

SOME ASPECTS OF CHEMOTAXONOMY

H. ERDTMAN

Institutionen för Organisk Kemi, KTH, Stockholm 70, Sverige

INTRODUCTION

During the First International Symposium on the Chemistry of Natural Products Lord Todd¹, 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 and, 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 nature; this is what Lord Todd wanted to emphasize. It is interesting to compare monographs on plant colouring matters, terpenes or alkaloids, written 15–20 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 alkoxy 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 their 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 headhunter used to depend upon the number of his trophies.

The elucidation of the structure of a natural product should no longer 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 Ružička. 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 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 *per se* the successful incorporation of a presumed precursor only shows that it can act as, and not that it necessarily constitutes, a true biosynthetic intermediate.

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 constituents.

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 served practical purposes. After Darwin, botanists proposed "natural systems" founded on real relationships but one is still unable to construct a true "phylogenetic" system. The main reason for this is that the vast majority of extinct species is unknown. The natural systems are based essentially 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 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 sometimes happens that unrelated groups of plants give rise to morphologically similar plants. This is called "convergence" or "parallel development". Conversely, related plants may give rise to very dissimilar descendants, "divergence". These phenomena sometimes cause considerable taxonomic difficulties.

Chemical contributions to plant classification are based on the chemical

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constituents of plants, *i.e.* on their "molecular characteristics". These characteristics are genetically controlled and have the advantage over morphological characteristics that they can be very exactly described in terms of definite structural and configurational chemical formulae.

The method of "chemical taxonomy" is 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 "secondary" products. This implies that some information may ultimately be gained from the investigation of the nucleic acids, but for the time being we will have to rely on the more trivial plant constituents.

The elucidation of the structures and configuration of naturally occurring organic compounds paves the way to an understanding of their biosynthesis which is a matter of fundamental systematic importance.

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 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 they 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. 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 taken over 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 interest²⁻⁵. 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 a single species are at present taxonomically useless if not biosynthetically related to plant constituents

of intermediate distribution. It is among the latter that we may expect to find substances 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 rearrangements 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 rearranged isoflavones, isoflavones and rotenoids, isoquinoline alkaloids and bisoquinoline alkaloids, diterpenes of the normal, regular labdane or pimarane structures and the rearranged, or otherwise modified, diterpenes of the abietane, totarane and podocarpene 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 indicate a relationship between the 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. (+)-Sparteine, (-)-sparteine and (\pm)-sparteine have been isolated from *Cytisus* and *Lupinus* and oenanthe toxin 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 result 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 unrelated plants would be in the possession of the enzymatic prerequisites for synthesizing the same "spectrum" of chemically unrelated compounds of intermediate distribution.

Chemotaxonomic studies should, therefore, include the investigation of the patterns of compounds occurring in plants and preferentially in 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 plant.

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 then this may be due to the lack of resin ducts. 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.

Compounds of considerable taxonomic value may be found in any part of a plant, but it is reasonable to assume that the most important ones occur in phylogenetically old, conservative, little specialized organs.

All living organisms are subject to variation and different specimens 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 soil conditions or to seasonal or climatic factors and one should, therefore, always examine several specimens of the same species, if possible specimens 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 Shirato⁶ 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 which 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, namely how to define a substance in chemotaxonomic contexts.

To a chemist, benzoic acid is C_6H_5-COOH regardless of whether it has been isolated from a natural source or obtained by a Grignard reaction, by hydrolysis of benzonitrile or by oxidation of toluene.

However, in chemotaxonomy the biosynthesis of benzoic acid is a matter of very great concern. Benzoic acid formed from shikimic acid, by degradation of a larger molecule, or by a cyclization reaction, is the result of quite different biochemical processes, and benzoic acid molecules having a different biosynthetic past 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 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 are 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. These reflections may at least serve to emphasize again the fundamental importance of biosynthesis in chemotaxonomy.

A few illustrations of some of these general points may be useful.

Separation of higher systematic categories

There are great differences between the chemistry of bacteria, fungi, and algae but there is no possibility of characterizing these categories chemically. 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.

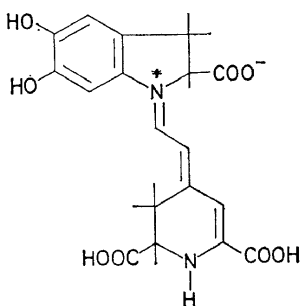
Bacteria, fungi and algae are all able to produce compounds according to the acetate and shikimic acid pathways but apparently they seldom combine them; flavenoids, for example, have never been found in any of these

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organisms. The alleged occurrence of rutin in *Chlamydomonas* has recently been withdrawn⁷.

