CHEMISTRY OF NATURAL PRODUCTS

RESUME

Thesis Submitted for the Degree of Doctor of Philosophy in CHEMISTRY

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**RESUME**

The theoretical part of the thesis includes a critical review of the chemistry of flavanoids and biflavanoids and highlights the recent advances in the analytical technique applied to their isolation and structure elucidation. A brief introduction of acylated glycosides along with few recently isolated examples of acylated flavone glycosides have also been included in the theoretical portion.

The work described in the thesis consists of isolation and characterisation of flavanoids, biflavanoids and acylated glycosides from the leaves/nuts of seven plants of different families. Taxonomical significance and medicinal importance in some cases have also been discussed in brief.

The list of plants, investigated for their phenolic constituents, are listed as under:

1. *Cupressus lawsoniana* A. Murr; (Syn. *C. nutkanus*) (Cupressaceae).
2. *Fitzroya patagonica* Hook.f. (Cupressaceae)
3. *Rhus punjabensis* Stew. ex Brand. (Anacardiaceae)
4. **Podocarpus taxofolia** Kunth (Podocarpaceae)
5. **Semecarpus praehit** King (Anacardiaceae)
6. **Semecarpus kurzii** Engler (Anacardiaceae)
7. **Lycopodium cernuum** Linn. (Lycopodiaceae)

1. **BIFLAVONES FROM THE LEAVES OF CUPRESSUS LAWSONIANA A. MURR. (CUPRESSACEAE)**

TLC examination (BPF, 36:9:5) of phenolic extractives of the leaves of *C. lawsoniana* after solvent fractionation and column chromatography revealed the presence of five bands which were labelled as CLI, CLII, CLIII, CLIV and CLV in order of increasing $R_f$ values. They were separated by preparative layer chromatography (silica gel, BPF, 36:9:5). The fractions CLI, CLIV and CLV on methylation with dimethyl sulphate gave the same methyl ether identified as amentoflavone hexamethyl ether. However, the chromatographically homogeneous fraction CLIII on methylation was found, on TLC examination, to be a mixture of amentoflavone, hinokiflavone pentamethyl ether. CCD separation of CLIII followed by acetylation showed the presence of sequoiaflavone and hinokiflavone. The following five biflavones and a monoflavone have been isolated and characterized by UV and $^1H$-NMR studies of their methyl
and acetyl derivatives and comparison with authentic samples (R_f values, m.p., m.m.p., characteristic fluorescence in UV light and 1_H-NMR) are listed as under:

CLI

(i) I-4',II-4',I-5,II-5,I-7,II-7-Hexahydroxy I-3',II-8_7 biflavone (amentoflavone).

CLII

(ii) 3,3',4',5,7-Pentahydroxyflavone (quercetin).

CLIII

(iii) I-4',II-4',I-5,II-5,II-7-Pentahydroxy I-7-O-methyl I-3',II-8_7 biflavone (sequoi aflavone).

(iv) II-4',I-5,II-5,I-7,II-7-Pentahydroxy I-4'-O-II-6_7 biflavone (hinokiflavone).

CLIV

(v) I-4',II-4',I-5,II-5-Tetrahydroxy I-7,II-7-di-O-methyl I-3',II-8_7 biflavone.

CLV

(vi) I-4',I-5,II-5-Trihydroxy I-7,II-7,II-4'-tri-O-methyl I-3',II-8_7 biflavone (heveaflavone).
Glycosidic fraction on usual workup and hydrolysis showed the presence of following two glycosides.

*(i) Quercetin-O-glycoside
*(ii) Kaempferol-O-glycoside.

The presence of 1-4',I-5,II-5-Trihydroxy-I-7, II-7,II-4'-tri-O-methylamentoflavone constitutes the first report of isolation and characterisation in the family cupressaceae and probably the second example for its occurrence in nature. The first one being from *hevea brasiliensis*. The compound I-7,II-7-di-O-methyl amentoflavone is also very rare in nature, and is being reported for the first time in *cupressus*. The absence of all the members of cupressusflavone group in *cupressus lawsoniana* and the occurrence of amentoflavone series in a systematic sequence of methylation (amentoflavone \(\rightarrow\) I-7 \(\rightarrow\) I-7,II-7 \(\rightarrow\) I-7,II-7,II-4') are also noteworthy.

2. **BIFLAVONES FROM THE LEAVES OF FITZROYA PATAGONICA HOOK,F. (CUPRESSACEAE)**

The phenolic extractives of the leaves of *Fitzroya patagonica*, the single species of monotypic genus *Fitzroya*, on solvent fractionation, column chromatography and preparative TLC (silica gel; BPF,
36:9:5) gave three compact bands which were labelled as FPI, FPII and FPIII in order of increasing Rf values. FPI and FPII although chromatographically homogeneous were found, on complete methylation and TLC examination (BPF, 36:9:5) to be mixtures of more than one components. The flavanoid and biflavanoid contents of each of the three fractions, characterised by UV and $^1$H-NMR studies of their methyl derivatives are listed below:

**FPI**

(i) I-4',II-4',I-5,II-5,I-7,II-7-Hexahydroxy [I-3',II-8] $^\perp$ biflavone (amentoflavone).

(ii) I-4',II-4',I-5,II-5,I-7,II-7-Hexahydroxy [I-8,II-8] $^\perp$ biflavone (cupressuflavone).

(iii) I-4',II-4',I-5,II-5,I-7,II-7-Hexahydroxy [I-3',II-6] $^\perp$ biflavone (robustaflavone)

**FPII**

(iv) II-4',I-5,II-5,I-7,II-7-Pentahydroxy [I-4'-0,II-6] $^\perp$ biflavone (hinokiflavone)

*(v)* Mono-0-methylamentoflavone.
**(vi)** 5,7,4′-Trihydroxyflavone (apigenin).

