prenylquinol-2-one (Birch, 1956; Price, 1961) I have included with them the isopropyldihydrofuroquinolines (for example, lunine (XVI), balfourodine (XVII)), the corresponding quaternary salts (lunasine (XVIII) and *O*-methylbalfourodinium salts (XIX)), the pyrano- and dihydropyrano-quinoline derivatives flindersine (XX) and isobalfourodine (XXI) and the bicyclic 3-prenylquinolines exempli-

* There are also two occurrences of a long-chain isobutylamide, neoherculin, the isobutylamide of dodeca-2,6,8,10-tetraenoic acid, in *Zanthoxylum clava-herculis* and *Z. piperitum*. There is some doubt concerning a second from *Z. piperitum*, sanshoamide, reported to be the 2-hydroxyisobutylamide of dodeca-2,4,8,10-tetraenoic acid.

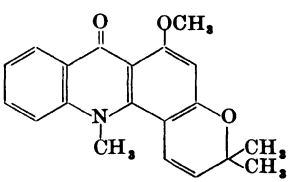
fied by orixine (XXII) and lunacridine (XXIII). The quinoline alkaloids, other than the furoquinoline group are mainly 2-phenyl- or 2-phenylethyl-quinoline derivatives with some simpler representatives. The benzylisoquinolines fall into two main subgroups, quaternary aporphines and protoberberines of derivatives of them, namely benzophenanthridines, protopines and one phthalide isoquinoline, narcotine (XXXV). This wide range of alkaloids has been isolated from rutaceous species representing thirty-six genera from five of the Engler and Prantl subfamilies.



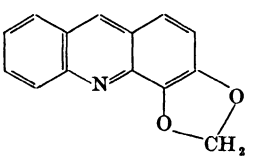
(X)
Evoxanthidine



(XI)
Melicopicine

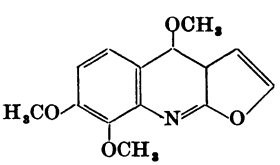


(XII)
Acronycine

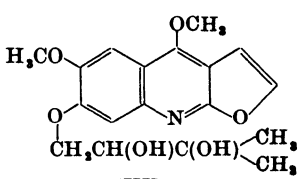


(XIII)
Dubamine

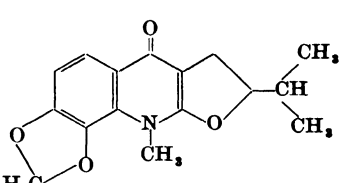
Acridine alkaloids



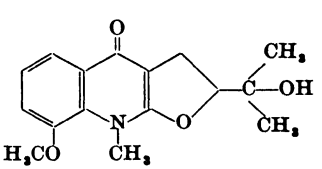
(XIV)
Skimmianine



(XV)
Evolatine

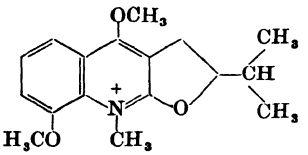


(XVI)
Lunine

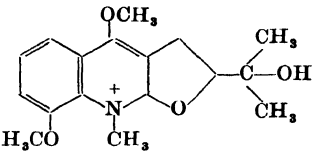


(XVII)
Balfourodine

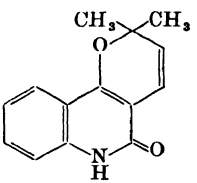
Furoquinoline alkaloids



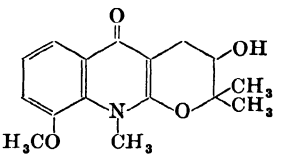
(XVIII)
Lunasine



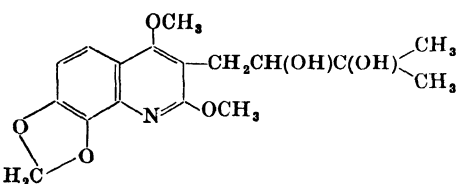
(XIX)
O-Methylbalfourodinium$^+$



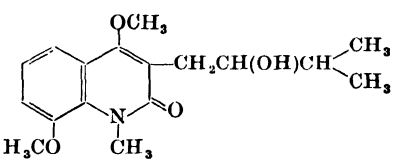
(XX)
Flindersine



(XXI)
Isobalfourodine

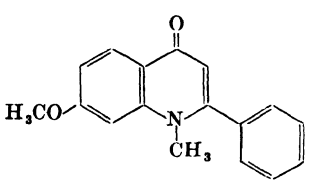


(XXII)
Orixine

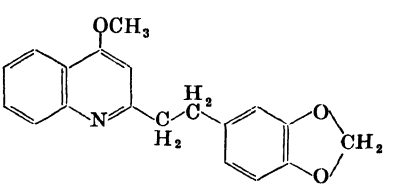


(XXIII)
Lunacridine

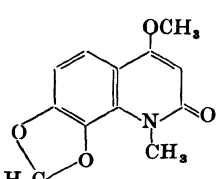
Furoquinoline and related alkaloids



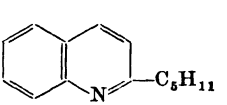
(XXIV)
Eduleine



(XXV)
Cusparine



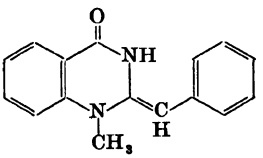
(XXVI)
Casimiroine



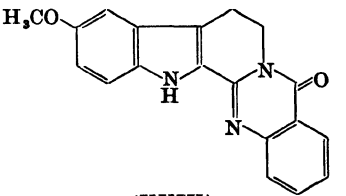
(XXVII)
2-Pentylquinoline

Quinoline alkaloids

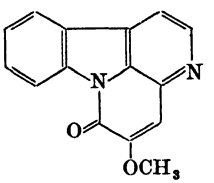
From a biogenetic point of view the number of classes can be reduced, but even so the Rutaceae presents an impressive picture of structural versatility. The acridines, quinolines of various types including the



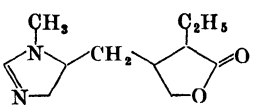
(XXVIII)
Arborine



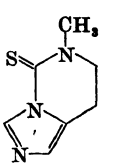
(XXIX)
Hortiacine



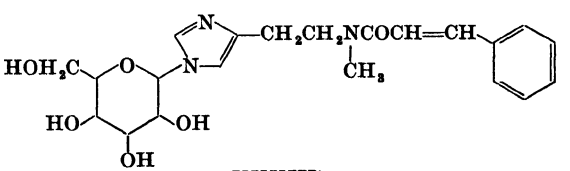
(XXX)
5-Methoxycanthin-6-one
Quinazoline, indoloquinazoline and canthinone alkaloids



(XXXI)
Pilocarpine



(XXXII)
Zapotidine

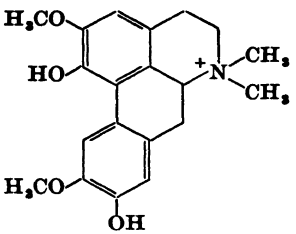


(XXXIII)
Casimiroedine
Imidazole alkaloids

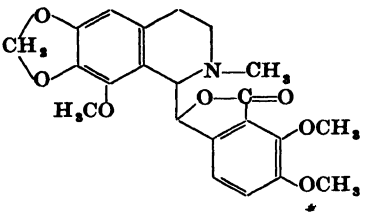
furoquinolines, and the quinazolines are considered to be a biogenetically homogeneous group based formally on the "anthranilic acid unit" common to all of them.* The anthranilic acid unit is also evident in the architecture of the indoloquinazolines.

The distribution as we know it, of these various classes of alkaloids

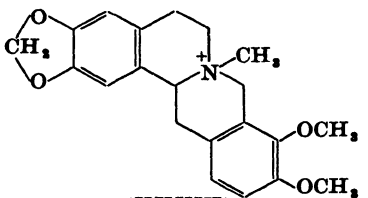
* Methyl anthranilate and *N*-methyl methyl anthranilate are present in certain rutaceous essential oils.



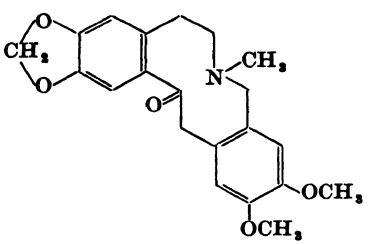
(XXXIV)
Laurifoline



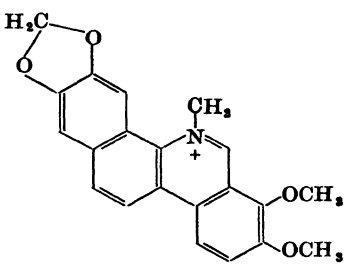
(XXXV)
Narcotine



(XXXVI)
N-Methylcanadine+

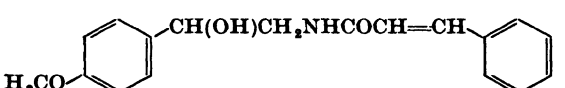


(XXXVII)
Fagarine II

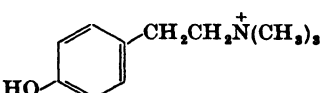


(XXXVIII)
Chelerythrine

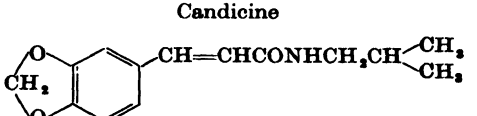
Benzylisoquinoline alkaloids



(XXXIX)
Aegeline



(XL)
Candicine



(XLI)
Fagaramide
Amines and amides

TABLE II

Distribution of alkaloids in the Rutaceae

Subfamily	Genus	Class of compound									
		Acridines	Furoquinolines	Quinolines	Quinazolines	Indolo-quinazolines	Canthinones	Imidazoles	Benzyliso-quinolines	Amines or Amides	
I. RUTOIDEAE	*Evodia*	+	+			+			+		
	Fagara		+						+	+	
	Melicope	+	+								
	Orixa		+								
	Pentaceras						+				
	Zanthoxylum		+			+	+		+	+	
	Lunasia		+	+							
	Choisya		+								
	Medicosma		+								
	Platydesma		+	+							
	Boenninghausenia		+								
	Ruta		+	+							
	Haplophyllum	+	+								
	Dictamnus		+								
	Boronia		+	+							
	Eriostemon		+								
	Geleznowia		+								
	Phebalium		+								
	Pilocarpus							+			
	Cusparia		+	+							
	Galipea			+							
II. DICTYOLOMATOIDEAE	*Dictyoloma*									+	
III. FLINDERSIOIDEAE	*Flindersia*		+								
	Chloroxylon		+								
V. TODDALIOIDEAE	*Phellodendron*								+	+	
	Balfourodendron	+	+	+							
	Acronychia	+	+								
	Casimiroa		+	+				+		+	
	Hortia		+			+					
	Skimmia		+								
	Toddalia								+		
	Vepris		+								
	Teclea	+	+								
VI. AURANTIOIDEAE	*Glycosmis*	+	+		+						
	Aegle		+		+					+	
	Citrus			+					+	+	

throughout the family is shown in Table II. The striking feature is that furoquinolines occur in twenty-nine genera of the thirty-six in which alkaloids have been identified, and these genera are distributed throughout four of the five subfamilies in which the occurrence of alkaloids has been reported. Moreover, neither the furoquinolines nor the biogenetically related acridines and indoloquinazolines have been isolated from any other natural source, while with one or two exceptions the simple quinoline derivatives are also confined to the Rutaceae. There is one other known occurrence of a canthinone (cf. XXX) which will be referred to later, but the small group of quinazoline alkaloids is found in five other families including the closely related Zygophyllaceae.