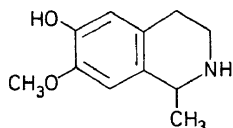
Flavonoids first appear in the *Bryophyta*. Quite recently, Bendz⁸ reported the isolation of crystalline anthocyanins from a red *Bryum* species which on hydrolysis gave luteolinidin. It is still uncertain whether the mosses contain compounds which could be regarded as lignins, but from *Polytrichum commune* Holmberg⁹ has obtained a product which appears to be of lignin type. It gave large amounts of *p*-hydroxybenzaldehyde, vanillin and syringaldehyde on oxidation with nitrobenzene and alkali. No lignins have been found in lower plants than mosses. Lignins and flavonoids are common in almost all plant groups higher than the bryophytes. Although ill-defined, the lignins appear to have some very general taxonomic interest since lignins from gymnosperms, mono- and dicotyledons usually exhibit characteristic chemical differences¹⁰.

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 Centrospermae¹¹ contain betacyanins, 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 Schmidt and Dreiding. The best known of these pigments is betanin from red beet (*Chenopodiaceae*), and Dreiding^{11a} has proposed the unique structure (I)—or a closely related alternative—for its aglycone, betanidin.



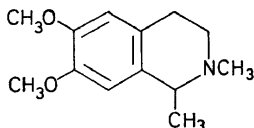
(I)

Betanidin



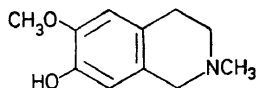
(II)

Salsoline



(III)

Carnegine



(IV)

Corypalline

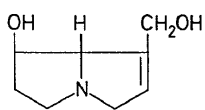
Betacyanins, even betanin itself, also occur in the order Opuntiales (Cactaceae) and this is important since it has long been assumed by botanists that the orders Centrospermae and Opuntiales are phylogenetically related.

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.

Similar enzyme systems in related plants producing analogous compounds

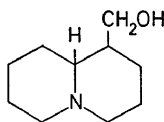
The alkaloids of the pyrrolizidine ("senecio") type and the analogous alkaloids of quinolizidine ("lupin") type are interesting since they have been found in some botanically related genera.

"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 *Crotalaria spectabilis*, retronecine (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).



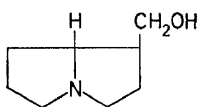
(V)

Retronecine



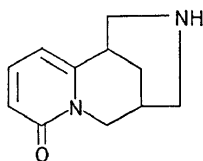
(VI)

Lupinine



(VII)

Laburnine



(VIII)

Cytisine

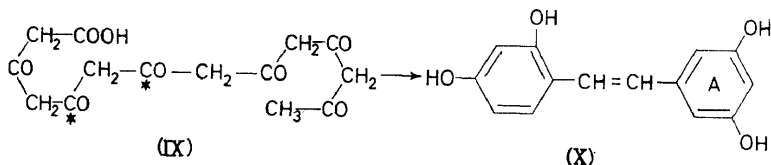
The furo- and pyranoquinolines of Rutaceae and the Amaryllidaceae alkaloids provide further examples.

Different pathways to similar products (?)

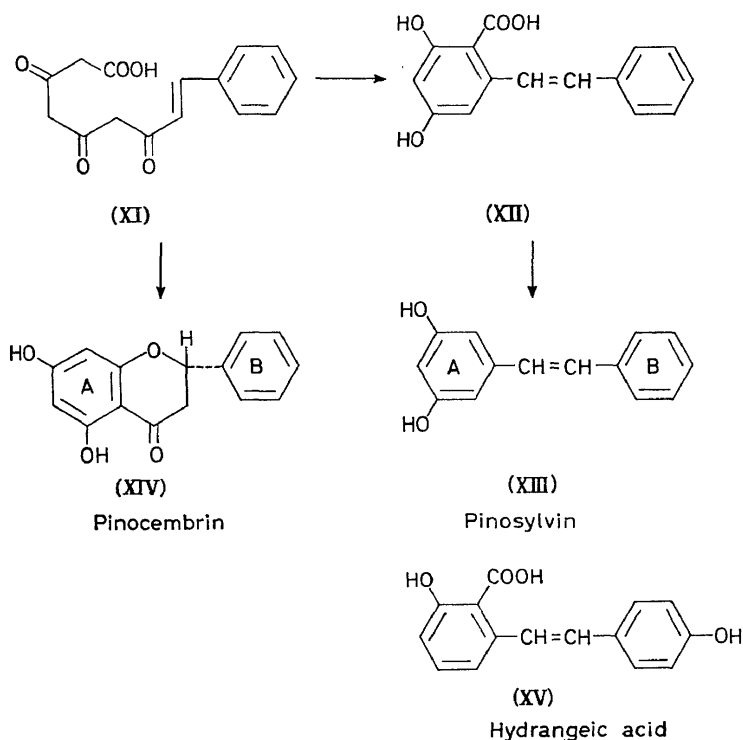
The natural stilbenes constitute a small but intriguing group and their structures and distribution in plants are given in *Table 1*. Two biosynthetic