### 3. BIFLAVONES FROM RHUS PUNJABENSIS STEW. EX BRAND. (ANACARDIACEAE)

TLC examination (BPF, 36:9:5) of phenolic extractives of the leaves of *Rhus punjabensis* after solvent fractionation and column chromatography revealed the presence of two bands, labelled as RPI and RPII in order of increasing Rf values. The following four biflavones have been isolated (PLC, silica gel, BPF, 36:9:5), identified and characterised by comparison of authentic samples with characteristic fluorescence in UV light, m.p., m.m.p. and 1H-NMR studies of their methyl and acetyl derivatives with those of authentic samples.

**RPI**

1. I-4′,II-4′,I-5,II-5,I-7,II-7-Hexahydroxy 1-6,II-8 biflavone (agathisflavone).

*(ii)* Amentoflavone

*(iii)* Robustaflavone
4. BIFLAVONES FROM PODOCARPUS TAGIFOLIA KUNTH
(PODOCARPACEAE)

The acetone extracts of the leaves of *P. taxifolia* after solvent fractionation, column chromatography and preparative TLC yielded five bands which were labelled as PT₁, PT₂, PT₃, PT₄ and PT₅ in order of increasing *R*ᵢ values. PT₁ and PT₂ were characterised earlier as amentoflavone and bilobetin, sequoiaflavone and podocarpsflavone-A respectively. The fraction PT₅ was characterised as I-5, II-5-dihydroxy-I-7,II-7,I-4',II-4'-tetra-O-methylamentoflavone by \(^1\)H-NMR studies of the parent compound and comparison of methyl and acetyl derivatives with those of authentic samples (*R*ᵢ value, characteristic fluorescence in UV light, m.p. and \(^1\)H-NMR). The biflavanoid contents of each of the last three fractions (PT₃, PT₄, and PT₅) are as under:

PT₅

(1) I-5,II-5-Dihydroxy-I-4',II-4',I-7,II-7-tetra-O-methyl \(^1\)I-3',II-8\) biflavone.
• (ii) Tri-O-methylamentoflavone

• (iii) Di-O-methylamentoflavone. The presence of tetra-O-methylamentoflavone constitutes the first report in the genus podocarpus.

**BIFLAVANOIDS FROM SEMECARPUS PRAINII KING (ANACARDIACEAE)**

The following two components were isolated and characterised from acetone extract of the defatted nuts of *S. prainii*.

(i) **SPI**: I-4',II-4',I-5,II-5,I-7,II-7-Hexahydroxy √I-3',II-8√ biflavaneone (tetrahydroamentoflavone).

(ii) **SPII**: 5,7,4'-Trihydroxyflavanone (naringenin).

The structure of SPI as I-3',II-8-binaringenin was established by UV, IR, mass, $^1$H-NMR and $^{13}$C-NMR studies.

The structure of tetrahydroamentoflavone (SPI) was further supported by its dehydrogenation with I$_2$-DMSO-H$_2$SO$_4$ reagent system followed by methylation/acetylation of the
dehydrogenated product and comparison with authentic samples of amentoflavone derivatives ($R_f$ value, m.p., characteristic fluorescence in UV light and $^1$H-NMR studies).

The isolation and characterisation of parent tetrahydroamentoflavone constitutes the first report in *Semecarpus* species.

**TETRAHYDROAMENTOFLAVONE FROM NUTS OF SEMECARPUS KURZII ENGLER (ANACARDIACEA)**

The acetone extract of defatted nuts of *S. kurzii* on solvent fractionation and column chromatography gave a single component labelled as SK, which was characterised as tetrahydroamentoflavone by dehydrogenation and spectral studies as described in *Semecarpus kurzii* prunifolia.

7. **ACYLATED GLYCOSIDES FROM THE LEAVES OF LYCOPODIUM CERNUUM LINN (LYCOPODIACEAE)**

TLC examination (TEF, 5:4:1) of phenolic extractives of the leaves of *L. cernuum* after solvent fractionation and column chromatography revealed the presence of three bands which were labelled as LCI (minor), LCII (minor) and LCIII (major) in order of increasing $R_f$ values. The structure of LCIII (major) was established by UV, IR,
mass, $^1$H-NMR, $^{13}$C-NMR studies of the parent, methyl and acetyl derivatives. The contents of each of the three fractions are given below:

**LCI**
*(i) Acylated apigenin-0-glycoside*

**LCII**
*(ii) Acylated apigenin-0-glucoside.

**LCIII**
*(iii) Apigenin 4'-0-(2',6'-di-O-p-coumaroyl-β-D-glucoside)*

(LCIII)
Acid hydrolysis of LCIII with 10% HCl yielded apigenin, p-coumaric acid and glucose. Treatment of LCIII with methanolic sodium methoxide yielded apigen-4'-O-glucoside and methyl-p-coumarate. Permethylation of LCIII gave hexa-O-methyl ether, the acid hydrolysis of which yielded 5,7-dimethoxy-4'-hydroxyflavone. This indicate that the acyl residue is linked to the glucose moiety. The high molecular weight of hexamethyl ether of LCIII m/e 808 shows the presence of two p-coumaroyl residues. The position of p-coumaroyl groups at the glucose moiety was assigned by $^{13}C$-NMR studies.
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Aligarh,
This is to certify that the work described in the thesis entitled "CHEMISTRY OF NATURAL PRODUCTS" is the original work of the candidate and is suitable for submission for the award of Ph.D. degree in Chemistry.

(M. ILWA3)
Supervisor.
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(QUAL AHMAD)
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THEORETICAL
but also because many members (e.g. coumestrol, phloridzin, rotenone) are physiologically active. They are universally distributed among vascular plants and found practically in all parts of plants.