In the higher plants the imidazoles are confined to the Rutaceae if one excludes the wide occurrence of histamine, the decarboxylation product of histidine.

Thus of our nine arbitrary classes of alkaloids in the Rutaceae, four are found in this family exclusively, while the occurrence outside the family of two others is very limited. But the remarkable feature of the alkaloid picture in the Rutaceae is not so much this exclusiveness, nor the diversity of biogenetic pathways followed, nor even the fact that certain genera are capable of producing alkaloids of several different classes—it is that an individual plant may do just this. In *Casimiroa edulis* for example, as well as the biogenetically related quinolines and furoquinolines, are found imidazoles and the amide *N*-benzyltyramine. From *Zanthoxylum ailanthoides*, Tomita and Ishii (1958) isolated the furoquinolines dictamnine and skimmianine (XIV) and the quaternary aporphines magnoflorine and laurifoline (XXXIV), while Chatterjee, Bose and Ghosh (1959) isolated chelerythrine (XXXVIII) and three indoloquinazolines from *Zanthoxylum rhetsa*. Considered in relation to the complexity of the overall metabolic picture this versatility is perhaps not so surprising, but it does distinguish the Rutaceae from other families and as such warrants the emphasis I have placed on it.

III. Taxonomic Implications

What does this alkaloid versatility mean, and has it any significance from the taxonomic point of view? It might well be argued that the synthesis of such a heterogeneous range of non-peptide nitrogenous compounds is evidence that the Rutaceae is a phylogenetically complex family and such an argument might be consistent with the statement of Willis (1960) that the groups of which the Rutaceae is made up differ among themselves to the extent that some have been regarded as independent families. Moore (1936) contends that, if floral anatomy is taken

as a criterion of relationship, the Rutoideae, at least, are a highly complex subfamily phylogenetically and that "the present classification of the Rutaceae is one which runs directly across the lines of specialization in floral anatomy". Despite this assessment, I believe that the picture presented by the alkaloids of those genera in which they have been identified suggests that the major subfamilies of the Rutaceae represent a highly homogeneous group. This rests primarily on the ubiquity of the furoquinolines which, as I have pointed out, are found throughout four of the five subfamilies for which data are available and which have not been reported from any other source. The acridines have been isolated from three of these subfamilies, and from no other source; quinolines from all four subfamilies; and benzylisoquinolines from three, as shown in Table II. The benzylisoquinolines are a particularly widespread group of alkaloids, in fact the most frequently encountered of all, but none has been reported from those families morphologically related to the Rutaceae—the Meliaceae, Burseraceae, Simarubaceae, Zygophyllaceae and Cneoraceae. There are no records of alkaloids occurring in the Cneoraceae or Burseraceae; it seems possible there may be some in the Meliaceae but no authentic structural identification has been made. The only reliable data on the Zygophyllaceae concern the quinazoline and harman alkaloids of *Peganum harmala* which Hutchinson (1926), on morphological grounds, originally placed in the Rutaceae though later transferring it to the Zygophyllaceae. In the Simarubaceae there is likewise only one authentic identification, that of 4,5-dimethoxycanthin-6-one in *Picrasma ailanthoides* which constitutes the third occurrence of canthinones referred to previously (Inamoto *et al.*, 1961).

Further support for the view that the Rutaceae is a distinct and homogeneous group is provided by its essential oils and coumarins. Reference has already been made to the common occurrence of essential oils in the Rutaceae; they are present in at least four subfamilies, Aurantioideae, Rutoideae, Toddalioideae and Flindersioideae. More detailed information is available concerning the coumarins. Karrer (1958) reports the occurrence of coumarins in representatives of 77 genera from 27 families. Two unpublished identifications which have been brought to my notice bring these figures to 79 genera from 27 families. Of these 79 genera, 18 belong to the Umbelliferae and 18 to the Rutaceae, the remainder being spread more or less sparsely over the remaining 25 families. Within the Rutaceae coumarins are distributed throughout the four subfamilies Aurantioideae, Rutoideae, Toddalioideae and Flindersioideae, as shown in Table III. On the other hand, though it is negative evidence, there is not one report of the isolation of a coumarin from the Meliaceae, Burseraceae, Simarubaceae, Zygophyllaceae or Cneoraceae.

Returning to consideration of the alkaloids let us look briefly below the family and subfamily level at what information is available concerning variation within genera. In many instances, as would be expected, either very few species in a genus have been examined, or very few of the species contain alkaloids in amounts sufficient for chemical examination. There are, however, some genera for which the evidence provided by alkaloid structures suggests a considerable degree of uniformity; these are *Eriostemon* (five species), *Flindersia* (thirteen species), *Haplophyllum*

TABLE III

Occurrence of coumarins in the Rutaceae

Rutoideae	Toddalioideae
*Eriostemon**	*Casimiroa*
Fagara	*Halfordia*
Geijera	*Skimmia*
Melicope	*Toddalia*
Phebalium	
Ruta	Aurantioideae
Zanthoxylum	*Aegle*
	Citrus
Flindersioideae	*Luvunga*
Chloroxylon	*Micromelum**
Flindersia	*Murraya*

* Jefferies (personal communication) reports the structure of the coumarin bruceol from *Eriostemon brucei*. The identification of osthol in *Micromelum minutum* is an unpublished report from the author's laboratory.

(five species), and *Pilocarpus* (five species). But this is not always so; the five *Evodia* species for which data are available differ appreciably among themselves and this variability is even more marked within the genera *Fagara* and *Zanthoxylum*. There is considerable nomenclatural confusion between these two genera, a number of *Fagara* species being alternatively named as *Zanthoxylum* and vice versa. To ascertain whether the alkaloid pattern might throw light on this situation the data are collected in Table IV. Excluding *Z. suberosum*, which stands apart, the twenty species for which data are available fall into two groups according to whether furoquinolines are or are not present. However, neither generically nor geographically is either of these groups of species homogeneous and it should certainly be of interest to acquire more chemical data concerning them. It may be pointed out that because of complete correspondence in the constituents of the two species, based on the examination of several samples from different areas, Cannon *et al.* (1953) suggested that *Z. brachyacanthum* and *Z. veneficum* are identical, and it was ascertained

subsequently that there is considerable doubt from the morphological point of view as to whether the two are distinct species.

The subfamily Rhabdodendroideae of Engler and Prantl (1931) appears to be even more confused than the genera *Fagara* and *Zanthoxylum*. The only genus, *Rhabdodendron*, is placed by Willis (1960) in the Rubiaceae, and by Record and Hess (1943) in the Phytolaccaceae. It is possibly a mixed genus with species belonging to each of the three families (Dadswell, personal communication). To my knowledge there are no chemical data whatsoever available for this genus which would seem to

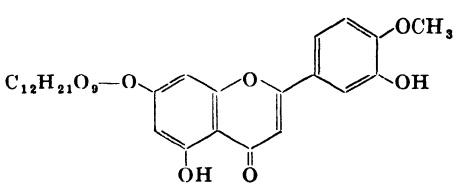
TABLE IV

Occurrence of alkaloids in the genera *Fagara* and *Zanthoxylum*

Species	Class of compound				
	Furoquinolines	Indolo-quinazolines	Canthinones	Benzyliso-quinolines	Amides
Z. avicennae				+	
Z. brachyacanthum				+	
Z. clava-herculis				+	+
Z. nitidum				+	
Z. piperitum				+	+
Z. veneficum				+	
F. semiarticulata				+	
F. tingoassuiba (= *Z. tingoassuiba*)				+	
F. macrophylla (= *Z. macrophyllum*)				+(?)	+
F. parvifolia				+(?)	
Z. ochroxylum				+(?)	
F. angolensis	+			+(?)	
F. xanthoxyloides (= *Z. senegalense*)	+			+(?)	
F. coco (= *Z. coco*)	+			+	
Z. ailanthoides	+			+	
Z. alatum	+			+	
F. mantchurica (= *Z. schinifolium*)	+				
F. viridis	+				
Z. rhetsa	+	+			
Z. suberosum			+		

provide an excellent opportunity for a chemical-taxonomical study. Dictyolomatoideae, consisting only of the genus *Dictyoloma*, is left in the Rutaceae by Willis but placed in the Simarubaceae by Metcalfe and Chalk (1950). The isolation of a tryptamine derivative from *D. incanescens* has little diagnostic value, all that can be stated is that this is not inconsistent with what we know of the Rutaceae.

I shall conclude with an example in which the chemical data seems more clearcut and useful. The relationship of one other Engler and Prantl subfamily, the Flindersioideae, to the remainder of the Rutaceae has been the subject of some difference of opinion. Hutchinson places it in the Meliaceae. Other authorities agree that it is out of place in the Meliaceae but Dadswell (quoted by Metcalfe and Chalk (1950)) considers it is not typical of either the Rutaceae or the Meliaceae and favours a separate family, the Flindersiaceae. The Flindersioideae contains two genera, *Flindersia* and *Chloroxylon*, and some chemical data are available for each of these, *Flindersia* possibly having been more thoroughly studied than any other genus in the Rutaceae. The one *Chloroxylon* species examined, and thirteen of a total of fourteen *Flindersia* species contain furoquinolines, no alkaloids being found in *F. brayleana*. It will be recalled that not only have furoquinolines not been isolated from any source other than the Rutaceae, but there is as yet not one definite identification of an alkaloid from the Meliaceae. Coumarins, which have been found in several genera in each of the rutaceous subfamilies Rutoideae, Toddalioideae and Aurantioideae, have been isolated from the one *Chloroxylon* species examined and from seven of the *Flindersia* species. As I have pointed out there is not one record of the isolation of a coumarin from the Meliaceae. Finally, several *Flindersia* species have yielded the flavanone glycoside hesperidin (XLII) a characteristic metabolite of *Citrus* species, also reported from *Zanthoxylum* but not, I think, from outside the Rutaceae.



