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A USEFUL DEVELOPMENT IN THE CHEMISTRY OF FLAVONOIDS AS NATURAL PRODUCTS

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IMPORTANCE OF *Ginkgo biloba* (MAIDEN-HAIR TREE)

Ginkgo biloba (maiden-hair tree) is a tree of great botanical and chemical interest (Fig. 1). It is the only surviving species among Ginkgoales which were widespread in the very distant past between 60 and 250 million years ago. It has apparently remained unchanged over geological ages and it has therefore been called a 'living fossil'. It has long been cultivated in temple gardens of Japan where it is considered sacred. Not only is the tree beautiful but the green fruits are eaten after frying. The flesh of the ripe fruits is found to contain toxic phenols. The tree has been introduced into European countries and the U.S.A.

The extract of the leaves of this tree (Fig. 2) has been reported to have medicinal properties and it is now being marketed in West Germany in forms suitable for oral administration and injection. It is considered to possess blood flow increasing and vascular widening properties and is recommended for use in hormonal, nervous, vascular, blood flow and nutritional disorders.

The special chemical components of these leaves have been studied intensively during the past ten years and their structures have been established. They are known as 'biflavonoids' in whose molecules two C_{15} units are linked together. In view of the important medicinal properties attributed to the leaves we have recently examined Indian plant sources for compounds of this type and have obtained some useful results.

Before proceeding further, it may be useful to mention earlier discoveries on the medicinal uses of flavonoids. These polyphenols were originally valued as mordant dyestuff and this use has become obsolete after the introduction of synthetic dyestuffs. But their importance has continued for other reasons. One of these is their vitamin P property. This was discovered by Professor Szent Georgyi more than 30 years ago. At the present time Rutin, which is obtained on a large scale from buck-wheat and eucalyptus leaves, is used in medicine. Similarly the bioflavonoids, hesperidin and naringin obtained as by-products of the citrus industry are employed for the maintenance

of capillary integrity, control of spontaneous abortion and to aid in surgery and wound healing.

Ginkgetin and related compounds

The leaves of *G. biloba* were first examined chemically by Furukawa in 1932. He proposed the structure of 5, 8-dihydroxy-4′-methoxy flavone (4′-methoxy primetin) for the chief component which he labelled as 'Compound B'. This was later on named 'ginkgetin' by Baker who also showed that it was different from synthetic—4′-methoxy primetin. The study was progressing for over 25 years without conclusive results. The main difficulties were

FIG. 1. *Ginkgo biloba*.

FIG. 2. *Ginkgo biloba*—Leaves.

as follows: (i) the leaves yielded a mixture of three closely related compounds and their separation was originally difficult; (ii) much of the earlier study was carried out with mixtures; (iii) there was also difficulty of correctly determining the molecular size of these compounds. Eventually it was concluded that they were biflavonoids containing two flavonyl units and their constitutions were arrived at by the very extensive work of Japanese and British workers.

The crude material contained at least three related natural products of which two major ones were ginkgetin and isoginkgetin. It was later shown

that these compounds could be comparatively easily isolated by taking advantage of Nakazawa's observation that ginkgetin formed a rather insoluble crystalline potassium salt. Treatment of the mixture with aqueous potassium carbonate gave the sparingly soluble potassium salt of ginkgetin and acidification of the mother liquor gave isoginkgetin. Later the surprising observation was made that the mixture could be easily separated by fractional crystallization from acetone. Since by the use of the Rast method for the determination of molecular weight satisfactory results were not obtained, the modified Menzies-Wright ebullioscopic method was used and the molecular formula of ginkgetin was found to be $C_{32}H_{22}O_{10}$ and it contained two methoxyl and four hydroxyl groups.

The UV spectra of ginkgetin tetraacetate and tetramethyl ether indicated that they are derivatives of apigenin. Moreover, the intensities of the absorption bands were approximately double those of the monomeric apigenin derivatives. This demonstrated the presence of two isolated flavonoid units per molecule. Further studies of spectra particularly IR and colour reactions gave more information about the location of groups. But definite information was obtained by chemical degradations. The most important of these are given in Chart 1.