The flavanoids belong to important group of naturally occurring compounds in which two benzene rings are linked by a propane bridge \((C_6-C-C-C-C_6)\) except in isoflavones in which the arrangement is \((C_6-C-C-C)\), and include chalcone (III), dihydrochalcones (II) aurones (I), flavanones (IV), flavones (VII), flavanonols (VI), flavonols (VIII), isoflavones (V), leucoanthocyanidins (XII), anthocyanidins (IX), proanthocyanins and catechins (I). In these compounds the oxidation level of \(C_3\) bridge varies from the lowest in catechin (I) to the highest in flavonol (II).
INTRODUCTION

Isolation and examination of natural products have drawn considerable attention of chemists from very early days. Many valuable products were obtained from the natural wealth and used for innumerable purposes in the past for human welfare and various other activities. Although the natural products have been obtained mostly from plant sources, still only a meagre percentage of the plant world has been explored for its chemical constituents, particularly in India where there is abundance of wide variety of vegetation since this country possesses different types of climatic conditions and soil etc.

The classes of organic compound generally associated with the term 'Natural Products' include mainly alkaloids, carbohydrates, fats, fatty acids, proteins, steroids, terpenoids, essential oils, carotenoids, vitamins, glycosides and numerous derivatives of heterocycles (e.g. flavanoids and coumarines).

The flavanoids, one of the most numerous and widespread groups of natural constituents, are important to man not only because they contribute to plant colour
Gabor has reviewed recent trends in research on the pharmacodynamic effects of flavanoids, mostly rutin and its derivatives. Flavone itself possesses coronary dilating action. With the extensive screening programmes of plant products for anticancer drugs, it is not surprising that claims have been made that flavanoids may contribute to, or be effective in combating, certain types of cancer. Numerous other physiological activities have been attributed to flavanoids. The potent uses of flavanoids may be listed as heart stimulants, contraceptive drugs, antibiotic effects coronary vasodilators, antiphlogistic, chloretic, spasmolytic and antihistamine activity, oestrogenic activity, antihelmitic activity, antiviral effects, anticonvulsant, analgesic and bronchodilator activity, treatment of allergic diseases, antitumor effects, vitamin P activity, effective inhibitors of blood cell aggregation and antioxidants. Since 1962, most plant surveyor
in taxonomically groups have included flavanoid studies
and indeed these pigments now occupy a pre-eminent position
as the most favoured of all plant constituents as taxonomic
makers.20c,22

During the last twelve years, a number of natural
products have been isolated, which are dimers of simpler
compounds known as biflavonoids. Biflavonoids are the
most recent addition to this class which are recognised
by having two flavanoid units and mostly isolated from
Gymnosperms. Among the angiosperms, some plants belonging
to Guttifaraceae, 23,24 Siphonospermae, 25,26 Caprifoliaceae, 27
Ochnaceae, 28 Casuarinaceae, 29 Rhamnaeae, 30 anacardiaceae 31
and some ferns belonging to sellaginellaceae 32 and
Psilotales 33 have been found to contain biflavonoids.

All the biflavonoids known to date may be classified
into two main groups.

A. C-C linked biflavonoids
B. C-O-C linked biflavonoids

A. C-C linked biflavonoids

Depending upon the nature of constituent monomeric
units and of the position of linkage we have different
series.
1. *Amentoflavone Series*

These are derived from two *apigenin* units with $\text{I-3',II-8}\}$ linkage and are represented by seventeen members with *amentoflavone* (XIII) as the parent compound.

\[
\begin{align*}
\text{(XIII)}
\end{align*}
\]

| (a) | Amentoflavone $^{34-36}$ | H | H | H | H | H | H | H |
| (b) | I-7-O-Methyl $^{37,38}$ (Sequoiaflavone) | Me | H | H | H | H | H | H |
| (c) | II-7-O-Methyl $^{39,40,44}$ (Sotetsuflavone) | Me | H | H | H | H | H | H |
| (d) | I-4'-O-Methyl (Bilobetin) $^{28,41}$ | H | H | H | H | Me | H |
| (e) | II-4'-O-Methyl (Podocarpus-flavone-A) $^{43,85}$ | H | H | H | H | H | H | Me |
(f) \( \text{I-7, I-4'-Di-O-methyl} \) (Ginkgetin)\(^3,4\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{H} \]

(g) \( \text{II-7, II-4'-Di-O-methyl} \) \(^4\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{H} \quad \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \]

(h) \( \text{I-7, II-7-Di-O-methyl} \) \(^3,4,7\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \]

(i) \( \text{I-4', II-4'-Di-O-methyl} \) (Isoginkgetin)\(^41,42\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{X} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{Me} \]

(j) \( \text{I-4', II-7-Di-O-methyl} \) \(^45\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{H} \]

(k) \( \text{I-7, II-4'-Di-O-methyl} \) (Podocarpus flavone-B)\(^42,45\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \]

(l) \( \text{I-7, I-4', II-4'-Tri-O-methyl} \) (Sciadopitysin)\(^3,41,42\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{Me} \]

(m) \( \text{I-7, II-7, II-4'-Tri-O-methyl} \) (Hevea flavone)\(^26,46\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{Me} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \]

(n) \( \text{I-4', II-4', II-7-Tri-O-methyl} \) (Kay flavone)\(^41,43,45\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{H} \quad \text{Me} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{Me} \]

(o) \( \text{I-4', I-7, II-7-Tri-O-methyl} \) \(^39\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{Me} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{H} \]

(p) \( \text{I-7, II-7, I-4', II-4'-Tetra-O-methyl} \) \(^47,48\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{Me} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{Me} \]

(q) \( \text{I-4', I-1', I-3, II-5, I-7, II-7-Hexa-O-methyl} \) (Dioon flavone)\(^49\)

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \quad R_6 \]
\[ \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \]
2. **I-2,3-Dihydroaemantoflavone Series**

These are derived from a naringenin and an apigenin units with flavanone \( I-3',II-8' \) linkage, and are represented by four members, with I-2,3-dihydroaemantoflavone as the parent compound while the other two are its partial methyl ethers.