(XLII)
Hesperidin
(Hesperetin 7-rhamnoglucoside)

I would not care to suggest whether the Flindersioideae should be designated a family or a subfamily. However, in the light of the evidence advanced, I do feel confident that the Flindersioideae, Aurantioideae,

Toddalioideae and Rutoideae, represent a homogeneous group distinct from the Meliaceae and other associated families. The only species of *Flindersia* diverging from the type is *F. brayleana*, which is being re-examined by Ritchie and his collaborators to check the absence of alkaloids, but which does contain coumarins.

IV. Conclusions

In conclusion, I would emphasize once again that useful as chemical data could be and will be in the study of plant relationships and evolution the data available at present are too limited and too unreliable to enable us to draw any far-reaching conclusions. The limitations of the data are exemplified by the preceding account of the distribution of alkaloids in the Rutaceae for which reliable information is available for only eighty-nine species, representing thirty-six genera out of over one thousand species belonging to one hundred and fifty genera. Nevertheless these data do imply that the four principal subfamilies of the Rutaceae constitute a homogeneous group and that the subfamily Flindersioideae has closer phylogenetic relations with the Aurantioideae, Toddalioideae and Rutoideae than with the Meliaceae.

Addendum

Since this chapter was written, further relevant data have been reported. The versatility of the Rutaceae in respect of the classes of alkaloids produced by members of the family is enhanced by the recognition of a further structural type, an oxazole alkaloid, from *Halfordia scleroxyla* (Crow and Hodgkin, 1963). On the other hand, the ubiquity of the furoquinoline alkaloids is further supported by the isolation of skimmianine (XIV) from two additional genera, Murraya (*M. omphalocarpa*) and Poncirus (*P. trifoliata*); see "Annual Index of the Reports on Plant Chemistry in 1959", ed. by T. Fariyone, 1962.

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CHAPTER 16

The Distribution of Sulphur Compounds

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I. Introduction

Sulphur is an indispensable element for all plants and enters the cellular system largely in the form of inorganic sulphate, which is subsequently reduced and transformed into a great variety of organic sulphur compounds. The sulphur requirements of plants have been thoroughly dealt with in numerous plant physiological treatises, but far less attention has been given to a systematic description of the type and distribution of the organic sulphur compounds encountered in the plant kingdom. Little is known also about the enzymic reduction of sulphate (Wilson, 1962), its subsequent incorporation into organic molecules and the metabolic pathways of the latter in green plants. However, it is beyond the scope of the present chapter to discuss these dynamic aspects, important though they are. The object will be to survey the more important organic sulphur compounds encountered in plants and, where possible, to indicate the taxonomic significance which may be attributed to such constituents. Emphasis will be placed on higher plants, but reference will be

made to other plants and micro-organisms where it is relevant to the discussion.

It should be remembered that comparative studies of the distribution within the plant kingdom of a certain type of compound can furnish nothing more than a first approximation to a chemical classification of living species. There can hardly be any doubt that a detailed insight into the individual enzymic reactions operating in the anabolism and catabolism of compounds in living cells will eventually provide a pattern of "chemical individuality" far more specific than that attainable solely from structural studies. Hence, dynamic biochemistry will always be of the utmost importance for the study of taxonomy and phylogeny. For example, it is quite conceivable that a certain compound present in different plant species arises through entirely different pathways, and hence, on the structural level, can contribute little to our understanding of the biological relationship of the taxa concerned. It is equally obvious, however, that knowledge of the molecular architecture of the plant constituent in question is usually a prerequisite for studies of its formation and degradation in living cells.

The chemistry of natural products can offer initial assistance to subsequent and undoubtedly more informative biochemical studies and an investigation of the chemical type and botanical distribution of a given class of compounds may contribute significantly to the task of classifying living species. Even the mere knowledge of the distribution pattern of a given compound, if critically evaluated and presented with due consideration of evidence provided by entirely different approaches, may frequently give considerable help in taxonomical problems. The organic sulphur compounds in plants provide an example.

The sulphur-containing protein amino acids, as well as coenzymes and vitamins having sulphur in their molecules, are of decisive importance in life-controlling processes, but provide little help in taxonomic studies because of their ubiquitous occurrence. In the following discussion emphasis will be placed on sulphur compounds that are not of universal distribution in living matter. A vast number of such organic sulphur compounds of great diversity with regard to chemical structure and biological function are known to occur in the plant kingdom. No efforts will be made here to give a complete coverage of these. Instead it is intended, through selected examples, to indicate the structural types represented in organic sulphur compounds and thereby to draw attention to a fascinating field of research where so many problems remain to be solved. Parallel with this outline, an attempt will be made, whenever possible, to comment on the possible taxonomic significance of the individual constituents discussed.

Apart from special review articles on selected classes of sulphur-containing plant constituents, a few more general surveys have appeared within the last few years (Young and Maw, 1958; Kjær, 1958; Challenger, 1959).

II. Thiols, Sulphides and Disulphides

All living matter contains a number of organic sulphur-compounds, equally important for the maintenance of the steady state in living cells as for cell division and growth. The sulphur amino acids cysteine and methionine have been long since recognized as essential entities of most proteins, including enzymes, and the simple peptide glutathione appears to be of rather universal distribution and considerable biochemical interest. Ergothioneine is another widely distributed sulphur-containing component, the existence of which in plants now seems reasonably well established (Melville, 1959). Furthermore, sulphur appears in several coenzymes such as thiamine pyrophosphate (cocarboxylase), coenzyme A, α-lipoic acid and biotin, all possessing vital functions in metabolism.

The ubiquitous distribution of the sulphur compounds mentioned above indicates their fundamental importance in basic life processes. From a chemotaxonomic viewpoint, however, their universal occurrence renders them less important.

A common characteristic of this series of biochemical key compounds is that the sulphur is present in the lowest oxidation step. They all contain thiol- or sulphide-groupings essential for the diverse biological functions. In addition, however, several other organic compounds containing sulphur in completely reduced form have been specifically reported from plant sources. These include thiols and their oxidation products (disulphides) as well as linear or cyclic sulphides and sulphonium compounds. A brief survey of these secondary sulphur-constituents of plants is presented below.

A. Thiols in Higher Plants

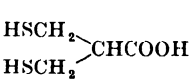
Most thiols are readily oxidized to disulphides, even by atmospheric oxygen. In addition, monofunctional thiols of low molecular weight are volatile substances, properties which often render their isolation somewhat difficult. Such thiols possess an obnoxious smell revealing their presence or formation in plant tissue. It appears that most plant thiols are not present as such in living cells, but instead arise as a result of enzymic or chemical cleavage from various precursors.

Several common cruciferous plants, such as cabbage, turnip and radish, are often associated with odoriferous components having a distinct "sulphur character". Thiols may, at least in part, be responsible for this smell.

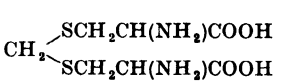
Nakamura (1925) proved that methanethiol was present in root material of *Raphanus sativus* L. It seems likely that in this case the thiol derives from a thioglucoside (deoxo-glucoraphenin, cf. Table I) which, according to unpublished results from the author's laboratory, is present in radish roots and gives rise to the production of methanethiol under the influence of acid and, possibly, enzymes. Koolhaas (1931) reported that leaves of species of the genus *Lasianthus* (Rubiaceae) were another source of methanethiol, whilst Sutherland (1947) presented evidence for its presence in *Coprosma foetidissima* Forst. of the same family. Nothing is known about the possible precursors in the last two species.

1-Propanethiol has been identified as a component present in disintegrated onions (*Allium cepa* L.) (Challenger and Greenwood, 1949) but its origin is uncertain. It may conceivably arise from enzymic transformations of *S*-propylcysteine sulphoxide which is an accepted constituent of *A. cepa* (Virtanen and Matikkala, 1959), or the corresponding sulphide which has not yet been observed in onions. Jansen (1948) reported the presence in *Asparagus* of 3,3'-dimercaptoisobutyric acid (I), so far a structurally unique plant constituent. This constituent is apparently not responsible for the readily detectable quantities of methanethiol which appear in human urine after eating asparagus.

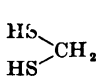
In this connection the long known formation of a "sulphur-like smell" from various species of the family *Mimosaceae* (Czapek, 1921; Warburg, 1923) should be noted. Recently, Gmelin *et al.* (1957) discovered that seeds of *Albizzia lophantha* Benth. of this family contain an enzyme which acts on djenkolic acid (II) present in the seeds, to give pyruvic acid, ammonia and a labile, garlic-smelling compound, presumably the labile compound methanedithiol (III). The distribution of this enzyme and substrate in the plant kingdom is as yet unknown.



(I)
Dimercaptoisobutyric acid



(II)
Djenkolic acid



(III)
Methanedithiol

The limited number of thiols reported so far in higher plants renders this class of natural products of little value for taxonomic purposes. It is quite likely, however, that the ability to produce mercaptans by enzymic

fission of suitable precursors is a highly specific property, characteristic for certain taxa. Further studies of the distribution of thiol-producing plants must therefore be awaited with considerable interest.

B. Sulphides and Sulphonium Compounds

A large number of sulphides, both aliphatic and cyclic, occur in plants. Several of these, such as the antibiotics penicillin, gliotoxin, bacitracin and others, are produced by micro-organisms and will not be discussed here. Others, such as the protein amino acid methionine and the sulphur-containing coenzymes, also fall outside the present discussion. A number of other plant amino acids with one or more sulphidic functions will be treated later (p. 459). The number of known plant constituents with thiophen rings is rapidly increasing. These cyclic sulphides are biogenetically closely related with acetylenes and hence should rather be treated in connection with this class of plant products (cf. Challenger, 1959, see Chapter 9).

The simplest sulphide, dimethyl sulphide, has been repeatedly reported from plant sources, though always as a product of enzymic or chemical degradation of non-volatile precursors. Thus, various marine algae readily produce dimethyl sulphide on exposure to air or alkali. Challenger and Simpson (1948) isolated dimethyl-β-propiothetin (IV) from the red alga *Polysiphonia fastigiata* and showed this sulphonium compound to be a likely precursor of the evolved dimethyl sulphide. Cantoni and Anderson (1956) later demonstrated the presence in these algae of an enzyme cleaving the thetin (IV) to dimethyl sulphide and acrylic acid. The same thetin was proved to be present in the green alga *Enteromorpha intestinalis*, and analogous sulphonium compounds have been found in *Acrosiphonia centralis*, *Ulva lactua*, *Enteromorpha compressa*, *Pelvetia caniculata*, *Halidrys siliquosa*, *Cladophora rupestris* and *Ceramium rubrum*, as well as in the freshwater algae *Oedogorium* spp., *Ulothrix* spp., and *Microspora amoena*. Other species, *Ascophyllum nodosum*, *Laminaria digitata* and *Corallina officinalis*, gave no dimethyl sulphide on alkali treatment (Challenger *et al.*, 1957).