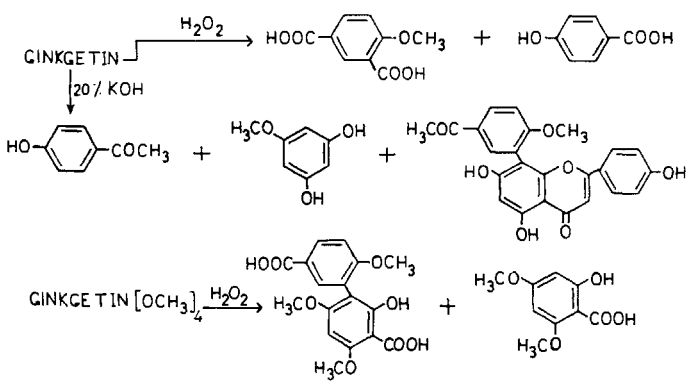


CHART 1. Chemical degradations of Ginkgetin.

They led to the conclusion that the flavonyl units had C–C linkage and were linked at 3′ and 8″-positions. The position of the methoxyls was not only indicated by the nature of the fission products but also confirmed by the study of UV spectra in the presence of bases. The constitution proposed by Baker and co-workers in the year 1959 was 4′, 7-O-dimethyl-3′, 8″-biapigeninyl (Chart 1*a*).

Nakazawa effected a synthesis of ginkgetin tetramethyl ether in 1959 by a mixed Ullmann reaction between 3′-iodo-5, 7, 4′-trimethoxyflavone and 8-iodo-5, 7, 4′-trimethoxyflavone using copper in dimethyl formamide medium. One of the products was found to be identical with the tetramethyl ether of

of natural ginkgetin. In 1963, Nakazawa synthesized ginkgetin itself as follows: He used for the protection of the free hydroxyl groups in the components benzyl and benzoyl units and these were conveniently removed later on to yield ginkgetin (Chart 2).

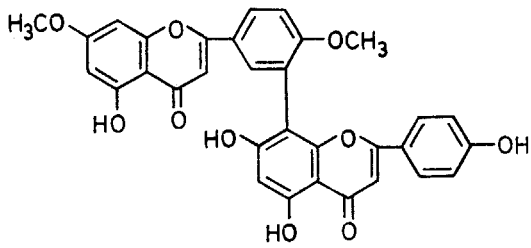


CHART 1*a*. Ginkgetin.

As already mentioned two more related compounds (partial methyl ethers) have been isolated from *G. biloba* leaves. They are isoginkgetin

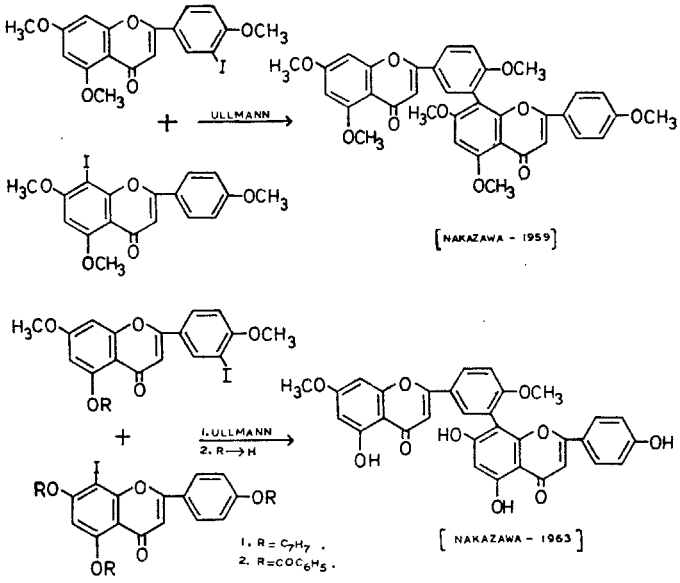
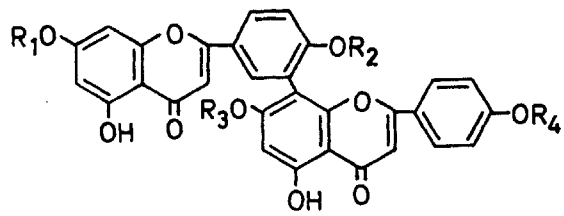


CHART 2. Synthesis of Ginkgetin and its methyl ether.

and bilobetin. The former is an isomeric dimethyl ether and the latter a related monomethyl ether. Soon many other methyl ethers of this group were isolated. The parent compound of all these ethers is called 'amentoflavone'. Originally it was considered to be rare; now several sources have been found. The trimethyl ether 'sciadopitysin' is of importance; it was isolated by Kariyone and Kawano in 1956 and their extensive work on it was complementary to the work of Baker *et al.* (1959) on ginkgetin (Chart 3).