![Chemical Structure](image)

\[(XIV)\]

<table>
<thead>
<tr>
<th></th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
<th>( R_4 )</th>
<th>( R_5 )</th>
<th>( R_6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) I-2,3-Dihydroaemantoflavone(^{80,92})</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>(b) I-7,II-7-D1-0-methyl(^{44,92})</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Me</td>
<td>Me</td>
</tr>
<tr>
<td>(c) I-7,II-4'-II-4'-Tri-0-methyl(^{44,92})</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Me</td>
<td>Me</td>
</tr>
<tr>
<td>(d) I-4',II-4',I-5,II-5, I-7,II-7-Hexa-0-methyl(^{44})</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
</tr>
</tbody>
</table>

* Synthetic
3. Tetrahydroamentoflavone series

I-2,3,II-2,3-Tetrahydroamentoflavone (XV) has been isolated from the nuts of Semecarpus species.51,53

![Chemical structure of Tetrahydroamentoflavone (XV)](image)

4. I-7-O-Methyl-I-6-C-methylamentoflavone

Rahman et al.54 have recently isolated I-7-O-methyl-I-6-C-methylamentoflavone (XVI) from the leaves extracts of Cephalotaxus harringtonia C. Koch. This is derived from 6-C-methylgenkwanin and apigenin with \[\text{I-3',II-8}\] linkage.
5. Cupressuflavone Series

These are derived from two apigenin units with $1-3,11-3$ linkage and are represented by nine members. Cupressuflavone (XVII) is the parent compound while the other seven are its partial methyl ethers.
(a) Cupressusflavone\textsuperscript{55,56} & R_1 & R_2 & R_3 & R_4 & R_5 & R_6 \\
(b) I-4',0-Methyl\textsuperscript{57} & H & H & H & H & H & H \\
(c) I-7-0-Methyl\textsuperscript{55,59} & Me & H & H & H & Me & H \\
(d) I-7,II-7-Di-0-methyl\textsuperscript{59,60} & Me & Me & H & H & H & H \\
(e) I-4',I-7 (or II-4',I-7) & Me & H & H & H & Me & Me \\
(f) I-4',I-7,II-7-Tri-0-methyl\textsuperscript{59} & Me & Me & H & H & Me & H \\
(g) I-4',II-4',I-7,II-7-Tetra-0-methyl\textsuperscript{60,61} & Me & Me & H & H & Me & Me \\
(h) I-4',II-4',I-5,II-7,II-7-Penta-0-methyl\textsuperscript{62} & Me & Me & Me & H & Me & Me \\
(i) I-4',II-4',I-5,II-5,II-7,II-7-Hexa-0-methyl\textsuperscript{55,63} & Me & Me & Me & Me & Me & Me \\

* Synthetic

5. Mesuaferrone-A and Mesuaferrone-B

Subramanyam et al.\textsuperscript{64} have isolated Mesuaferrone-A (XVIII) and Mesuaferrone-B (XII) from the stamens of Mesuaferrrea. Mesuaferrone-A (Tetrahydrocupressusflavone) is derived from two naringenin units with $[\overline{I-8,II-8}]$ linkage and Mesuaferrone-B (Dihydrocupressusflavone) is a dimer of a naringenin and an apigenin units through $[\overline{I-8,II-8}]$ linkage.
7. Agathisflavone Series

These are derived from two apigenin units with
\([1-6,1-2] \text{ linkage}\) and are represented by five members
with agathisflavone (XII) as the parent compound.
The Rhusflavanone (Tetrahydroagathisflavone) has been isolated from seed kernels of Rhus succedanea. This is derived from two naringenin units with [I-6,II-8] linkage.
11. **Succedanea flavanone**

This is derived from two naringenin units with \( I-6, II-6 \) linkage and isolated from seed kernel of *Rhus Succedanea*. However, its hexamethyl ether has, very recently been synthesised by Parthasarthy et al.\(^6\)
12. **Taiwaniaflavone Series**

A new series of naturally occurring biflavones have been isolated from *Taiwania cryptomerioides Hayata* as the parent and its partial methyl ethers. These are derived from two apigenin units with $[I-3,II-3']$ linkage. Although $[I-3,II-3']$ bisapigenin has already been reported as one of the oxidative coupling products of apigenin.

![Chemical Structure](image)

\[(XXV)\]

<table>
<thead>
<tr>
<th></th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>$R_5$</th>
<th>$R_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Taiwaniaflavone $^{72}$</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>(b) I-7-O-Methyl $^{72}$</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>(c) I-7,II-4'-O-Methyl $^{72}$</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Me</td>
</tr>
<tr>
<td>(d) I-4',II-4',I-5,II-5, I-7,II-7-Hexa-O-methyl $^{72}$</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
</tr>
</tbody>
</table>

* Synthetic
13. **G.B-Series** \(^{24,155-157}\)

This series comprises of reduced heterocyclic system. Six members are reported to occur in nature. These are derived from a naringenin linked with naringenin or aromadendrin a taxifolin or eriodictyol through \(\angle 1-3, II-8 \angle\) linkage.

![Diagram](image)

(XXVI)

(a) **Manniflavanone** \(^{99}\)  \(R_1=R_2=R_3=R_4=O\)H
(b) **G2-1a** \(^{24,155-157}\)  \(R_1=O\)H;  \(R_2=R_4=E\)
(c) **G2-1a** \(^{24,155-157}\)  \(R_3=O\)H;  \(R_1=R_2=E\)
(d) **G2-2a** \(^{24,155-157}\)  \(R_1=R_3=O\)H;  \(R_2=H\)
(e) **G2-2a** \(^{24,155-157}\)  \(R_3=R_4=O\)H;  \(R_1=R_2=H\)
(f) **Kolaflavanone** \(^{100}\)  \(R_1=R_3=O\)H;  \(R_3=OMe; R_2=H\)

14. **BGH Series**

These are derived from a naringenin and an apigenin or luteolin unit with flavanone \(\angle 1-3, II-8 \angle\) flavone
linkage and are represented by BGE-II (XXVIIa) and BGE (XXVIIg) as the parent compounds respectively.