It is interesting that asparagus, various species of *Equisetum*, and the fronds and leaves of *Pteridium aquilinum*, evolve dimethyl sulphide on hot alkali treatment (Bywood *et al.*, 1951), suggesting that they contain sulphonium compounds. In the case of asparagus, *S*-methylmethionine (V) was found to be responsible for the production of dimethyl sulphide (Challenger and Hayward, 1954). This sulphonium compound, first isolated from cabbage juice but also shown to be present in parsley, lettuce and turnip greens, seems to be of rather wide natural distribution

(McRorie *et al.*, 1954) and in many cases may explain the observed formation of dimethyl sulphide on treating plant materials with hot alkali.

$(CH_3)_2\overset{+}{S}CH_2CH_2COOH$

(IV)

Dimethyl-β-propiothetin

$(CH_3)_2\overset{+}{S}CH_2CH_2CH(NH_2)COOH$

(V)

S-Methylmethionine

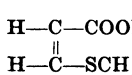
Divinyl sulphide was reported to be a component of the essential oil from *Allium ursinum* L. (Semmler, 1887) but older reports to the effect that garlic oil contains diallyl sulphide could not be confirmed (Semmler, 1892). More recently, however, the latter sulphide has been reported as a constituent of the volatile fraction from the leaves of *Diplotaxis tenuifolia* (Pottiez, 1921) and the roots of *Armoracia lapathifolia* (Guillaume and Shajik, 1951), in both cases accompanied by allyl isothiocyanate. More rigorous characterization is needed, however, before it can be accepted that divinyl and diallyl sulphide as such occur in plants. It is quite possible that unknown precursors are responsible for the production of these sulphides.

A number of methyl sulphides with additional functional groups have been encountered in higher plants. Thus, the essential oil from *Ananas sativus* contains methyl 3-methylthiopropionate (VI) (Haagen-Smit *et al.*, 1945), and the acid moities of the petasolesters B and C (Stoll *et al.*, 1956), as well as the *S*-petasitolides A and B (Novotný *et al.*, 1962), occurring in *Petasites officinalis* Moench, have been identified as *cis*- and *trans*-3-methylthioacrylic acid (VII, VIII). Again, several isothiocyanate-producing glucosides in cruciferous species are characterized by having a methylthio-grouping in their side-chains (cf. Table I). The above observations are, however, far too few and scattered to permit any speculations as to their chemotaxonomic importance. Insight into the biogenesis of the various sulphides should prove profitable for such considerations.

$CH_3SCH_2CH_2COOCH_3$

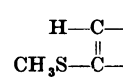
(VI)

Methyl 3-methylthiopropionate



(VII)

cis



(VIII)

trans

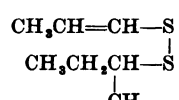
3-Methylthioacrylic acid

C. Di- and Polysulphides

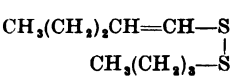
A series of more or less well-defined di- and polysulphides has been reported in the volatile products from higher plants. Undoubtedly, most of these are of secondary origin, formed by disproportionation of com-

pounds themselves derivable from non-volatile precursors in the plant. Nevertheless, the identity of these sulphides may provide a clue to the nature of these latter constituents and a brief discussion of their occurrence will therefore be presented.

By fractional distillation of garlic oil, Semmler (1892) obtained allyl propyl disulphide, diallyl disulphide, diallyl trisulphide, and possibly, diallyl tetrasulphide, all of which, according to present day knowledge, should be regarded as artifacts originating from alliin and other progenitors, possibly of analogous structure. Although there is no obvious botanical relationship, the essential oil of *Ferula foetida* (asafœtida) has been reported as another source of unsaturated disulphides (Semmler, 1891), the major component of which was reported by Mannich and Fresenius (1936) to be an optically active 1-propenyl 2-butyl disulphide (IX). The scattered distribution of disulphides in higher plants also appears from the reported presence in the essential oil of *Agathosma apiculata* Meyer, a South-African species of the family *Rutaceae*, of butyl 1-pentenyl disulphide (X) (Smith and Rivett, 1946). Nothing seems to be known about the origin of such disulphides carrying different substituents. They may well originate from precursors of an entirely new type.



(IX)
1-Propenyl 2-butyl disulphide



(X)
Butyl 1-pentenyl disulphide

III. Non-protein Sulphur Amino Acids of Higher Plants

Besides the protein-building units cysteine, cystine and methionine, a number of interesting sulphur amino acids have been encountered in higher plants, either free or conjugated as γ-glutamyl derivatives. It is noteworthy that all of these are derivatives of cysteine.

S-Methyl-L-cysteine (XI) was discovered by Thompson *et al.* (1956) as a constituent of seeds of *Phaseolus vulgaris*. Its γ-glutamyl derivative was subsequently identified as an important seed component of *Phaseolus lunatus* (Rinderknecht *et al.*, 1958) and *P. vulgaris* (Zacharius *et al.*, 1959). The corresponding (+)-*S*-methyl-L-cysteine sulphoxide (XII) was isolated independently from cabbage juice (Synge and Wood, 1956) and turnip roots (Morris and Thompson, 1956). The occurrence of this amino acid outside the genus *Brassica* is evidenced by its reported isolation from *Allium cepa* and *Capsella bursa-pastoris* (Virtanen and Matikkala,

1959), and from the analytical demonstration of its presence in *Cheiranthus cheiri* L. and *Sinapis alba* L. among a series of cruciferous plants investigated (Synge and Wood, 1956). Recently, Arnold and Thompson (1962) provided evidence for the biological formation in crucifers of (+)-*S*-methyl-L-cysteine sulphoxide by enzymic oxidation of the corresponding thiol *S*-methyl-L-cysteine.

The analogous (+)-*S*-propyl-L-cysteine sulphoxide (XIII) is another established constituent of *Allium cepa* (Virtanen and Matikkala, 1959; Carson and Wong, 1961), structurally related to *S*-propenyl-L-cysteine sulphoxide (XIV) which also occurs in onions, both free (Virtanen and Spåre, 1961) and in γ-glutamyl-bonded form (Virtanen and Matikkala, 1961). There is reasonably good evidence that (XIV) constitutes the

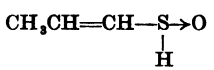
$CH_3SCH_2CH(NH_2)COOH$
(XI)
S-Methyl-L-cysteine

$CH_3SOCH_2CH(NH_2)COOH$
(XII)
(+)-*S*-Methyl-L-cysteine sulphoxide

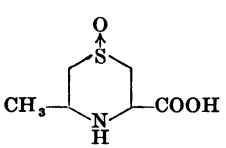
$CH_3CH_2CH_2SOCH_2CH(NH_2)COOH$
(XIII)
(+)-*S*-Propyl-L-cysteine sulphoxide

$CH_3CH{=}CHSOCH_2CH(NH_2)COOH$
(XIV)
S-Propenyl-L-cysteine sulphoxide

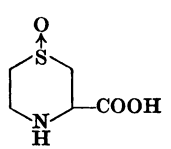
progenitor of the lachrymatory principle in onion (Virtanen and Spåre, 1962) which, according to mass-spectroscopic data, seems to be propenylsulphenic acid (XV) (Moisio *et al.*, 1962). A related amino acid, cycloalliin (XVI), easily produced from (XIV) in alkaline medium, is also believed to be a genuine constituent of onions (Virtanen and Matikkala, 1961). Of interest in this connection is an amino acid, yuanine, isolated from the seaweed *Chondria crassicaulis*, to which the structure (XVII) was assigned (Takemoto, 1960).



(XV)
Propenylsulphenic acid



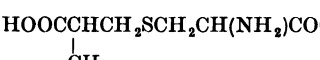
(XVI)
Cycloalliin



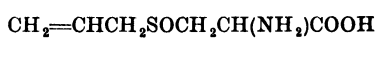
(XVII)
Yuanine

Besides the above described sulphur-containing amino acids, a number of γ-glutamyl derivatives have been found in *Allium cepa*, one of which contains *S*-(β-carboxypropyl)-L-cysteine (XVIII), apparently not occurring in free form (Virtanen and Matikkala, 1960). Before these comprehensive studies on onions, Stoll and Seebeck (1951) contributed significantly to our knowledge of sulphur-containing amino acids in plants

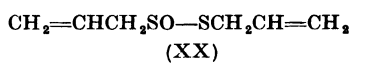
by their discovery of alliin in *Allium sativum* L. (garlic). This amino acid (XIX) readily undergoes enzymic fission to ammonia, pyruvic acid and allicin (XX), the latter being responsible for the typical, but not repulsive, odour of garlic. The unpleasant smell of garlic oil is attributable to diallyl disulphide produced by decomposition of allicin during steam distillation. Besides alliin, garlic contains γ-L-glutamyl-*S*-allyl-L-cysteine, but apparently not the unconjugated deoxo-alliin (Virtanen and Mattila, 1961), and γ-L-glutamyl-*S*-propylcysteine (Virtanen *et al.*, 1962) none of which have been encountered in onions. Other γ-glutamyl derivatives, however, occur in both species (Suzuki *et al.*, 1961). The numerous sulphur compounds contained in onion and garlic have recently been reviewed (Virtanen, 1962).



(XVIII)
S-(β-Carboxypropyl)-L-cysteine

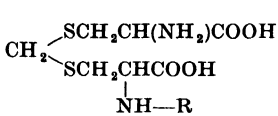


(XIX)
Alliin

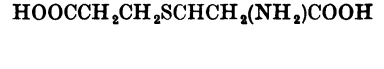


(XX)
Allicin

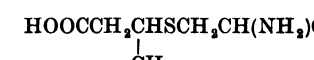
A botanically different family where cysteine derivatives occur is the *Mimosaceae*. Some thirty years ago, djenkolic acid (XXI, R = H) was isolated from *Pithecolobium lobatum* and *P. bigeminum* (van Veen and Hyman, 1935), an observation which was recently supplemented by the discovery of *N*-acetyl-L-djenkolic acid (XXI, R = CH_3CO) in seeds of *Acacia farnesiana* Willd., *A. horrida* Willd., *A. karroo* Hayne, and *Mimosa acanthocarpa* Benth. (Gmelin *et al.*, 1962). Seeds of *Albizzia lophanta* Benth. contain as much as 1·1% djenkolic acid, functioning as substrate for the enzyme that cleaves this amino acid to pyruvic acid, ammonia and methanedithiol (Gmelin *et al.*, 1957). *S*-(β-Carboxyethyl)-L-cysteine (XXII) was reported by Gmelin *et al.* (1958) as a seed constituent



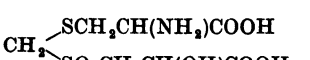
(XXI)
R = H, Djenkolic acid
R = CH_3CO, *N*-Acetyl-L-djenkolic acid



(XXII)
S-(β-Carboxyethyl)-L-cysteine



(XXIII)
S-(β-Carboxyisopropyl)-L-cysteine



(XXIV)
Dichrostachic acid

of *Albizzia julibrissin* Durazz., and is structurally related to *S*-(β-carboxyisopropyl)-L-cysteine (XXIII), another acid obtained from seeds of *Acacia millefolia* and *A. Willardiana* but present also in several other species of the family *Mimosaceae* (Gmelin and Hietala, 1960). The latest addition to the series of sulphur amino acids in this family is dichrostachic acid (XXIV), present in seeds of *Dichrostachys glomerata* (Forsk.) Hutch. & Dalz. and *Neptunia oleracea* Lour., both belonging to the tribe *Adenanthereae* (Gmelin, 1962). Obviously, this amino acid represents a modified form of djenkolic acid.