No	Name	R_1	R_2	R_3	R_4
1.	Amentoflavone	H	H	H	H
2.	Bilobetin	H	CH_3	H	H
3.	Podocarpusflavone A	H	H	H	CH_3
4.	Sotetsuflavone	H	H	CH_3	H
5.	Ginkgetin	CH_3	CH_3	H	H
6.	Isoginkgetin	H	CH_3	H	CH_3
7.	Podocarpusflavone B	CH_3	H	H	CH_3
8.	Sciadopitysin	CH_3	CH_3	H	CH_3
9.	Kayaflavone	H	CH_3	CH_3	CH_3
10.	Amentoflavone tetramethyl ether	CH_3	CH_3	CH_3	CH_3

Chart 3. Amentoflavone series.

Hinokiflavone

A different type of biflavonoid was isolated by Kariyone and Sawada in 1958 from *Chamaecyparis obtusa*. It was named 'hinokiflavone' based on the Japanese name of the plant 'hinoki' (Chart 4). The constitution of this

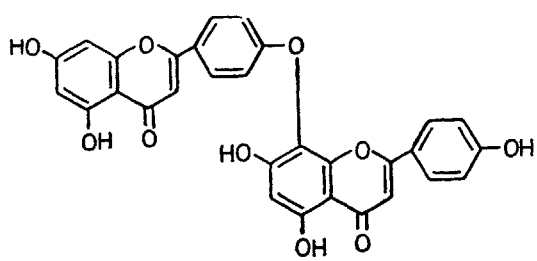


Chart 4. Hinokiflavone.

compound was arrived at by Kawano in 1960. It was also a dimer of apigenin, the molecular formula being $C_{30}H_{18}O_{10}$; it had no methoxyl groups. It formed a pentamethyl ether and pentaacetate. The UV pattern was

different from that of the earlier known biflavonyls but the UV of the complete methyl ether agreed with the UV of ginkgetin tetramethyl ether. By ferric colour reaction and IR data two hydroxyls were inferred to be in 5 and 5″-positions. From the degradations of hinokiflavone and its pentamethyl ether a diphenyl ether constitution was arrived at (Chart 5).

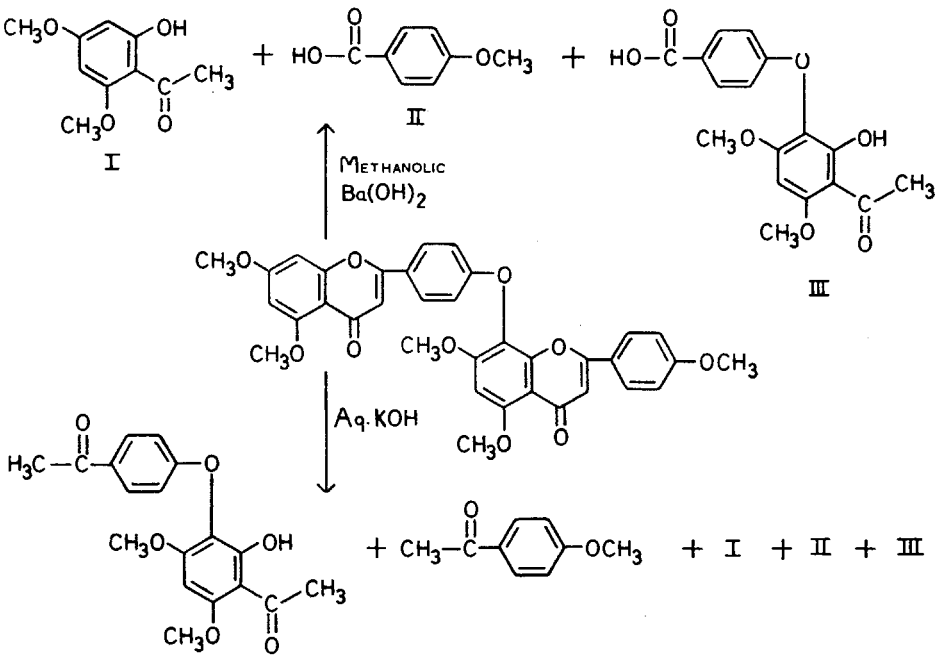


CHART 5. Chemical degradation of Hinokiflavone methyl ether.