![Molecular structure diagram](image)

(XXVII):

\[
\begin{array}{cccccccccc}
R_1 & R_2 & R_3 & R_4 & R_5 & R_6 \\
(a) & \text{BGE-II (Moraflavone - 29, 74, 75)} & \text{Me} & \text{H} & \text{H} & \text{H} & \text{Me} \\
(b) & \text{II-3'-O-Methyl-75} & \text{Me} & \text{Me} & \text{Me} & \text{Me} & \text{Me} \\
(c) & \text{I-4', II-6', III-2', IV-3'} & \text{Me} & \text{Me} & \text{Me} & \text{Me} & \text{Me} \\
(d) & \text{I-4', II-2', III-2', IV-2'} & \text{Me} & \text{Me} & \text{Me} & \text{Me} & \text{Me} \\
(e) & \text{I-4', II-6', III-2', IV-3'} & \text{Me} & \text{Me} & \text{Me} & \text{Me} & \text{Me} \\
(f) & \text{I-4', II-2', III-2', IV-2'} & \text{Me} & \text{Me} & \text{Me} & \text{Me} & \text{Me} \\
\end{array}
\]
15. WGH Series 23, 77

Two new biflavones, WGH-II and WGH-III have been synthesised by dehydrogenation of BGH-II and BGH-III respectively.

\[ \text{(XXIII)} \]

(a) \( R = \text{CH}; 1-4',11-2',12-6',1-5,11-5,1-7,11-7- \)
Kepthydroxy \( \int_{11-9,1-5}^{11-8} \) biflaven (WGH-II or Sanaranflavone)

(b) \( R = \text{K} ; 1-9,1-4',12-5,1-7,11-7- \) Hexahydroxy \( \int_{1-3,11-8}^{1-3,11-8} \) biflaven (WGH-III)

16. \( \int_{1-2,11-6}^{1-2,11-6} \) chromone 80

This compound has been isolated from the leaves of *Geranium dulcis* Kurz. It is a dimer of naringenin and 5,7-dihydroxychromone linked through \( \int_{1-3,11-8}^{1-3,11-8} \). Its isolation has introduced a new series comprising of flavonone-carotene structure.
B. 

1. Tinokiiflavone Series

These are derived from two apigenin units with \[\gamma-4'\-0-II-6'\] linkage. Tinokiiflavone (XXX) is the parent compound with six others as its partial methyl ethers. Earlier tinokiiflavone and its derivatives were assigned \[\gamma-4'\-0-II-5\] linkage which has later been revised to \[\gamma-1'-0-II-3\].

[Diagram of chemical structures]
2. I-2,3-Dihydro hinokiflavone$^{44,92}$

The sole member (XXXI) has been isolated from *Metasequoia glyptostroboides* and cycas species.$^{44,92}$
3. **Ochnaflavone Series**

This class is represented by five members with ochnaflavone as the parent compound. They are derived from two apigenin units linked through \(\text{I-3'}-\text{O-II-4'}\).

\[
\begin{align*}
\text{(a) Ochnaflavone}^{28} & \quad H \quad H \quad H \quad H \quad H \\
\text{(b) I-4'-O-methyl}^{28} & \quad H \quad H \quad H \quad H \quad \text{Me} \\
\text{(c) II-7'-O-methyl}^{93} & \quad H \quad \text{Me} \quad H \quad K \quad H \\
\text{(d) I-7',II-5'-O-methyl-}^{28,94} & \quad \text{Me} \quad H \quad K \quad H \quad \text{Me} \\
\text{* (e) I-4',I-7,II-7'-Tri-O-methyl-}^{94} & \quad \text{Me} \quad \text{Me} \quad H \quad H \quad \text{Me} \\
\text{* (f) I-4',I-7,II-7',I-3,II-3'-Penta-O-methyl-}^{94} & \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \\
\end{align*}
\]

*Synthetic*
9. **Rhusflavone**

This flavanoneflavone is derived from naringenin and apigenin units linked through \(\text{I-3',II-8}\).

\[\text{Rhusflavone}^{69b}\]

These are derived from two apigenin units with \(\text{I-3',II-8}\) linkage. Before robustaflavones had been isolated from a natural source, its hexamethyl ether had been prepared by Waasely-Moser rearrangement of amentoflavone hexamethyl ether (XXIc).
Miscellaneous Synthetic Biflavanoids

This comprises several biflavanoids which might occur naturally, since their interflavonoyl links are situated between atoms which take part in the interflavonoyl link of naturally occurring biflavanoids.

\[(XXXIII)\]
\[I-5,II-5\text{-Dideoxycupressuflavone}\]

\[(XXXIV)\]
\[\text{I-3,II-3\text{-biapigenin}}^{73}\]

\[(XXXV)\]
\[I-5,II-5,I-7,II-7,II-4',I-4',\text{-pentahydroxy I-4',II-8\text{-bi flavone}}^{82}\]
Biflavonoid Glycosides

Guttiferae and anacardiaceae are the only families of angiosperms in which biflavonoid glycosides have been found. No biflavonoid glycoside so far has been reported from gymnosperms.

1. M. Konoshima et al. have isolated fukuside (XXXVIIa) and spicataside (XXXVIIb) from Garcinia spicatae and Xanthochymusside (XXXVIII) from Garcinia xanthochymus.
(XXXVII)
(a) Fukugiside; $R_1 = \text{OH}$, $R_\beta = \text{D-Gluc}$
(b) Spicataside; $R_1 = \text{H}$, $R_\beta = \text{D-Gluc}$.

(XXXVIII)
Xanthochymusside, $R_\beta = \text{D-Gluc}$. 
2. \(\left[1-3,11-8\right]\)-Binarigenin-11-7-0-\(\beta\)-Glucoside\(^96\)

A new biflavonone glucoside (XXXIX) has very recently been isolated from Garcinia multiflora.

\[
\begin{align*}
\text{(XXXIX)} \\
R = \text{Glucosyl}
\end{align*}
\]

3. Amentoflavone-0-glucoside

Wallace and Markham\(^97\) have reported very recently, a series of amentoflavone-0-glucosides from three species of Psilotales, an order that shows one of the most primitive organization of any living vascular plant. However, these authors could not establish the glucosyl pattern in the compound and suggested the structure as mono-, di-, tri- and tetra-0-glucosides of amentoflavone.
Murthy et al. \textsuperscript{93} have very recently isolated Occidentoside, a biflavanoid-C-glucoside in which a flavanone unit is linked with a chalcone unit via C-O-C linkage from defatted nuts of Anardium occidentale Linn. This constitutes the first example of biphenyl ether type biflavone glucoside with reduced heterocyclic ring system.