The number of plants studied so far with regard to their contents of sulphur-containing amino acids is too limited to permit any chemotaxonomic conclusions to be drawn. It seems likely, however, that the accumulation of analogous amino acids in two so distantly related families as *Liliaceae* and *Mimosaceae* reflects nothing more than a certain parallelism in the biochemical combinations of cysteine with fragments from basal metabolism. Valuable help from chemistry in the systematic classification within each group may, however, be visualized when more comprehensive and detailed analytical data become available.

IV. Isothiocyanate-producing Glucosides

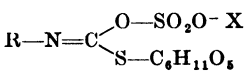
A. General Remarks

A uniform but far from universally distributed group of plant constituents is composed of thioglucosides, possessing the property of undergoing hydrolysis to glucose, sulphuric acid and isothiocyanates induced by the enzyme myrosinase, which usually accompanies the thioglucosides in plant tissues. The classical representatives of this group of compounds are sinigrin (XXVIa) and sinalbin (XXVIb), isolated from black and white mustard, respectively, more than a hundred years ago and even then correctly considered to be the principles responsible for the biting taste and pungent smell of disintegrated mustard seeds. Several observations, scattered through the older literature, showed that the *Cruciferae* were a family particularly rich in species furnishing isothiocyanates on enzymic hydrolysis. At the same time, Guignard (1890, 1893) demonstrated that the enzyme myrosinase was widely distributed within the same family, but was also found in species of *Capparidaceae*, *Resedaceae*, *Tropaeolaceae* and *Limnanthaceae*. Numerous recorded applications of plants of these families as condiments, potherbs and remedies are undoubtedly due to their containing isothiocyanate-producing glucosides, a fact which has made studies of their applications to folk medicine a useful incentive in the search for new botanical sources of these glucosides.

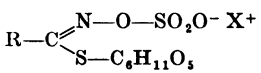
The properties and chemistry of this type of glucoside have recently been reviewed in detail (Kjær, 1960). In the present section emphasis will be placed on the botanical distribution of the individual glucosides.

B. Chemistry

The crystalline compounds sinigrin and sinalbin were early subjects of structural speculations. Gadamer (1897) concluded that they possessed the general structure (XXV) which apparently accounted for the production of isothiocyanates, glucose and sulphate on enzymic hydrolysis. This structure was generally accepted until a few years ago when Ettlinger and Lundeen (1956a) proved (XXVI), rather than (XXV), to be the correct expression for all isothiocyanate-producing glucosides known thus far.



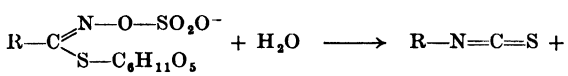
(XXV)



(XXVI)

(a) R = CH_2=CH—CH_2, $X^+ = K^+$; sinigrin
(b) R = *p*-$HOC_6H_4CH_2$, X^+ = sinapine, sinalbin

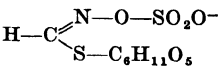
In this structure, the thioglucosidic linkage is β and susceptible to hydrolytic attack by myrosinase. Of at least two enzymes, present in seeds of *Sinapis alba* and catalysing the reaction shown below, one is remarkable in having vitamin C as a coenzyme, the first clearly demonstrated physiological function of ascorbic acid (Ettlinger *et al.*, 1961).



Within the last decade much progress has been made in the study of the various side-chains (R, XXVI) encountered in thioglucosides of higher plants. Present knowledge indicates that all the forty or so individual compounds of this type reported so far possess the same general structure (XXVI), with β-attached glucose and ester-bound sulphuric acid as invariable structural components. The glucoside ions have mostly been isolated as potassium salts but other cations, such as quaternary organic bases, occasionally afford more readily crystallizable salts. Alternatively, acetylation has been frequently used as a procedure for obtaining crystalline products.

Apart from the long-established names sinigrin and sinalbin, it has become customary to name the various glucosides of this type by individual designations, e.g. glucocochlearin, glucocapparin, etc., derived

from the Latin name of the first botanical source in which they were recognized, preceded by the prefix gluco. In view of the rapidly increasing number of glucosides this practice may soon lead to rather unwieldy designations of questionable value. On the other hand, the structures are too complicated to make fully systematic names practical. Hence, the present author should like to advocate adoption of the designation glucosinolate, introduced by Ettlinger and Dateo (1961) for the ion (XXVII) in which $C_6H_{11}O_5-$ denotes a β-D-1-glucopyranosyl residue. In a semi-systematic nomenclature the nature of the cation and the side-chain can then be conveniently specified; e.g. sinigrin (XXVIa) becomes identical with potassium allylglucosinolate, sinalbin (XXVIb) with sinapine 4-hydroxybenzylglucosinolate, etc.



(XXVII)

In Table I are presented the isothiocyanate-producing glucoside ions with established chemical structures known today as plant constituents. Only a few of these have been obtained in a crystalline state. In many cases the structures have been deduced through identification of the isothiocyanates resulting from the enzymic hydrolysis, supplemented with experimental proof for the production of hydroxylamine and glucose on acid hydrolysis.

C. Analysis and Results

Rapid chemical analysis of a substantial number of plant extracts is a recurring problem with chemotaxonomic work, and requires simple and efficient methods. In the area of isothiocyanate-producing glucosides, paper chromatography was put to good use for separation of the individual thioglucosides in crude plant extracts by Schultz and colleagues (cf. Schultz and Wagner, 1956c). Alternatively, enzymic hydrolysis of the parent compounds, followed by conversion of the resulting isothiocyanates into thioureas and paper chromatography of the latter, form the basis of another useful method for serial analysis (Kjær and Rubinstein, 1953). In more recent years, gas chromatography of the isothiocyanates has proved to be an efficient adjunct to other analytical methods (Kjær and Jart, 1957; Jart, 1961).

Essentially by means of the above tools, an estimated number of about 350 botanical species have been assayed so far for their content of isothiocyanate-producing glucosides. It is beyond the scope of the present chapter, however, to enumerate all species investigated. A recently

TABLE I

Isothiocyanate-producing glucosides of established structure from higher plants

Glucoside	Side-chain (R, XXVI)	References[a]
Glucocapparin	CH_3	Kjær *et al.* (1955); Kjær and Gmelin (1956a)
Glucolepidiin[b]	CH_3CH_2	Kjær and Larsen (1954)
Glucoputranjivin	$CH_3CH(CH_3)$	Puntambekar (1950); Kjær and Conti (1953)
Glucocochlearin	$CH_3CH_2CH(CH_3)$[c]	Hofmann (1874); Gadamer (1899a)
Glucojiaputin	$CH_3CH_2CH(CH_3)CH_2$	Kjær and Friis (1962)
Glucoerypestrin	$CH_3OOCCH_2CH_2CH_2$	Kjær and Gmelin (1957a)
Glucocapangulin	$CH_3CH_2CH_2COCH_2CH_1CH_2$	Kjær and Thomsen (1960)
Glucocappasalin	$CH_3CH_2CH_2COCH_2CH_2CH_2CH_2$	Kjær and Thomsen (1962a)
Gluconorcappasalin	$CH_3CH_2COCH_2CH_2CH_2CH_2$	Kjær and Thomsen (1963b)
Sinigrin	$CH_2{=}CHCH_2$	Will and Körner (1863)[d]
Gluconapin	$CH_2{=}CHCH_2CH_2$	Ettlinger and Hodgkins (1955); Kjær *et al.* (1953)
Glucobrassicanapin	$CH_2{=}CHCH_2CH_2CH_2$	Kjær and Boe Jensen (1956a)
Glucoibervirin	$CH_3SCH_2CH_2CH_2$	Kjær *et al.* (1955a)
Glucoiberin	$CH_3SOCH_2CH_2CH_2$[e]	Schultz and Gmelin (1954)
Glucocheirolin	$CH_3SO_2CH_2CH_2CH_2$	Schneider (1910)
Glucoerucin	$CH_3SCH_2CH_2CH_2CH_2$	Kjær and Gmelin (1955)
Glucoraphanin	$CH_3SOCH_2CH_2CH_2CH_2$[e]	Procházka (1959a)
Glucoraphenin	$CH_3SOCH{=}CHCH_2CH_2$[e]	Schmid and Karrer (1948)
Glucoerysolin	$CH_3SO_2CH_2CH_2CH_2CH_2$	Schneider and Kaufmann (1912)
Glucoberteroin	$CH_3SCH_2CH_2CH_2CH_2CH_2$	Kjær *et al.* (1955b)
Glucoalyssin	$CH_3SOCH_2CH_2CH_2CH_2CH_2$[e]	Kjær and Gmelin (1956b); Schultz and Wagner (1956a)
Glucolesquerellin	$CH_3SCH_2CH_2CH_2CH_2CH_2CH_2$	Daxenbichler *et al.* (1961)
Glucohirsutin	$CH_3SO(CH_2)_8$[e]	Kjær and Christensen (1958)
Glucoarabin	$CH_3SO(CH_2)_9$[e]	Kjær and Gmelin (1956c)
Glucocamelinin	$CH_3SO(CH_2)_{10}$[e]	Kjær *et al.* (1956a)