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routes have been discussed for these compounds. Robinson¹² 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 and its geranyl derivative, chlorophorin, found in Moraceae. According to this hypothesis, oxyresveratrol (X) would be formed as shown in the accompanying formulae.


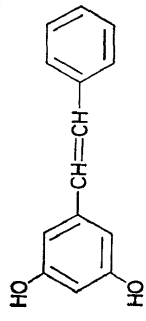
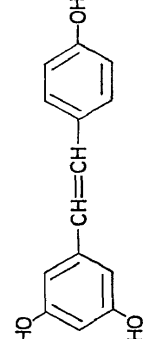
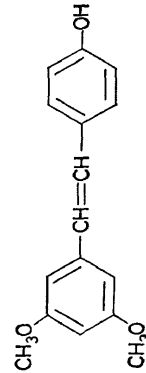
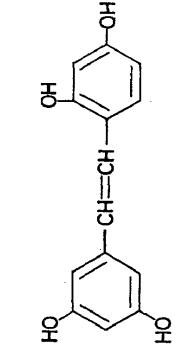


The cyclization of the hypothetical intermediate (IX) is followed by reduction of the two carbonyl groups marked leaving, after aromatization, the remaining hydroxyl groups correctly oriented. Pinosylvin (XIII) could be formed in a similar manner, but in this case it is necessary to assume that two more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

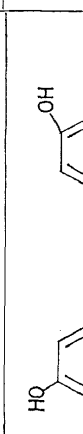
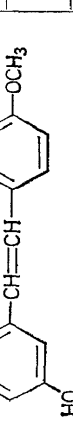


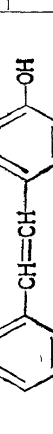




Birch¹³ has suggested that the stilbenes result from a combination of the acetic acid and shikimic acid pathways. He assumes that, for example, in the biosynthesis of pinosylvin (XIII) one molecule of cinnamic acid is

Table 1. Distribution of natural stilbenes

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

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Chlorophora One out of 3 species Heartwood	Moraceae	Urticales		
In 3 or more out of about 20 species Root	Polygonaceae	Polygonales	 <p>R = $\text{CH}_2-\text{CH}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-\text{CH}=\text{C}(\text{CH}_3)_2$</p>	Rhapontigenin
In one of about 300 species Rhizome	Polygonaceae	Polygonales		Rhapontigenin
Leaves	Myrtaceae	Myrtiliflorae		3,5,3',4'-Tetrahydroxystilbene
In one out of 3 species Heartwood	Leguminosae (Papilionatae)	Rosales		3,5,3',4',5'-Pentahydroxystilbene
In 2 out of about 30 species Heartwood	Leguminosae (Papilionatae)	Rosales		Hydrangeic acid
In one species Leaves	Saxifragaceae	Rosales		Phylloidalic acid
In one species Leaves	Saxifragaceae	Rosales		

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 phyllostilbic 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 the pinosylvins and pinocembrin (XIV) and other flavonoids in the heartwood of all pines from some of which even cinnamic acid has been isolated. This hypothesis has recently received experimental support from Billek and Kindl¹⁴ as well as Ibrahim and Towers¹⁵ who studied the biosynthesis of pinosylvin and hydrangeic acid using radioactively labelled acetate and glucose. The former was incorporated into the A-ring 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 a *meta*-oriented hydroxyl group in ring B, has been isolated from *Morus* and *Artocarpus* species. If this is true then the hydroxyl group in position 2' would result from 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.

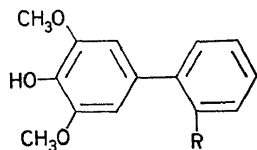
The biosynthesis of 4-hydroxystilbene is not clear. It is probable that it follows a route similar to that of pinosylvin since the compound occurs in pine heartwood, but then one has to postulate, for example, both hydroxylation and reduction of a common intermediate. As a matter of fact, it is at present 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 ether¹⁶.