\textit{Acylated glycosides}

The term glycoside was adopted to embrace a large and remarkably varied group of organic compounds which on hydrolysis yield, in addition to sugar other substances, frequently of aromatic nature. The non-sugar part (aglycone) may include a wide variety of compounds occurring in nature. In the case of flavanoid glycosides this moiety is generally a phenolic compound. A large number of glycosides which were isolated from natural sources were characterised either as \textit{O}-linked or C-C linked glycosides.

The flavanoid glycosides also occur in acylated form with acid such as p-coumaric, caffeic, sinapic, ferulic, gallic, benzoic, p-hydroxybenzoic, acetic and
malonic. Of these, the most frequently found are p-coumaric and ferulic acid. Acylated glycosides may be recognised by their high chromatographic mobility on paper in solvents 15% acetic acid and phenol and low mobility in water, when compared with the corresponding unacylated glycoside.

Acylated glycosides have also distinctive spectral properties; those acylated with aromatic acids are readily distinguished by UV spectroscopy, since the aromatic acid absorption is superimposed on the normal flavanoidic spectral bands. The acyl group can then be removed by mild alkaline hydrolysis and the acid present recovered and identified by standard procedures.

In acylated glycosides, there is usually only one acyl group and this is almost invariably attached to one of the sugar hydroxyls and is not directly linked to the flavanoid skeleton.

(1) Quercetin-Galactoside-Gallate

Quercetin-3-β-D-galactopyranoside-2"-gallate (XLa) and quercetin-3-β-D-galactopyranoside-6"-gallate (XLb) have been isolated from Euphorbiaceae verrucosa and E. platiphylos respectively.
The flavanol-O-acyl glycoside (XLI) has been isolated from Salix viminalis.
(iii) Naringenin-7-O-\(^{6''}\)-O-p coumaroyl-\(\beta\)-D-glucoside

Rahman et al.\(^{103}\) have recently isolated an acylated flavanone glycoside (XLII) from nutshell of *Anacardium occidentale*.

![Chemical structure of Naringenin-7-O-\(^{6''}\)-O-p coumaroyl-\(\beta\)-D-glucoside](image)

(iv) Acylated Allose-containing 8-hydroxyflavone glycoside\(^{104}\)

A novel flavone glycoside has been isolated from the whole plant of *Vernonia zilliformis* and identified as *isosoulierein-4'-methyl ether 7-O-\(\beta\)-(6''-O-acetyl-2''-O-allosyl glucoside)*.

![Chemical structure of Acylated Allose-containing 8-hydroxyflavone glycoside](image)

\[ R = 6-O\text{-acetyl-}\beta\text{-D-allosyl-}\beta\text{-D-glucosyl} \]
(v) **Acylated Kaempferol Glycosides from Aconitum**

Four acylated kaempferol glycoside have been recently isolated from *Aconitum novoboraceense* and *A. columbianum*.

(i) Kaempferol 3-(Caffeoyl glucoside)-7-glucoside.

(ii) Kaempferol 3-gentiobioside-7-(cafeylarabinosyl thioside).

(iii) Kaempferol 3-glucoside-7-(p-coumaryl glucoside).

(iv) Kaempferol 3-(p-coumaryl rutinoside)-7-glucoside.

(vi) **Vitamin-2\(^{\text{\prime\prime}}\)-O-p-coumarate**

Vitamin-2\(^{\text{\prime\prime}}\)-O-p-coumarate (XLIq) has been isolated from *Trigonella foenum-graecum* (Leguminaceae).

(XLIq)
(vii) **Naringenin-7-<b>β</b>-O(6<sup>″</sup>-O-galloyl)-glucopyranoside**<sup>107</sup>

The flavanone-<b>β</b>-acylglycoside has been isolated from the pods of *Accacia farnesiana*. Thus the 7-<b>β</b>-glucoside of naringenin in which gallic acid is attached with 6<sup>″</sup> hydroxyl of glucose.

(viii) **Linarin-<b>α</b>-2-methylbutyrate**<sup>108</sup>

This has recently been isolated from *Valeriana wallichii*.

\[(a) \quad R_1 = \text{OH}; \quad R_2 = \text{Et(Me)} \quad \text{CH-CO} \\
(b) \quad R_2 = \text{Et}; \quad R_1 = \text{Et(Me)} \quad \text{CH-CO} \]
Acylated flavanone glycoside from *Mierembergia Hippomonica* (Solanaceae) 109.

Two acylated flavanone glycosides namely Pinocembrin 7-O-\(\beta\)(3'-O-acetyl) neohesperidoside (XLVIa) and Pinocembrin 7-O-\(\beta\)-(6'-O-acetyl) neohesperidoside (XLVIa) have been very recently isolated from *Mierembergia Hippomonica*.

\[
\text{(XLVIa) } R_1 = R_3 = \text{H}; \quad R_2 = \text{Ac}
\]
\[
\text{(XLVIb) } R_1 = R_2 = \text{H}; \quad R_3 = \text{Ac}
\]

**Optical activity in Biflavonoids**

A large number of optically active biflavonoids have been reported (Table 1). The optically active biflavones belonging to amantoflavone, cupressusflavone and agathisflavone series incorporate a biphenyl system.
in which at least three out of four ortho positions are substituted. These ortho substituents interfere with one another in coplanar positions and are comfortable only in non-planar position. Complete rotation is therefore, prevented and optical resolution becomes possible. This phenomenon of restricted rotation leading to optical activity in biphenyl system, is known as 'atropisomerism'. However, in fukugetin and zanthochymnuside, the optical activity may either be due to the asymmetric centre \( (C_2) \) alone or to both the asymmetric centre and restricted rotation.