TABLE I—*continued*

Glucoside	Side-chain (R, XXVI)	References[a]
Glucotropaeolin	$C_6H_5CH_2$	Gadamer (1899a, b)
Sinalbin	(*p*)-$HOC_6H_4CH_2$	Salkowski (1899)
Glucoaubrietin	(*p*)-$CH_3OC_6H_4CH_2$	Kjær *et al.* (1956b)
Glucolimnanthin[b]	(*m*)-$CH_3OC_6H_4CH_2$	Ettlinger and Lundeen (1956b)
Gluconasturtiin	$C_6H_5CH_2CH_2$	Gadamer (1899c)
Glucomalcolmiin	$C_6H_5COOCH_2CH_2CH_2$	Kjær and Gmelin (1956d)
Glucobenzosisymbrin	$C_6H_5COOCH_2CH(CH_3)$	Kjær and Christensen (1961)
Glucobenzsisaustricin	$C_6H_5COOCH_2CH(CH_2CH_3)$	Kjær and Christensen (1962a)
Glucobrassicin	3-Indolyl-CH_2	Gmelin and Virtanen (1961)
Neoglucobrassicin	1-CH_3O-3-Indolyl-CH_2	Gmelin and Virtanen (1962)
Progoitrin (Glucorapiferin[f])	CH_2=$CHCHOHCH_2$[g]	Greer (1962); Kjær *et al.* (1956c); Schultz and Wagner (1956b)
Glucoconringiin	$(CH_3)_2C(OH)CH_2$	Gmelin and Virtanen (1959)
Glucocleomin	$(CH_3CH_2)(CH_3)C(OH)CH_2$	Kjær and Thomsen (1962b)
Glucosisymbrin	$HOCH_2CH(CH_3)$	Kjær and Christensen (1959)
Glucosisaustricin	$HOCH_2CH(CH_2CH_3)$	Kjær and Christensen (1962b)
Glucobarbarin	$C_6H_5CHOHCH_2$	Kjær and Gmelin (1957b; 1958)

[a] Attempts are made to present the most pertinent references rather than a complete list.
[b] This name was not proposed in the original paper.
[c] For absolute configuration, see Kjær and Hansen (1957).
[d] Includes references to previous papers.
[e] For configuration, see Klyne *et al.* (1960).
[f] Alternative name proposed by Schultz and Wagner (1956b).
[g] For absolute configuration, see Kjær *et al.* (1959).

published survey by the present author (Kjær, 1960) lists about 200 species studied, together with the analytical results, the type of evidence on which these are based, and pertinent references.

Frequently, several of the individual thioglucosides listed in Table I occur together in a given species, and give rise to a characteristic "pattern". However, quantitative variations in the latter may occasionally be observed, depending on environmental factors such as soil, climate, etc. Although in general only minor deviations have been observed in the "thioglucoside pattern" of different parts of a given plant, examples are on record of even qualitative variations between roots, leaves and seeds. This fact illustrates the importance of precise specifications as to the employed parts of a given plant. Again, a similar warning may be appropriate in connection with the problem of botanical authenticity. It is important to realize that in phytochemical studies, one relates precise chemical information with botanical individuality. It is not always appreciated that the latter must be defined equally as well as the former for the combination to be meaningful and reproducible.

The chromatographic methods, however rapid and simple, have their obvious limitations. Within the present group of plant constituents only a few differential-diagnostic reagents have been developed for paper chromatography of the individual representatives of the forty known thioglucosides. Hence, many identifications of the observed thioglucosides are based solely on R_F-values and are therefore prone to be fallacious. Supplementary chromatograms of the corresponding thiourea derivatives, of course, provides a higher degree of reliability, but still more selective analytical methods are desirable.

D. Botanical Distribution

According to our present knowledge the natural occurrence of members of the characteristic group of thioglucosides discussed above is restricted to a few families of dicotyledons. There seems to be no indications of the presence of the parent glucosides or the derived isothiocyanates in micro-organisms or lower plants.

By far the most abundant source of thioglucosides has been the family *Cruciferae*, in which, compounds of this type were first discovered. Of more than 200 cruciferous species investigated so far, hardly any has been devoid of thioglucosides. It therefore seems safe to conclude that glucosides of this type are regular constituents of species of this family. That the glucosides, on the other hand, are not exclusively found in *Cruciferae* has long been recognized. Recent results in the author's laboratory (Kjær, 1960; Kjær and Thomsen, 1963a) have demonstrated

that the large family *Capparidaceae* is an equally constant source of thioglucosides. Again, the limited number of species of *Resedaceae* that have been studied all contained thioglucosides of the same general type (cf. Kjær, 1960). In addition to these three families with their constant contents of thioglucosides, a number of sporadic occurrences in species belonging to other families have been noted. These are presented in Table II.

The unigeneric family *Moringaceae* is generally considered to possess close affinity to *Capparidaceae* and *Resedaceae*, whereas no obvious relationship exists between the other families of Table II. To what extent thioglucosides occur in other species of these remains to be seen. From unpublished data in the author's laboratory it is clear, however, that thioglucosides are not typical constituents of all members of *Euphorbiaceae*, *Phytolaccaceae* and *Plantaginaceae* but instead are highly sporadically distributed. Of the remaining families in Table II, too few species have been studied to make any general assessment possible.

On basis of the present knowledge the interesting conclusion can be drawn that all species belonging to *Capparidaceae*, *Cruciferae*, *Moringaceae* and *Resecadeae* are sources of thioglucosides. As also pointed out by Hegnauer (1961) this fact lends support to the belief that the four families above represent a natural group and should remain together, as they have long been placed in the order *Rhoeadales* (Wettstein, 1935; Harms, 1936). According to this classification, however, the four families are grouped together with *Papaveraceae* (Fig. 1), a linkage which has been questioned from time to time over the years. In the author's laboratory a few species of *Fumaria* as well as several representatives of various genera of *Papaveraceae* have been assayed for thioglucosides, though with consistently negative results (unpublished). Hegnauer (1961) rightly stresses the importance of considering the combined chemical characters in attempts to make phytochemical speculations useful for phylogeny. By so doing the conclusion is reached that *Papaveraceae sensu* Wettstein is of deviating ancestry, possibly deriving directly from *Ranales*, and hence should be removed from the *Rhoeadales*. This chemically motivated alteration has recently been supported by botanical considerations by Takhtajan (1959) who, mainly on morphological grounds, suggested the same limitation of Wettstein's *Rhoeadales* (Fig. 1). The alternative opinion, expressed by Hutchinson (1959) (Fig. 1), that *Capparidaceae* and *Moringaceae* should have an ancestry entirely different from that of *Papaveraceae*, *Cruciferae* and *Resedaceae* is certainly not supported by chemistry.

It is interesting that the thioglucosides in about 40 species of *Capparidaceae* investigated thus far are mainly different from those encountered

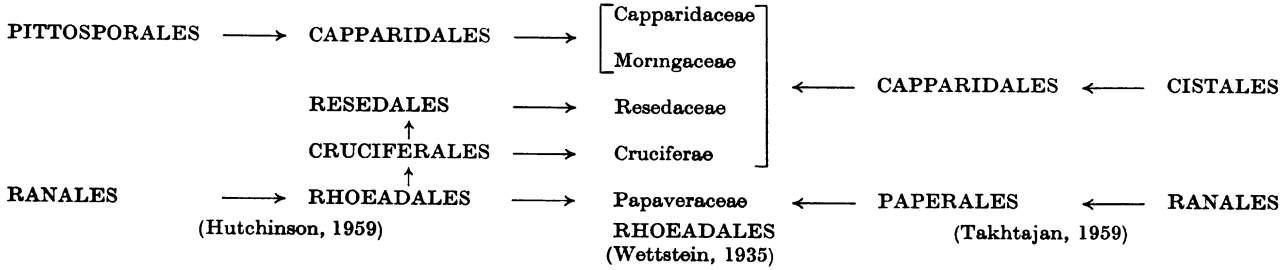


FIG. 1. Different classification systems of families grouped together in *Rhoeadales sensu* Wettstein.

TABLE II

Thioglucosides in species not belonging to *Capparidaceae*, *Cruciferae* and *Resedaceae*

Species	Family	Type of thioglucoside	References
Carica papaya L.[a]	*Caricaceae*	Glucotropaeolin	Ettlinger and Hodgkins (1956)
Jatropha multifida L.[b]	*Euphorbiaceae*	Glucotropaeolin (?)	Freise (1935)
Putranjiva roxburghii Wall.[a]		Glucoputranjivin, glucocochlearin Glucojiaputin, glucocleomin	Kjær and Friis (1962)
Limnanthes douglasii R. Br.[a]	*Limnanthaceae*	Glucolimnanthin	Ettlinger and Lundeen (1956b)
Moringa pterygosperma Gaertn.[c]	*Moringaceae*	Glucotropaeolin	Kurup and Rao (1954)
Codonocarpus cotinifolius F. Muell.[d]	*Phytolaccaceae*	Glucocochlearin	Bottomley and White (1950)
Plantago major L.	*Plantaginaceae*	Glucoraphenin	Procházka (1959b)
Salvadora oleoides Den.[a]	*Salvadoraceae*	Glucotropaeolin	Patel *et al.* (1926)
Tropaeolum majus L.[a,d]	*Tropaeolaceae*	Glucotropaeolin	Gadamer (1899b)
T. peregrinum hort.[a]		Glucoputranjivin Glucocochlearin	Kjær *et al.* (1953b)

[a] Seed material. [b] Latex. [c] Root material. [d] Green parts.

in crucifers. Thus, glucocapparin (Table I), which has not yet been recognized with certainty in any cruciferous taxon, seems to be the most widely distributed thioglucoside of the caper family. Another interesting feature is that the three caper thiolgucosides with keto-substituted side-chains (glucocapangulin, glucocappasalin and gluconorcappasalin, Table I) have solely been observed in South American species, in which glucocapparin is far less prominent. The possible phylogenetic consequences of this observation cannot yet be evaluated but current studies on a more extensive botanical material may provide further information along this line.

Attempts to apply the thioglucoside patterns for taxonomic problems on taxa below family rank have so far been merely preliminary. The paper chromatographic analysis of a series of *Arabis* species for thioglucosides illustrates the potential usefulness of a phytochemical approach to taxonomic problems in this area (Kjær and Hansen, 1958).

Despite the considerable number of taxa studied for their content of thioglucosides, it appears premature to discuss the possible implications with regard to taxonomy. It will be necessary here, as in many other areas of this field, to accumulate further data on the relationship between chemical constituents and botanical identity before speculations can be made really fruitful. It appears likely, however, that phytochemistry in future will provide an increasingly important auxiliary tool in the formidable task of disentangling the countless threads binding living species together.