It is seen from the *Table 1* 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 heartwood¹⁷, and pinocembrin from the related genus *Sarothamnus*¹⁸. It is possible that they contain similar enzyme systems but that in the former genus the routes to flavones are blocked, and in the latter those leading to stilbenes.

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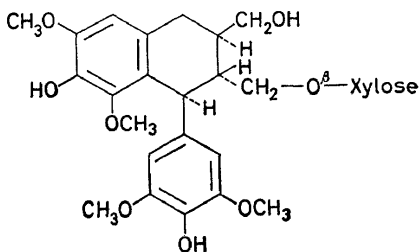
From the heartwood of *Sorbus aucuparia* (mountain ash, Rosaceae, Pomoi-deae) we have recently isolated some compounds which may be mentioned in connection with the stilbenes. These are the biphenyl derivatives aucuparin (XVI, R = H) and methoxyaucuparin (XVI, R = OCH₃)¹⁹.



(XVI)

R = H: Aucuparin

R = OCH₃: Methoxyaucuparin



(XVII)

Lyonia-xyloside

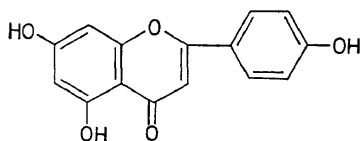
The sapwood contained a completely different compound which turned out to be a lignan xyloside²⁰ (XVII) previously found in some unrelated plants *Lyonia* (Ericaceae) and *Alnus* (Betulaceae). *Sorbus intermedia* was next investigated and the *Lyonia*-xyloside was isolated but no aucuparins. This is 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 *Sorbus 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.

Structural complexity and restricted occurrence

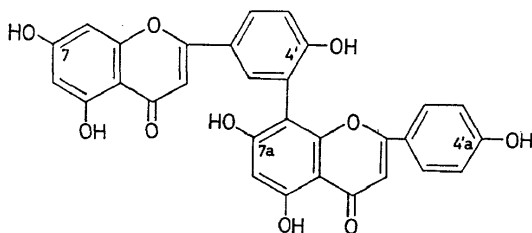
Many examples could be mentioned from several groups of compounds, including the alkaloids, which demonstrate relationships between the complexity of a compound and its systematic value. The biflavonyls provide a good illustration.

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

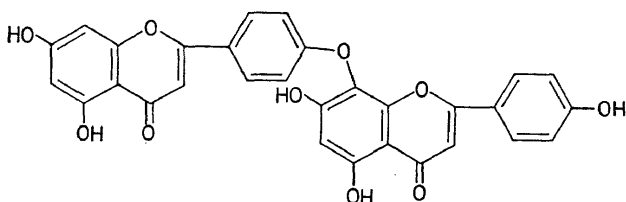
isolated later by Japanese chemists, especially Kariyone and his collaborators. The structures of these compounds have been investigated and finally elucidated by Baker, Nakazawa, Kariyone and their co-workers²¹. The two fundamental structures of the biflavonyls are (XIX) and (XX) representing respectively the recently discovered amentoflavone²² and hinokiflavone which was first isolated by Kariyone and Sawada.



(XVIII)
Apigenin



(XIX)
Amentoflavone



(XX)
Hinokiflavone

Several methyl ethers of amentoflavone are known. Sotetsuflavone is a monomethyl ether (CH_3O at 7a), ginkgetin and isoginkgetin are dimethyl ethers (CH_3O at 7,4' viz. 4',4'a), and sciadopitysin and kayaflavone are trimethyl ethers (CH_3O at 7,4',4'a viz. 4',7a,4'a). No methyl ethers derived from hinokiflavone have yet been found. The biphenyls have mainly been found in leaves of Gymnosperms. Hsü has found apigenin together with amento- and sotetsuflavone in *Selaginella tamariscina*. From *Cycas* species the occurrence of sotetsuflavone has been reported. *Ginkgo* contains 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 been studied and one contained sciadopitysin and an apigenin glycoside and the other kayaflavone.

SOME ASPECTS OF CHEMOTAXONOMY

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 contained kavaflavone. Biflavonyls are common in the order Cupressales, both in the heterogeneous family Taxodiaceae and in Cupressaceae. In these families hinokiflavone is a very common biflavonyl and has been found in all genera except *Cryptomeria* (Taxodiaceae) which contains sciadopitysin, kavaflavone and sotetsuflavone. In some other genera 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.