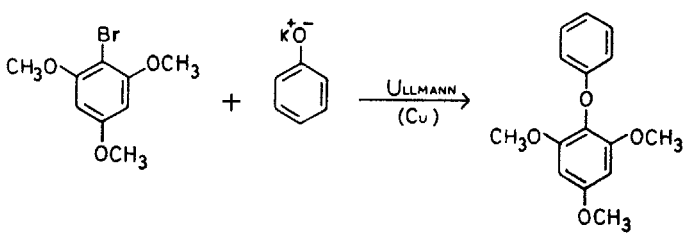


CHART 6. Synthesis of 2, 4, 6-trimethoxy diphenyl ether.

Among the products of degradation compound (III) having a diphenyl ether structure was important. It could be converted by methylation followed by oxidation and decarboxylation into 2, 4, 6-trimethoxy diphenyl ether. This could be synthesized by the Ullmann condensation of 2, 4, 6-trimethoxy-bromobenzene and potassium phenoxide. Thus the diphenyl ether structure was proved (Chart 6).

Since compound (III) gave positive reaction with Gibbs' reagent there was indication that the diphenyl ether link was ortho to the hydroxyl and the alternative possibility was eliminated. We attempted the synthesis of hinokiflavone pentamethyl ether through the Ullmann condensation of 8-iodoapigenin trimethyl ether and potassium salt of 4′-hydroxy-5, 7-dimethoxyflavone. There was partial demethylation in this reaction and remethylation was done to obtain the pentamethyl ether. The resulting product was identical with hinokiflavone pentamethyl ether. However, Nakazawa has recently claimed that he has synthesized both 4′, 8″ and 4′, 6″-isomers by unequivocal methods indicated in the formulae given below and only the 4′, 6″-compound agrees with the natural sample. This is explicable if in the above Ullmann synthesis the partial demethylation that takes place leads to isomeric change of the biflavonoid system. This is supported by his observation that synthetic 4′, 8″-biflavonyl compound gives natural hinokiflavone when boiled with hydroiodic acid. On this basis, the structure of hinokiflavone and the partial methyl ethers require revision (Chart 7).

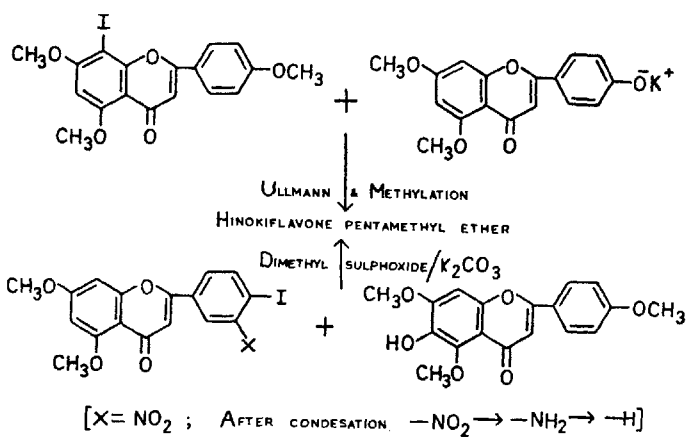


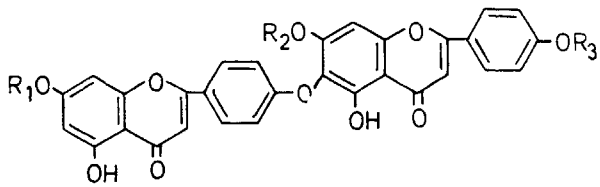
CHART 7. Synthesis of Hinokiflavone methyl ether.

Hinokiflavone is now found to occur fairly widely in nature; several of its mono- and dimethyl ethers are also found (Chart 8).