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Appendix

Arrangements of Orders and Families in Spermatophyta

I. Gymnospermae*

Orders	Families		
1. Cycadales	1. Cycadaceae		
2. Ginkgoales	1. Ginkgoaceae		
3. Coniferales	1. Pinaceae	3. Cupressaceae	5. Cephalotaxaceae
	2. Taxodiaceae	4. Podocarpaceae	6. Araucariaceae
4. Taxales	1. Taxaceae		
5. Gnetales	1. Gnetaceae	2. Ephedraceae	3. Welwitschiaceae

II. Angiospermae†

A. MONOCOTYLEDONEÆ

1. Pandanales	1. Typhaceae	2. Pandanaceae	3. Sparganiaceae
2. Helobiae	1. Potamogetonaceae	4. Scheuchzeriaceae	6. Butomaceae
	2. Najadaceae	5. Alismaceae	7. Hydrocharitaceae
	3. Aponogetonaceae		
3. Triuridales	1. Triuridaceae		
4. Glumiflorae	1. Gramineae	2. Cyperaceae	
5. Principes	1. Palmae		
6. Synanthae	1. Cyclanthaceae		
7. Spathiflorae	1. Araceae	2. Lemnaceae	
8. Farinosae	1. Flagellariaceae	6. Eriocaulaceae	10. Commelinaceae
	2. Restionaceae	7. Thurniaceae	11. Pontederiaceae
	3. Centrolepidaceae	8. Rapateaceae	12. Cyanastraceae
	4. Mayacaceae	9. Bromeliaceae	13. Philydraceae
	5. Xyridaceae		
9. Liliiflorae	1. Juncaceae	4. Haemodoraceae	7. Taccaceae
	2. Stemonaceae	5. Amaryllidaceae	8. Dioscoreaceae
	3. Liliaceae	6. Velloziaceae	9. Iridaceae
10. Scitamineae	1. Musaceae	3. Cannaceae	4. Marantaceae
	2. Zingiberaceae		
11. Microspermae	1. Burmanniaceae	2. Orchidaceae	

* After R. G. West, "Classification of Plants". Cambridge University Press, 1961. Fossil forms omitted.

† After Engler as given in J. C. Willis, "A Dictionary of Flowering Plants and Ferns", 6th Ed. Cambridge University Press, 1957.

Orders	Families		
	B. DICOTYLEDONEÆ		
I. *Archichlamydeae*			
1. Verticillatae	1. Casuarinaceae		
2. Piperales	1. Saururaceae	3. Chloranthaceae	4. Lacistemaceae
	2. Piperaceae		
3. Salicales	1. Salicaceae		
4. Garryales	1. Garryaceae		
5. Myricales	1. Myricaceae		
6. Balanopsidales	1. Balanopsidaceae		
7. Leitneriales	1. Leitneriaceae		
8. Juglandales	1. Juglandaceae		
9. Batidales	1. Batidaceae		
10. Julianiales	1. Julianiaceae		
11. Fagales	1. Betulaceae	2. Fagaceae	
12. Urticales	1. Ulmaceae	2. Moraceae	3. Urticaceae
13. Proteales	1. Proteaceae		
14. Santalales	1. Myzodendraceae	4. Grubbiaceae	7. Loranthaceae
	2. Santalaceae	5. Olacaceae	8. Balanophoraceae
	3. Opiliaceae	6. Octoknemataceae	
15. Aristolochiales	1. Aristolochiaceae	2. Rafflesiaceae	3. Hydnoraceae
16. Polygonales	1. Polygonaceae		
17. Centrospermae	1. Chenopodiaceae	4. Cynocrambaceae	7. Portulacaceae
	2. Amarantaceae	5. Phytolaccaceae	8. Basellaceae
	3. Nyctaginaceae	6. Aizoaceae	9. Caryophyllaceae
18. Ranales	1. Nymphaeaceae	7. Berberidaceae	13. Eupomatiaceae
	2. Ceratophyllaceae	8. Menispermaceae	14. Myristicaceae
	3. Trochodendraceae	9. Magnoliaceae	15. Gomortegaceae
	4. Cercidiphyllaceae	10. Calycanthaceae	16. Monimiaceae
	5. Ranunculaceae	11. Lactoridaceae	17. Lauraceae
	6. Lardizabalaceae	12. Anonaceae	18. Hernandiaceae
19. Rhoeadales	1. Papaveraceae	3. Cruciferae	5. Resedaceae
	2. Capparidaceae	4. Tovariaceae	6. Moringaceae
20. Sarraceniales	1. Sarraceniaceae	2. Nepenthaceae	3. Droseraceae
21. Rosales	1. Podostemaceae	7. Pittosporaceae	13. Eucommiaceae
	2. Tristichaceae	8. Brunelliaceae	14. Platanaceae
	3. Hydrostachyaceae	9. Cunoniaceae	15. Crossosomataceae
	4. Crassulaceae	10. Myrothamnaceae	16. Rosaceae
	5. Cephalotaceae	11. Bruniaceae	17. Connaraceae
	6. Saxifragaceae	12. Hamamelidaceae	18. Leguminosae
22. Pandales	1. Pandaceae		
23. Geraniales	1. Geraniaceae	8. Cneoraceae	15. Vochysiaceae
	2. Oxalidaceae	9. Rutaceae	16. Tremandraceae
	3. Tropaeolaceae	10. Simarubaceae	17. Polygalaceae
	4. Linaceae	11. Burseraceae	18. Dichapetalaceae
	5. Humiriaceae	12. Meliaceae	19. Euphorbiaceae
	6. Erythroxylaceae	13. Malpighiaceae	20. Callitrichaceae
	7. Zygophyllaceae	14. Trigoniaceae	

Orders	Families		
24. Sapindales	1. Buxaceae	8. Corynocarpaceae	15. Icacinaceae
	2. Empetraceae	9. Aquifoliaceae	16. Aceraceae
	3. Coriariaceae	10. Celastraceae	17. Hippocastanaceae
	4. Limnanthaceae	11. Hippocrateaceae	18. Sapindaceae
	5. Anacardiaceae	12. Salvadoraceae	19. Sabiaceae
	6. Cyrillaceae	13. Stackhousiaceae	20. Melianthaceae
	7. Pentaphylacaceae	14. Staphyleaceae	21. Balsaminaceae
25. Rhamnales	1. Rhamnaceae	2. Vitaceae	
26. Malvales	1. Elaeocarpaceae	4. Tiliaceae	7. Sterculiaceae
	2. Chlaenaceae	5. Malvaceae	8. Scytopetalaceae
	3. Gonystilaceae	6. Bombacaceae	
27. Parietales	1. Dilleniaceae	11. Frankeniaceae	21. Turneraceae
	2. Eucryphiaceae	12. Tamaricaceae	22. Malesherbiaceae
	3. Ochnaceae	13. Fouquieriaceae	23. Passifloraceae
	4. Caryocaraceae	14. Cistaceae	24. Achariaceae
	5. Marcgraviaceae	15. Bixaceae	25. Caricaceae
	6. Quiinaceae	16. Cochlospermaceae	26. Loasaceae
	7. Theaceae	17. Winteranaceae	27. Datiscaceae
	8. Guttiferae	18. Violaceae	28. Begoniaceae
	9. Dipterocarpaceae	19. Flacourtiaceae	29. Ancistrocladaceae
	10. Elatinaceae	20. Stachyuraceae	
28. Opuntiales	1. Cactaceae		
29. Myrtiflorae	1. Geissolomataceae	8. Punicaceae	14. Myrtaceae
	2. Penaeaceae	9. Lecythidaceae	15. Melastomaceae
	3. Oliniaceae	10. Rhizophoraceae	16. Onagraceae
	4. Thymelaeaceae	11. Nyssaceae	17. Haloragidaceae
	5. Elaeagnaceae	12. Alangiaceae	18. Hippuridaceae
	6. Lythraceae	13. Combretaceae	19. Cynomoriaceae
	7. Sonneratiaceae		
30. Umbelliflorae	1. Araliaceae	2. Umbelliferae	3. Cornaceae
II. *Sympetalae* (= *Metachlamydeae*)			
1. Ericales	1. Clethraceae	3. Lennoaceae	5. Epacridaceae
	2. Pyrolaceae	4. Ericaceae	6. Diapensiaceae
2. Primulales	1. Theophrastaceae	2. Myrsinaceae	3. Primulaceae
3. Plumbaginales	1. Plumbaginaceae		
4. Ebenales	1. Sapotaceae	3. Symplocaceae	4. Styracaceae
	2. Ebenaceae		
5. Contortae	1. Oleaceae	3. Gentianaceae	5. Asclepiadaceae
	2. Loganiaceae	4. Apocynaceae	
6. Tubiflorae	1. Convolvulaceae	8. Solanaceae	15. Columelliaceae
	2. Polemoniaceae	9. Scrophulariaceae	16. Lentibulariaceae
	3. Hydrophyllaceae	10. Bignoniaceae	17. Globulariaceae
	4. Boraginaceae	11. Pedaliaceae	18. Acanthaceae
	5. Verbenaceae	12. Martyniaceae	19. Myoporaceae
	6. Labiatae	13. Orobanchaceae	20. Phrymaceae
	7. Nolanaceae	14. Gesneriaceae	

Orders	Families		
7. Plantaginales	1. Plantaginaceae		
8. Rubiales	1. Rubiaceae	3. Adoxaceae	5. Dipsacaceae
	2. Caprifoliaceae	4. Valerianaceae	
9. Cucurbitales	1. Cucurbitaceae		
10. Campanulatae	1. Campanulaceae	3. Brunoniaceae	5. Calyceraceae
	2. Goodeniaceae	4. Stylidiaceae	6. Compositae

Alphabetical List of Orders and Families

I. Orders*

Aristolochiales	A 15	Juglandales	A 8	Ranales	A 18
Balanopsidales	A 6	Julianiales	A 10	Rhamnales	A 25
Batidales	A 9	Leitneriales	A 7	Rhoeadales	A 19
Campanulatae	S 10	Liliiflorae	M 9	Rosales	A 21
Centrospermae	A 17	Malvales	A 26	Rubiales	S 8
Coniferales	G 3	Microspermae	M 11	Salicales	A 3
Contortae	S 5	Myricales	A 5	Santalales	A 14
Cucurbitales	S 9	Myrtiflorae	A 29	Sapindales	A 24
Cycadales	G 1	Opuntiales	A 28	Sarraceniales	A 20
Ebenales	S 4	Pandales	A 22	Scitamineae	M 10
Ericales	S 1	Pandanales	M 1	Spathiflorae	M 7
Fagales	A 11	Parietales	A 27	Synanthae	M 6
Farinosae	M 8	Piperales	A 2	Taxales	G 6
Garryales	A 4	Plantaginales	S 7	Triuridales	M 3
Geraniales	A 23	Plumbaginales	S 3	Tubiflorae	S 6
Ginkgoales	G 2	Polygonales	A 16	Umbelliflorae	A 30
Glumiflorae	M 4	Primulales	S 2	Urticales	A 12
Gnetales	G 5	Principes	M 5	Verticillatae	A 1
Helobiae	M 2	Proteales	A 13		

II. Families†

A. GYMNOSPERMAE

Araucariaceae	3·6	Ephedraceae	5·2	Podocarpaceae	3·4
Cephalotaxaceae	3·5	Ginkgoaceae	2·1	Taxaceae	4·1
Cycadaceae	1·1	Gnetaceae	5·1	Taxodiaceae	3·2
Cupressaceae	3·3	Pinaceae	3·1	Welwitschiaceae	5·3

B. MONOCOTYLEDONEÆ

Alismataceae	2·5	Bromeliaceae	8·9	Centrolepidaceae	8·3
Amaryllidaceae	9·5	Burmanniaceae	11·1	Commelinaceae	8·10
Aponogetonaceae	2·3	Butomaceae	2·6	Cyanastraceae	8·12
Araceae	7·1	Cannaceae	10·3	Cyclanthaceae	6·1

* A = Archichlamydae (Dicotyledonæ). G = Gymnospermae. M = Monocotyledoneae. S = Sympetalae (Dicotyledonæ). Figures refer to order number (see Key, p. 480).