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

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 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 e.g. quercetin have been found in many plants, even in the leaves of some conifers but no corresponding biflavonyls.

Integrated investigations of groups of plants

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, as most of the plant groups are discouragingly large.

By a compilation of the literature some such information can be gained, e.g. 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.

CONIFERAE

We have concentrated our work on the conifers²⁻⁵, 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.

Natural Order Pinales

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

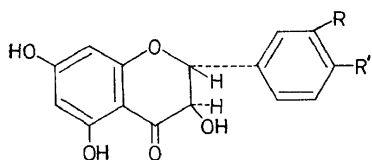
Our investigations started with the phenolic constituents of the heartwood of a large number of pine species (over fifty per cent of the recent species). A series of new or known compounds were isolated including stilbenes, flavanones and flavones. The results of this work, which was largely carried out by Dr Lindstedt²³, are recorded in *Table 2*.

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, R = R' = H), 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, strobopin 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 the pinosylvins in the latter, and thereby concluded that heartwoods are no good for the chemical characterization of pines.

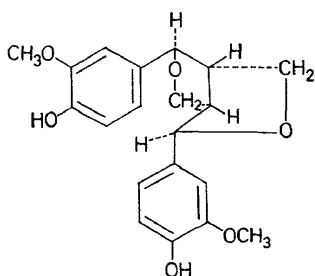
Other pine substances of interest are the lignan pinosresinol (XXII), which is also found in *Picea*, and resin acids such as those of pimarane (XXIII, XXIV) and abietane (XXV) type. These acids have also been found in *Abies*, *Larix*, *Picea*, and *Pseudotsuga*^{24a}. (The acids of pimarane type have 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,

SOME ASPECTS OF CHEMOTAXONOMY



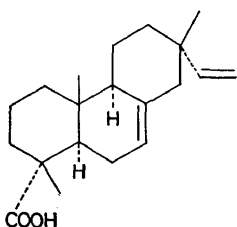
(XXI)

R = R' = H: Pinobanksin
 R = H, R' = OH: Aromadendrin
 R = R' = OH: Taxifolin



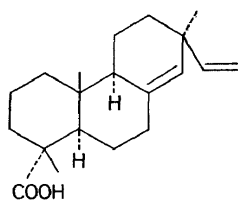
(XXII)

Pinoresinol



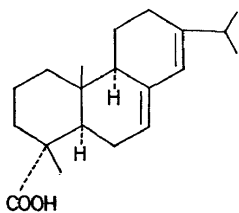
(XXIII)

Isopimaric acid



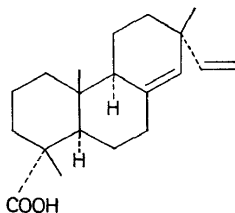
(XXIV)

Pimaric acid



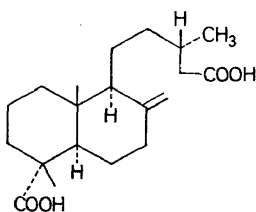
(XXV)

Abietic acid



(XXVI)

Sandaracopimaric acid



(XXVII)

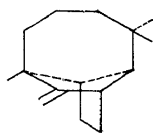
Pinifolic acid

Table 2. Heartwood constituents of pines (classification according to Shaw)

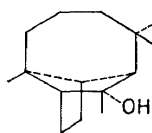
Hophloxylon		Pinus Species	Pinosylvin = P	P-monomethyl ether = PSM	Dihydro-P	Dihydro-PSM	Pinoembrin	Chrysin	Pinostrobin	Tectochrysin	Pinobanksin	Strobobanksin	Strobo-pin	Cryptostrobin	
Section	Sub-section														
Cembra	Cembrae	1 koratensis S. and Z.	+++	xxx	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	
		2 cembra L.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
		3 albicaulis Eng.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		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.	?	+	+	+	+	+	+	+	+	+	+	+	

pimaradiene, from the oleoresin of the wood of *P. silvestris*^{24b}. From the leaves of *Pinus silvestris* a new bicyclic resin acid, pinifolic acid²⁵ (XXVII) 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) are restricted to *Pinus* but this is by no means certain. The same applies to thunbergene (cembrene), a novel macrocyclic diterpene²⁶. Several overlaps 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*²⁷ 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.