Cupressuflavone

While looking for Indian sources of biflavonoids, we isolated from the leaves of *Cupressus torulosa* and *Cupressus sempervirens* (Figs. 3, 4) a new biflavone which belonged to a C–C type and called it cupressuflavone. In 1964, it was assigned the constitution of 8, 8″-biapigeninyl. Its UV spectrum was similar to that of apigenin (peaks at 226, 274 and 330 mμ). The intensity of the UV maxima suggested that cupressuflavone was a biflavone. IR spectrum indicated the presence of chelated hydroxyl (s). Its molecular

formula was found to be $C_{30}H_{18}O_{10}$. It did not contain any methoxyl or C-methyl groups. It gave a hexamethyl ether and hexaacetate. On oxidation cupressuflavone hexamethyl ether yielded anisic acid as the only



No.	Name	R_1	R_2	R_3
1.	Cryptomerin A	H	H	CH_3
2.	Isocryptomerin	H	CH_3	H
3.	Neocryptomerin	CH_3	H	H
4.	Chamaecyparin	CH_3	CH_3	H
5.	Cryptomerin B	H	CH_3	CH_3

CHART 8. Hinokiflavone series.

FIG. 3. *Cupressus torulosa.*

FIG. 4. *Cupressus sempervirens.*

isolable entity. The NMR spectrum of the complete methyl ether of cupressuflavone was symmetrical. From the coupling pattern and the chemical shifts of the aromatic protons, it was inferred that oxygenation was at 5, 7, and 4′-positions. On these considerations the following constitution was proposed (Chart 9).

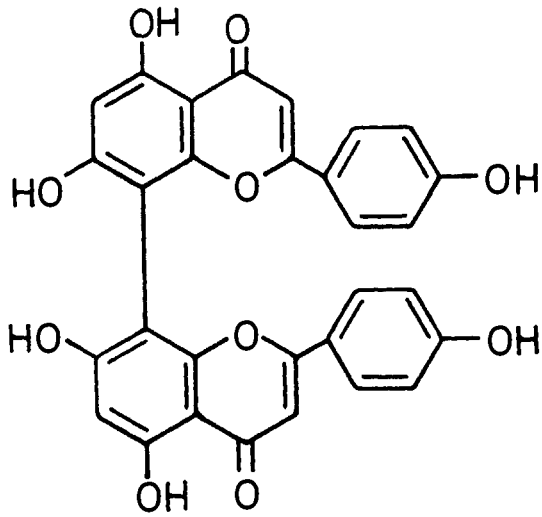


CHART 9. Cupressuflavone.

The decision between these two structures was sought to be made by the synthesis starting from 8-iodoapigenin trimethyl ether and subjecting this to Ullmann condensation. The synthetic hexamethyl ether was identical with the natural sample and from it cupressuflavone itself and its hexaacetate were obtained. But the possibility of rearrangement during these reactions has not been fully eliminated (Chart 10).

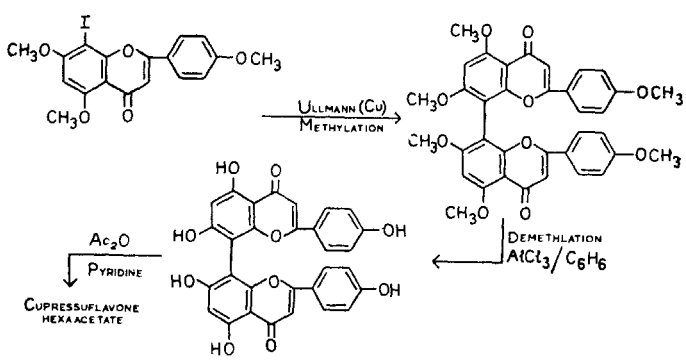


CHART 10. Synthesis of Cupressuflavone.

Recently cupressuflavone tetramethyl ether (7, 7″, 4′, 4‴-) and dimethyl ether (4′, 4‴-) were isolated by Rahman *et al.* (1968) from the leaves of

Araucaria cunnighamii and *A. cookii* (Fig. 5). These compounds are important because they exhibit optical activity arising from restricted rotation.

FIG. 5. *Araucaria columnaris* (Syn. *A. cooki*).

Biogenesis

The biogenesis of the biflavonoids seems to involve oxidative coupling of polyphenols. It has been well known for a very long time that phenols are readily oxidized by many different reagents. The products are complicated mixtures of dimeric, polymeric and quinonoid types of compounds. This method is useful for the synthesis of simple and complex compounds when coupled with efficient methods for the separation of the complex mixtures.

Phenol coupling has been suggested as an important step in the biogenesis of different classes of natural products and has stimulated intensive research. In general, the dimeric and polymeric oxidation products can be formulated by abstracting one hydrogen atom from the starting phenol and coupling together these intermediates by C–C and C–O bonds at active nuclear positions o- and p- to the hydroxyl. We owe mainly to Barton (1956) the development of these ideas leading to the synthesis of usnic acid and many others by oxidative phenol coupling.