**TABLE - I**

Optically active biflavonoids

<table>
<thead>
<tr>
<th>Biflavonoids</th>
<th>( \Delta \gamma_{a,b,c,d,e} ) (pyridine)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amentoflavone (XIIIa)</td>
<td>+9°</td>
<td>Podocarpus gracilicusp.</td>
</tr>
<tr>
<td>2. Amentoflavone (XIIIa)</td>
<td>+100°</td>
<td>Thuja orientalis</td>
</tr>
<tr>
<td>3. Cupressuflavone (XVIIa)</td>
<td>+63°</td>
<td>Cupressus occidentalis</td>
</tr>
<tr>
<td>4. 7,7'-Di-O-methyl-cupressuflavone (XVIIc)</td>
<td>+65°</td>
<td>Araucaria cunninghamii</td>
</tr>
<tr>
<td>5. 7,7'-Di-O-methyl-cupressuflavone (XVIIc)</td>
<td>+37.5°</td>
<td>Araucaria cunninghamii</td>
</tr>
<tr>
<td>6. 3',4'-7,7'-Tetra-O-methyl cupressuflavone (XVIIg)</td>
<td>+30°</td>
<td>Araucaria cunninghamii</td>
</tr>
<tr>
<td>Biflavonoids</td>
<td>$\sum_{a,b,c,d,e} \alpha_{D(\text{pyridine})}$</td>
<td>Source</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>7. 4',4''7,7''-Tetra-O-methyl-amentoflavone (XIIp)</td>
<td>+ 41°</td>
<td>Araucaria cookii ( ^47 )</td>
</tr>
<tr>
<td>9. Kayafavone (XIIIa)</td>
<td>+ 18°</td>
<td>Araucaria cookii ( ^47 )</td>
</tr>
<tr>
<td>9. Fukuzetin (XXVIIa)</td>
<td>+ 17°</td>
<td>Garcinia spicata ( ^75 )</td>
</tr>
<tr>
<td>10. Podocarpus flavone-A (XIIIa)</td>
<td>- 8°</td>
<td>Podocarpus gracilior ( ^43 )</td>
</tr>
<tr>
<td>11. 7,4''-Di-O-methyl agathisflavone (XXd)</td>
<td>- 55°</td>
<td>Agathis palmerstonii ( ^65 )</td>
</tr>
<tr>
<td>12. 7-O-methyl-agathisflavone (XXb)</td>
<td>- 50°</td>
<td>Agathis palmerstonii ( ^65 )</td>
</tr>
<tr>
<td>13. 7,7''-Di-O-methyl-agathisflavone (XXc)</td>
<td>- 12.5°</td>
<td>Araucaria bidwillii ( ^96 )</td>
</tr>
<tr>
<td>14. Xanthochymusside (XXVIII)</td>
<td>- 40°</td>
<td>Garcinia xanthochymus ( ^85 )</td>
</tr>
<tr>
<td>15. 7''-O-Methyl-amentoflavone (XIIIb)</td>
<td>- 15.2°</td>
<td>Araucaria cunninghamii ( ^43 )</td>
</tr>
<tr>
<td>16. 4',7''-Di-O-methyl amentoflavone (XIIIj)</td>
<td>+ 22.7°</td>
<td>Araucaria cunninghamii ( ^45 )</td>
</tr>
</tbody>
</table>

\( a = 40°, b = 34°, c = 29° \) and \( e = 20° \)
STRUCTURE DETERMINATION
Structure Determination of Biflavonoids

The problem of structure determination of biflavonoids is a complex one because of (a) occurrence of more than one biflavone in chromatographically homogeneous fractions with the consequent difficulty in their isolation in pure form, (b) insolubility in usual organic solvents, (c) the difficulty in exact location of O-methyl in partially methylated derivatives of biflavones and (d) the intricate problem of establishing the interflavanoid linkage.

There are various methods generally used for structure determination such as colour reaction, degradation, physical methods and syntheses. The physical methods and syntheses are of key importance for complete structure elucidation of biflavonoids.

Physical methods

The physical methods generally employed in the identification and structural analysis of plant pigments are chromatography, UV, IR, NMR spectroscopy and mass spectrometry. Among the physical methods nuclear magnetic resonance spectroscopy and mass spectrometry are most sophisticated dependable tools for
the structure determination of flavanoids and these will be described in detail.

Proton Magnetic Resonance (\textsuperscript{1}H-NMR) Spectroscopy

The application of PMR spectroscopy proved to be the most powerful tool in the structure determination of flavanoids. By the use of PMR studies of silyl derivatives, double irradiation technique, solvoint induced shift studies, lanthanide induced shift studies (LIS),\textsuperscript{122} nuclear overhauser effect (n.o.e.),\textsuperscript{122} and \textsuperscript{13}C-NMR spectroscopy,\textsuperscript{123} it has been possible to elucidate fully the structure of flavanoids occurring even minor quantities without resort to tedious and time consuming chemical degradation and synthesis. The valuable contribution in this field have been made by Batterham and Mighet,\textsuperscript{126} Mabry,\textsuperscript{115,125} Kassicot,\textsuperscript{126} C.-ara-Lewis,\textsuperscript{127} Kawano\textsuperscript{42,85,131} and Peliter and Rahman.\textsuperscript{94,60,47,62}

The chemical shifts of the protons of ring A and B prove to be independent of each other, but are affected by the nature of ring C\textsuperscript{51} (XLVII).

\begin{center}
\textsuperscript{XLVII}
\end{center}
The peaks arising from ring A in most flavanoids occur upfield from the other peaks and are readily recognised. Thus examination of an unfamiliar spectrum will commonly start by the recognition of these peaks, which will often allow the nature ring A and C, and the class of compound, in hand to inferred. The remaining peaks in the aromatic region will reveal the pattern of oxygen substitution of ring B, and confirm the nature of the ring C.