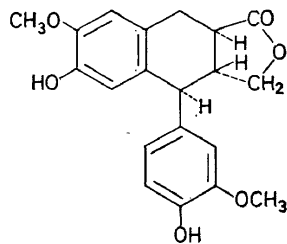
Flavanones such as pinobanksin and the related aromadendrin and taxifolin provide bridges between most of the genera. It is possible that some novel tetraline compounds from spruce bark, e.g. piceatannol²⁸ (XXXI), are restricted to *Picea* and the himachalenes (e.g. XXXII)²⁹, sesquiterpenes of a novel type, to *Cedrus*.



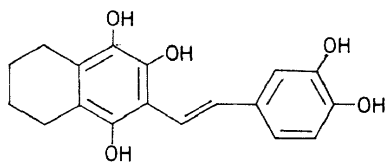
(XXVIII)
Longifolene



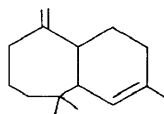
(XXIX)
Juniperol
(Longiborneol)



(XXX)
Conidendrin



(XXXI)
Piceatannol



(XXXII)
 α -Himachalene

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.

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 Podocarpaceae, e.g. eudesmane, pimarane and phyllocladane³⁰ derivatives.

Athrotaxis is a small southern genus (3 species from Tasmania) and *Taiwania* is northern (1 species from Formosa and China) and they are considered to be botanically related. The wood of *Taiwania* contains a cadinol and hinokiol³¹ and we have also found cadinol and hinokiol in the *Athrotaxis*³² species. This does not mean very much but at least it is in agreement with the views of the botanists.

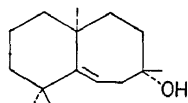
During our studies of the family Cupressaceae we have isolated many compounds, mainly of terpene nature. Some of these were new and we have elucidated the structures of several of them. Other compounds were already well known. Examples of new substances or compounds of incompletely known structure or configuration, or which had previously been assigned erroneous structures are: tropolones of thujaplicin—"C₁₀ type", tropolones of nootkatin—"C₁₅-type", thujic, chamic, and chaminic acids, cuparene and cuparenic acid, widdrol³³ (XXXIII), thujopsene³⁴ (XXXIV), and hinokiic acid, cedrolic acid³⁵ (XXXV), nootkatone³⁶ (XXXVI), occidentalol³⁷ (XLIV), communic acid³⁸ (XXXVII), torulosol³⁹ (XXXVIII), hinokiol⁴⁰ (XXXIX), and totarolone⁴¹ (XL).

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 Thujopsidae. Callitroideae is essentially southern, an exception being *Tetraclinis* (N. Africa). Some of our results on the heartwood constituents of Cupressaceae and those of several others are summarized in *Tables 3 and 4*. In order to make these tables more easy to follow, only skeletons of the sesquiterpenes are given, 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.

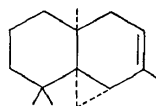
Of the tribe Cupresseae, the genus *Chamaecyparis* still appears to be heterogeneous from a chemical point of view. Most sesquiterpenes are of the "cis-farnesyl type", e.g. cadinane, thujopsane, widdrane, cuparene, and cedrane derivatives but there are also some of "trans-farnesyl type", e.g. eudesmane derivatives. The most aberrant species is certainly *Ch. nootkatensis* which contains C₁₅-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*.

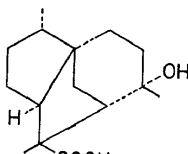
The heartwood of *Ch. pisifera* contains a phenolic substance, sawaranin, which has recently been elucidated by Imamura⁴². Its structure (XLI)



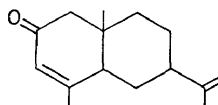
(XXXIII)
Widdrol



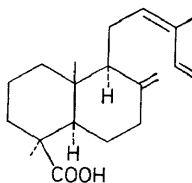
(XXXIV)
Thujopsene



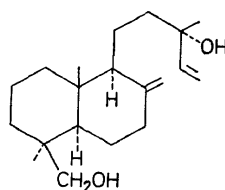
(XXXV)
Cedrolic acid



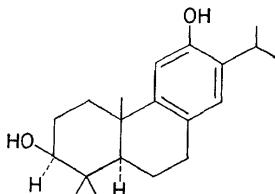
(XXXVI)
Nootkatone



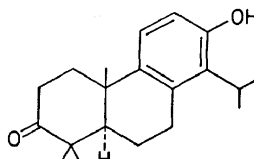
(XXXVII)
Communic acid



(XXXVIII)
Torulosol



(XXXIX)
Hinokiol



(XL)
Tatarolone

seems to indicate that sawaranin has been formed from a biphenyl derivative by cleavage of one of the rings.