The above considerations make it probable that the biflavonyl structure could arise by oxidative coupling of the monoflavonoid precursor apigenin. The oxygenation pattern of the precursor restricts the interflavonyl linkage to 6, 8 and 3′-nuclear positions (Chart 11). In the formation of ethers, each of these positions may be linked with any of the three oxygens of the hydroxyl groups. However, among the large number of possibilities only a few are so far known and this may be due to some factor of preferential activation. A later stage of methylation can yield the known methyl ethers. Support for this scheme is provided by the fact that in a number of plants apigenin is also present along with the biflavonyl pigments. Some experiments in our laboratory using one electron transfer reagents confirm the scheme.

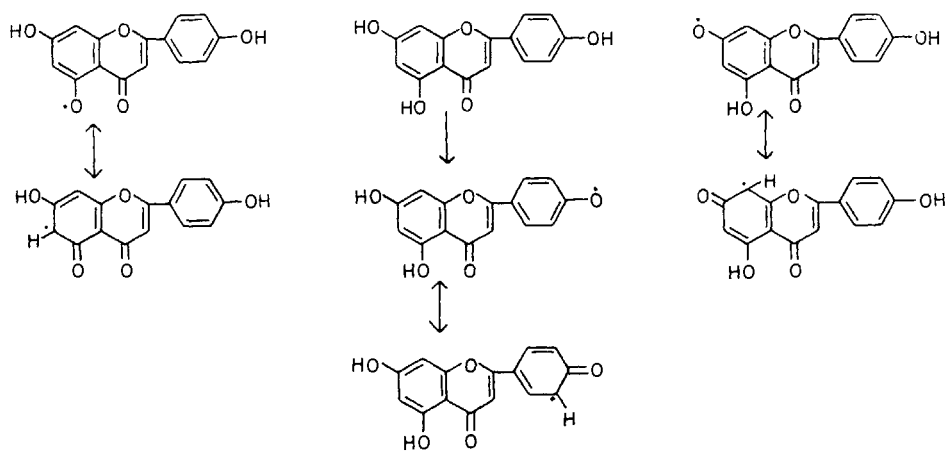


CHART 11. Free radical intermediates of oxidative coupling.

Study of Casuarinaceae

The study of the chemical components of the gymnosperms, particularly the conifers, will be useful for their possible application as drugs. There is also interest in regard to the taxonomy of the plants since they contain biflavonoids as special components. I may mention the study of *Casuarina* as a typical example. This genus has about 40 species chiefly found in Australia and in Indo-Malayan region. Of these, *Casuarina equisetifolia* is commonly

cultivated in India (Fig. 6). There is some difference of opinion regarding the taxonomic position of the genus. Sawada examined the leaves of *Casuarina stricta* and found that hinokiflavone is present in them. Earlier investigations had shown that the leaf-wax of this plant gives on hydrolysis juniperic acid (16-hydroxy palmitic acid) another typical gymnosperm constituent. The

FIG. 6. *Casuarina equisetifolia.*

occurrence of these two gymnosperm compounds in the leaves of *C. stricta* lent support to the view that Casuarinaceae is close to the gymnosperms. However, Batesmith (1962) reported that *C. cunnighamii* contains only kaempferol and quercetin. Our re-examination of the leaves of Indian trees

revealed the presence of cupressuflavone. We also examined *Casuarina suberosa* and it contained hinokiflavone. These results may help to settle the taxonomic position of the genus (Chart 12).

No	Source	Biflavones Identified
	Cupressaceae	
1.	C. funebris	C, H, A, H-7″OCH$_3$.
2.	C. sempervirens	C, A.
3.	C. torulosa	C, A, H.
4.	Biota orientalis	H.
	Casuarinaceae	
1.	C. cunninghamiana	C.
2.	C. suberosa	H.

C–Cupressuflavone H-7″OCH$_3$–Isocryptomerin
A– Amentoflavone H–Hinokiflavone

Chart 12. Chemical components of some species of Cupressaceae and Casuarinaceae.