† Figures refer to order and family number (see Key, p. 480).

Cyperaceae	4·2	Marantaceae	10·4	Restionaceae	8·2
Dioscoreaceae	9·8	Mayacaceae	8·4	Scheuchzeriaceae	2·4
Eriocaulaceae	8·6	Musaceae	10·1	Sparganiaceae	1·3
Flagellariaceae	8·1	Najadaceae	2·2	Stemonaceae	9·2
Gramineae	4·1	Orchidaceae	11·2	Taccaceae	9·7
Haemodoraceae	9·4	Palmae	5·1	Thurniaceae	8·7
Hydrocharitaceae	2.7	Pandanaceae	1·2	Triuridaceae	3·1
Iridaceae	9·9	Philydraceae	8·13	Typhaceae	1·1
Juncaceae	9·1	Pontederiaceae	8·11	Velloziaceae	9·6
Lemnaceae	7·2	Potamogetonaceae	2·1	Xyridaceae	8·5
Liliaceae	9·3	Rapateaceae	8·8	Zingiberaceae	10·2

C. DICOTYLEDONÆ*

Acanthaceae	S	6	18	Campanulaceae	S	10	1	Dipsacaceae	S	8	5
Aceraceae	A	24	16	Capparidaceae	A	19	2	Dipterocarpaceae	A	27	9
Achariaceae	A	27	24	Caprifoliaceae	S	8	2	Droseraceae	A	20	3
Actinidiaceae†	A	27	–	Caricaceae	A	27	25	Ebenaceae	S	4	2
Adoxaceae	S	8	3	Caryocaraceae	A	27	4	Elaeagnaceae	A	29	5
Aizoaceae	A	17	6	Caryophyllaceae	A	17	9	Elaeocarpaceae	A	26	1
Akaniaceae†	A	24	–	Casuarinaceae	A	1	1	Elatinaceae	A	27	10
Alangiaceae	A	29	12	Celastraceae	A	24	10	Empetraceae	A	24	2
Amarantaceae	A	17	2	Cephalotaceae	A	21	5	Epacridaceae	S	1	5
Anacardiaceae	A	24	5	Ceratophyllaceae	A	18	2	Ericaceae	S	1	4
Ancistrocladaceae	A	27	29	Cercidiphyllaceae	A	18	4	Erythroxylaceae	A	23	6
Anonaceae	A	18	12	Chenopodiaceae	A	17	1	Eucommiaceae	A	21	13
Apocynaceae	S	5	4	Chalaenaceae	A	26	2	Eucryphiaceae	A	27	2
Aquifoliaceae	A	24	9	Chloranthaceae	A	2	3	Euphorbiaceae	A	23	19
Araliaceae	A	30	1	Cistaceae	A	27	14	Eupomatiaceae	A	18	13
Aristolochiaceae	A	15	1	Clethraceae	S	1	1	Fagaceae	A	11	2
Asclepiadaceae	S	5	5	Cneoraceae	A	23	8	Flacourtiaceae	A	27	19
Balanophoraceae	A	14	8	Cochlospermaceae	A	27	16	Fouquieriaceae	A	27	13
Balanopsidaceae	A	6	1	Collumelliaceae	S	6	15	Frankeniaceae	A	27	11
Balsaminaceae	A	24	21	Combretaceae	A	29	13	Garryaceae	A	4	1
Basellaceae	A	17	8	Compositae	S	10	6	Geissolomataceae	A	29	1
Batidaceae	A	9	1	Connaraceae	A	21	17	Gentianaceae	S	5	3
Begoniaceae	A	27	28	Convolvulaceae	S	6	1	Geraniaceae	A	23	1
Berberidaceae	A	18	7	Coriariaceae	A	24	3	Gesneriaceae	S	6	14
Betulaceae	A	11	1	Cornaceae	A	30	3	Globulariaceae	S	6	17
Bignoniaceae	S	6	10	Corynocarpaceae	A	24	8	Gomortegaceae	A	18	15
Bixaceae	A	27	15	Crassulaceae	A	21	4	Gonystilaceae	A	26	3
Bombacaceae	A	26	6	Crossosomataceae	A	21	15	Goodeniaceae	S	10	2
Boraginaceae	S	6	4	Cruciferae	A	19	3	Grubbiaceae	A	14	4
Brunelliaceae	A	21	8	Cucurbitaceae	S	9	1	Guttiferae	A	27	8
Bruniaceae	A	21	11	Cunoniaceae	A	21	9	Haloragiaceae	A	29	17
Brunoniaceae	S	10	3	Cynocrambaceae	A	17	4	Hamamelidaceae	A	21	12
Burseraceae	A	23	11	Cynomoriaceae	A	29	19	Hernandiaceae	A	18	18
Buxaceae	A	24	1	Cyrillaceae	A	24	6	Himantandraceae†	A	18	–
Cactaceae	A	28	1	Datiscaceae	A	27	27	Hippocastaneaceae	A	24	17
Callitrichaceae	A	23	20	Diapensiaceae	S	1	6	Hippocrateaceae	A	24	11
Calycanthaceae	A	18	10	Dichapetalaceae	A	23	18	Hippuridaceae	A	29	18
Calyceraceae	S	10	5	Dilleniaceae	A	27	1	Humiriaceae	A	23	5

* A = Archichlamydaceae; S = Sympetalae.

† Additional families given in Willis (*loc. cit.*).

Hydnoraceae	A	15	3	Nyctaginaceae	A	17	3	Sabiaceae	A	24	19
Hydrophyllaceae	S	6	3	Nymphaeaceae	A	18	1	Salicaceae	A	3	1
Hydrostachyaceae	A	21	3	Nyssaceae	A	29	11	Salvadoraceae	A	24	12
Icacinaceae	A	24	15	Ochnaceae	A	27	3	Santalaceae	A	14	2
Juglandaceae	A	8	1	Octoknemataceae	A	14	6	Sapindaceae	A	24	18
Julianiaceae	A	10	1	Olacaceae	A	14	5	Sapotaceae	S	4	1
Labiatae	S	6	6	Oleaceae	S	5	1	Sarraceniaceae	A	20	1
Lacistemaceae	A	2	4	Oliniaceae	A	29	3	Saururaceae	A	2	1
Lactoridaceae	A	18	11	Onagraceae	A	29	16	Saxifragaceae	A	21	6
Lardizabalaceae	A	18	6	Opiliaceae	A	14	3	Scrophulariaceae	S	6	9
Lauraceae	A	18	17	Orobanchaceae	S	6	13	Scytopetalaceae	A	26	8
Lecythidaceae	A	29	9	Oxalidaceae	A	23	2	Simarubaceae	A	23	10
Leguminosae	A	21	18	Pandaceae	A	22	1	Solanaceae	S	6	8
Leitneriaceae	A	7	1	Papaveraceae	A	19	1	Sonneratiaceae	A	29	7
Lennoaceae	S	1	3	Passifloraceae	A	27	23	Stachyuraceae	A	27	20
Lentibulariaceae	S	6	16	Pedaliaceae	S	6	11	Stackhousiaceae	A	24	13
Limnanthaceae	A	24	4	Penaeaceae	A	29	2	Staphyleaceae	A	24	14
Linaceae	A	23	4	Pentaphylacaceae	A	24	7	Sterculiaceae	A	26	7
Loasaceae	A	27	26	Phrymaceae	S	6	20	Stylidiaceae	S	10	4
Loganiaceae	S	5	2	Phytolaccaceae	A	17	5	Styracaceae	S	4	4
Loranthaceae	A	14	7	Piperaceae	A	2	2	Symplocaceae	S	4	3
Lythraceae	A	29	6	Pittosporaceae	A	21	7	Tamaricaceae	A	27	12
Magnoliaceae	A	18	9	Plantaginaceae	S	7	1	Theaceae	A	27	7
Malesherbiaceae	A	27	22	Platanaceae	A	21	14	Theophrastaceae	S	2	1
Malpighiaceae	A	23	13	Plumbaginaceae	S	3	1	Thymelaeaceae	A	29	4
Malvaceae	A	26	5	Podostemaceae	A	21	1	Tiliaceae	A	26	4
Marcgraviaceae	A	27	5	Polemoniaceae	S	6	2	Tovariaceae	A	19	4
Martyniaceae	S	6	12	Polygalaceae	A	23	17	Tremandraceae	A	23	16
Melastomataceae	A	29	15	Polygonaceae	A	16	1	Trigoniaceae	A	23	14
Meliaceae	A	23	12	Portulacaceae	A	17	7	Tristichaceae	A	21	2
Melianthaceae	A	24	20	Primulaceae	S	2	3	Trochodendraceae	A	18	3
Menispermaceae	A	18	8	Proteaceae	A	13	1	Tropaeolaceae	A	23	3
Monimiaceae	A	18	16	Punicaceae	A	29	8	Turneraceae	A	27	21
Moraceae	A	12	2	Pyrolaceae	S	1	2	Ulmaceae	A	12	1
Moringaceae	A	19	6	Quiinaceae	A	27	6	Umbelliferae	A	30	2
Myoporaceae	S	6	19	Rafflesiaceae	A	15	2	Urticaceae	A	12	3
Myricaceae	A	5	1	Ranunculaceae	A	18	5	Valerianaceae	S	8	4
Myristicaceae	A	18	14	Resedaceae	A	19	5	Verbenaceae	S	6	5
Myrothamnaceae	A	21	10	Rhamnaceae	A	25	1	Violaceae	A	27	18
Myrsinaceae	S	2	2	Rhizophoraceae	A	29	10	Vitaceae	A	25	2
Myrtaceae	A	29	14	Rosaceae	A	21	16	Vochysiaceae	A	23	15
Myzodendraceae	A	14	1	Rubiaceae	S	8	1	Winteranaceae	A	27	17
Nepenthaceae	A	20	2	Rutaceae	A	23	9	Zygophyllaceae	A	23	7
Nolanaceae	S	6	7								

A = Archichlamydeae. S = Sympetalae.

Key—Orders: Spathiflorae M 7 ≡ Monocotyledoneae, order 7. Families: Ochnaceae A 27 3 ≡ Archichlamydaceae (Dicotyledoneae), order 27 (Parietales), family 3.

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