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 manool (XLII), earlier found in *Dacrydium* (Podocarpaceae); it has not been found in any of the American species investigated. *Cupressus torulosa* also contained the hydroxy-manool torulosol

SOME ASPECTS OF CHEMOTAXONOMY

Wood (species)	Carvareol	Hydrothymoquinone derivatives	C ₁₀ -trypolones	C ₁₆ -trypolones	1-Rhodinic acid	Dehydrogeranic acid	Myricenic acid	Chamiric, chamirinic acid	Thujic acid	Shonanic acid	Cadinanes	Thujopsanes	Widdrines	Cuparanes	Cedranes	Eudesmanes	Ocidanes	Guaianes	Manool	Feruginol	Hinokiol	Totarol
Cupressaceae	<i>Chamaecyparis laussoniana</i>																					
	<i>thyoides</i>																					
	<i>obtusata</i>																					
	<i>pisifera</i>																					
	<i>formosensis</i>																					
	<i>taiwanensis</i>																					
	<i>nootkatensis</i>																					
	<i>Cupressus bakeri</i>																					
	<i>macnabiana</i>																					
	<i>macrocarpa</i>																					
<i>ari-zonica</i>																						
<i>sempervirens</i>																						
<i>torulosa</i>																						
Juniperaceae	<i>Juniperus communis</i>																					
	<i>cedrus</i>																					
	<i>foetidissima</i>																					
	<i>virginiana</i>																					
	<i>californica</i>																					
	<i>utahensis</i>																					
	<i>thurifera</i>																					
	<i>phoenicea</i>																					
	<i>procera</i>																					
	<i>chinensis</i>																					
Thujiopsidaceae	<i>Thuja plicata</i>																					
	<i>occidentalis</i>																					
	<i>standishii</i>																					
	<i>Thujiopsis</i>																					
	<i>Platycladus (Biota)</i>																					
	<i>Calocedrus decurrens</i>																					
<i>formosana</i>																						
<i>Fokienia hodginsii</i>																						

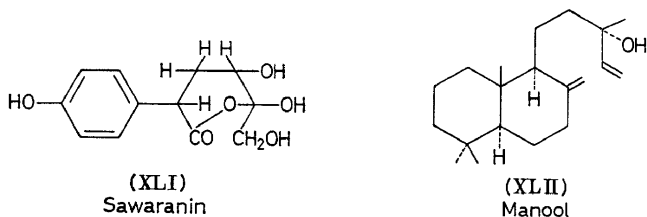
Table 3. Some heartwood constituents of the sub-family Cupressoidae

Table 4. Some heartwood constituents of the sub-family Callitroideae

Wood (species)	Carvacrol	Hydrothymoguinone derivatives	C ₁₀ -tropolones	C ₁₅ -tropolones	1-Rhodinic acid	Dehydrogeranic acid	Myricenic acid	Chamic, chaminic acid	Thujic acid	Shonanic acid	Cadinanes	Thujopsanes	Widdrantes	Cuparanes	Cedranes	Eudesmanes	Occidanes	Guaianes	Manool	Ferruginol	Hinokiol	Torol
<i>Callitris</i>					+++	+					+					++		+++++				
<i>calcarata</i>																						
<i>glauca</i>																						
<i>Intratropica</i>																						
<i>morrisonii</i>																						
<i>princei</i>																						
<i>prohispida</i>																						
<i>rhoni</i>																						
<i>rhomboidalis</i>																						
<i>verrucosa</i>																						
<i>maclayana</i>																						
<i>sulcata</i>																						
<i>Neocallitropsis</i>																						
<i>Widdringtonia</i>																						
<i>agressoides</i>																						
<i>dracmontana</i>																						
<i>juniperoides</i>																						
<i>schwarzii</i>																						
<i>sublycii</i>																						
<i>Tetractelis</i>	+	+																			+	
<i>Pilegerodendron</i>																						
<i>Austrocedrus</i>	+																					
<i>Popanacedrus</i>																						

SOME ASPECTS OF CHEMOTAXONOMY

(XXXVIII) (and the corresponding aldehyde) in which the CH_2OH -group is axially oriented as the COOH group at C-4 in agathene dicarboxylic (XLIII, $\text{R} = \text{CO}_2\text{H}$) and podocarpic acids.



Of the tribe Juniperae several species of *Juniperus* have recently been investigated by Runeberg⁴³ and, so far, they appear to be more similar to *Cupressus* than to *Chamaecyparis*. This has recently caused us to investigate some juniper barks and the results of this very preliminary study⁴⁴, which also includes one *Cupressus* species, are given in Table 5. It was interesting to find that the bark of *Cupressus arizonica* like that of the *Juniperus* species contained communic acid and the closely related agathene dicarboxylic acid (XLIII, $\text{R} = \text{CO}_2\text{H}$). Both have axial carboxyl groups at C-4.