Study of mass-spectral fragmentation

During our study of the biflavonyl pigments of Cupressaceae we have found the presence of partial methyl ethers of the three known types of biflavones and also new types of biflavones. They are present in very minor amounts and chemical degradation cannot be carried out for arriving at their structures. Mass spectra can be utilized in these cases and we have therefore made a critical study of the three types of biflavones known. Since the hydroxy compounds are very high melting the spectra of their complete methyl ethers have been recorded. From such a study, we can distinguish diphenyl and diphenyl ether type of biflavones and the position of the methoxyls can also be fixed.

Other biflavonyl compounds

Other modes of biflavonyl linking has been shown to be possible. Recently Jackson *et al.* (1967) isolated three compounds of a new dimer that could be called flavanonyl flavanone (biflavanone) from the heartwood of *Garcinia buchanii*. This involves the linking of two flavanone units at 3 and 8 positions. Their constitutions were arrived at by UV, IR, NMR and mass spectra. In these compounds both the units consist of flavanone units. Morelloflavone, a flavonyl-flavanone, isolated by Venkataraman *et al.* (1967) from the pericarp of the seeds of *Garcinia morella* contains a linking between a flavanone and flavone. Xanthorrhone and 14-hydroxy xanthorrhone which

were isolated by Pelter *et al.* (1967) from Australian xanthorrhea contain a flavanone and flavan units (Chart 13).

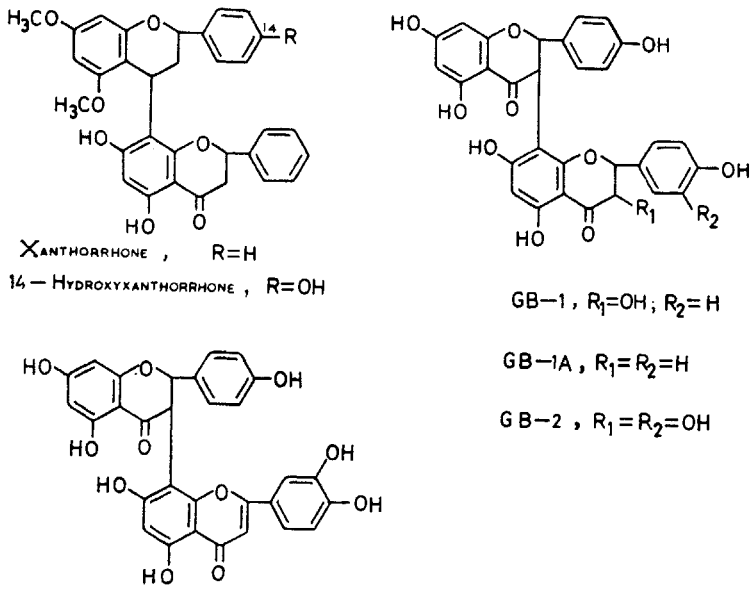


CHART 13. Recent biflavonyl compounds.

More widespread and important is another group of this type which has also been studied with some clarity during the past ten years. They are the proanthocyanidins (Chart 14). Under the name leucoanthocyanidins, they

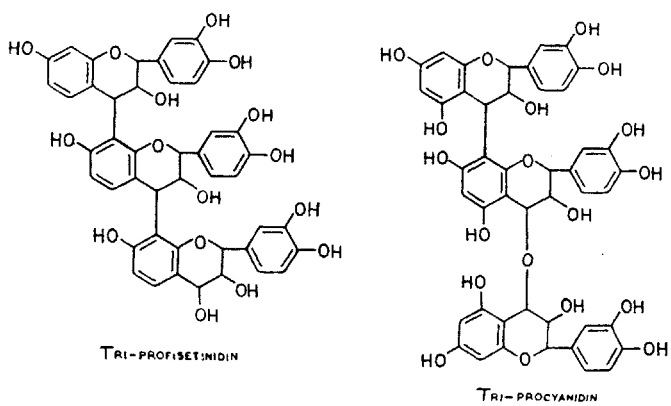


CHART 14. Proanthocyanidins.

had earlier attracted considerable attention as components of tanning materials and as astringent principles in foods and drugs. They are also capable of readily forming polymeric resins and a number of them have been isolated

in recent years. They are made up of either two leucoanthoanthocyanidin units or of leucoanthocyanidin and catechin. But the linking is easily broken by mineral acids, yielding eventually the corresponding anthocyanidin and catechin. Some typical examples are given in Chart 14. Frequently we obtain trimers and higher polymers.

ACKNOWLEDGEMENTS

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