Table 5. Bark constituents of *Juniperus* and *Cupressus* species

<i>Substance</i>	<i>J. communis</i>	<i>J. foetidissima</i>	<i>J. procera</i>	<i>J. californica</i>	<i>J. utahensis</i>	<i>J. mexicana</i>	<i>C. arizonica</i>
Longifolene	+						
Juniperol	+						
Hinokiic acid				+			
Communic acid	+	+	+	+	+	+	+
Agathenedicarboxylic acid		+			+	+	+
Ferruginol							+
Sugiol						+	+
Totarol	+						

The Thujopsidae 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.

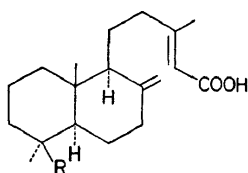
Passing over to the Callitroideae it is seen that *Callitris* and *Neocallitropsis* (in the chemical literature often "*Callitropsis*") have guaiol and eudesmol in common. Guaiol is a sesquiterpene which has not been found in any other conifers. The two genera are botanically related. The heartwood of *Neocallitropsis* has been claimed to contain cadinols⁴⁵.

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 different⁴⁶. *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.

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 Hartwell⁴⁷ to contain the tumour damaging lignans, podophyllotoxin and deoxypodophyllotoxin.

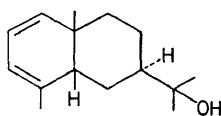
It is easy to see how very different the orders Pinales and Cupressales are from a chemical point of view. They have several constituents in common but there are also certain compounds of specific "Cupressales type" such as the tropolones and several sesquiterpenes. Some of these and other



(XLIII)

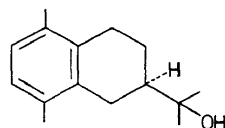
R = CO₂H : Agathene dicarboxylic acid

R = CH₂OH : Agatholic acid



(XLIV)

Occidentalol



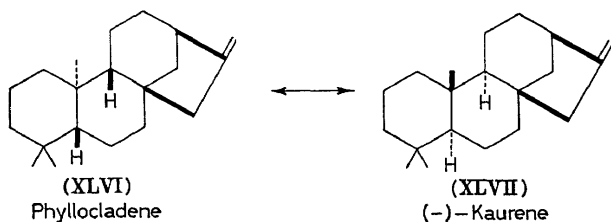
(XLV)

Occidol

compounds are fairly widely distributed in the order, others are restricted to a few genera. There are also some substances, such as occidentalol³⁷ (XLIV) and occidol⁴⁸ (XLV), 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*.

Natural Orders Araucariales and Podocarpaceles

The orders Araucariales and Podocarpaceles 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. The leaves contain (–)-kaurene (XLVII), a compound of the phyllocladene type but having the opposite configuration at C-5, C-9 and C-10.



The order Podocarpaceles 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-CH₂OH, CHO and COOH at C-4⁴⁹⁻⁵¹) have been isolated from *Podocarpus* species belonging to the sections *Eupodocarpus*, *Stachycarpus* and *Dacrycarpus*. 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 *Podocarpus spicatus* (matai) is completely aberrant. The wood contains a series of non-terpeneic substances such as aromadendrin, taxifolin and the isoflavones, genistein and podospicatin, as well as lignans, e.g. matairesinol and conidendrin. The leaves of *Podocarpus ferrugineus* contain phyllocladene and (+)-kaurene but those of *P. macrophylla* contain (–)-kaurene.

Surprises are also to be found in the *Dacrydium* species. The woods of *D. bidwillii* and *D. biforme* contain manool and 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 *Dacrydium 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, elemicin⁵² and several other phenolic compounds.

Our knowledge of the chemistry of the conifers is still very incomplete and unexpected discoveries will certainly be made in the future.

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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 Karrer⁵³, 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 desk⁵⁴. 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. International collaboration is greatly needed in this field. Many herbs can be raised from seeds obtainable from botanical gardens all over the world, but 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 convenient starting materials useful for the isolation

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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.

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, naively, 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. "L. Endl." for example is not a chemist; L. stands for Linneus and Endl. for Endlicher, two prominent botanists⁵⁵.

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*⁵⁶ and Kariyone's recent *Annual Index of the Reports on Plant Chemistry*⁵⁷.

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