The observation and interpretation of spin-spin splitting are the means by which the sequence of groups in the molecule is established by PMR. However, the process of establishing sequence of groups in molecules even on high resolution $^1$H-NMR frequently fails, because, it may be possible to observe a discrete multiplet from one group of protons it may be possible to recognise the absorptions of protons to which this group is coupled, since they may be obscured by absorption of other protons in the molecule. An ancillary technique known as spin decoupling, double resonance often helps to overcome this difficulty. By the help of double irradiation technique it has been possible to assign each and every proton in biflavonoids. The recent technique of preparing silyl derivatives, for PMR studies has not only overcome solubility problem but also has contributed towards the simplification of spectra.
In the structure elucidation of biflavonoids certain useful information can be obtained by comparison of their PMR spectra with those of corresponding monomers. Such a choice, however, is compelling but by no means infallible. Comparison of the PMR spectra of methyl and acetyl derivatives of a biflavonoid with those of biflavonoids of the same series as well as with those of biflavonoids of other series in which at least one monoflavonoid unit is similarly constituted, is very helpful in assigning each and individual proton and the position of the methoxy groups. The problem of interflavonoid linkage has successfully been solved by solvent induced shift studies of methoxy resonance and lanthanide induced shift studies.

In biphenyl type biflavones such as amentoflavone, eupressiflavone, agathisflavone etc., the peak of the ring protons involved in interflavonoidic linkage appear at somewhat lower field (~0.5 ppm) as compared with the peaks of the same protons in monomer due to extended conjugation.

It has been observed, both in biphenyl as well as biphenyl ether type biflavonoids that the 5-methoxy group of an 8-linked monoflavonoid unit in a biflavonoid shows up below ~2.00 in deuterochloroform in all the cases examined so far (Table II). This observation may be explained on the basis of extended conjugation. 5-Methoxy
group of an 8-linked monoflavonoid unit in biflavonoids of BGH-series, WGH-series and GB-series does not show up below \( \tau \geq 6.00 \) as the linkage is through heterocyclic ring.

**TABLE - II**

Methoxy protons shift (\( \tau \)-values) of fully methylated biflavonoids

<table>
<thead>
<tr>
<th>Biflavonoids</th>
<th>5-OMe</th>
<th>5&quot;-OMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupressuflavone ( \begin{array}{l} \text{I-9,II-8} \end{array} )</td>
<td>5.85</td>
<td>5.85</td>
</tr>
<tr>
<td>Amentoflavone ( \begin{array}{l} \text{I-3',II-8} \end{array} )</td>
<td>6.13</td>
<td>5.94</td>
</tr>
<tr>
<td>Agathisflavone ( \begin{array}{l} \text{I-6,II-8} \end{array} )</td>
<td>6.41</td>
<td>5.95</td>
</tr>
<tr>
<td>( \begin{array}{l} \text{I-4'-O-II-8} \end{array} ) biflavone</td>
<td>6.00</td>
<td>5.92</td>
</tr>
</tbody>
</table>

* Synthetic

By examining the methoxy and acetoxy shifts certain useful correlation emerge but they should be used only as the supporting evidence. It is only by looking at the full series (parent, fully methylated and acetylated derivatives) and comparing multiplicities and chemical shifts of the aromatic protons that safe assignments can be made.

Aromatic protons are completely self-consistent in cupressuflavone, amentoflavone, agathisflavone (assumed
values of ring II-B protons) and hinokiflavone series. The protons of ring I-8 appear consistently lower than those of ring II-B. 47, 60, 65.

The protons at II-8 in hinokiflavone \( \{I-4', 0-II-6\} \) methyl ether and at I-8 in agathisflavone \( \{1-6, II-8\} \) methyl ether appear at exceptionally low field, \( \gamma \) 2.95 and 3.09, respectively. This may be diagnostic of H-8 of a 6-substituted ring in biflavanoid methyl ether both of biphenyl and biphenyl ether types.

The methoxy at 0-5 (ring I-A) of agathisflavone methyl ether (\( \gamma \) 6.41) and one methoxyl in chalocone-flavone corresponding to BGH-III methyl ether (\( \gamma \) 6.80) and WGH-II methyl ether (\( \gamma \) 6.56) show up at exceptionally higher field than the other methoxy groups. This internal shielding effect is also evident in the case of chalocones BGH-III heptaacetate and BGH-II octaacetate in which the protons of one acetoxy group appear at 8.08 whereas those of other at 7.25-7.30.

The dependency of H-6 of II-A upon its mode of bonding with the other half of the biflavanoid has been observed.
<table>
<thead>
<tr>
<th>Biflavonoid methyl ether</th>
<th>H-6 (Ring II-A) (δ value)</th>
<th>C-8 (Ring II-A) bonded to</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGH-III</td>
<td>3.82</td>
<td>reduced heterocyclic ring</td>
</tr>
<tr>
<td>GH-II</td>
<td>3.74</td>
<td>&quot;</td>
</tr>
<tr>
<td>WGH-III</td>
<td>3.55</td>
<td>heterocyclic ring</td>
</tr>
<tr>
<td>WGH-II</td>
<td>3.49</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cupressusflavone</td>
<td>3.41, 3.42</td>
<td>aromatic ring I-A</td>
</tr>
<tr>
<td>Amentoflavone</td>
<td>3.38</td>
<td>aromatic ring I-B</td>
</tr>
<tr>
<td>Agathisflavone</td>
<td>3.36</td>
<td>aromatic ring I-A</td>
</tr>
</tbody>
</table>

Solvent induced shift in ²H-NMR spectroscopy

Williams and co-workers have observed that the methoxyls at C-3, C-7, C-2' and C-4' exhibit large positive Δ values (Δ = δCDCl₃ - δ C₆H₆ > 0.5-0.8 ppm) in the absence of methoxy or hydroxyl substituents ortho to these groups. This means that the aforesaid methoxy signals move upfield in benzene relative to deuterochloroform. The observation is consistent with the formal ability of all these methoxy groups to conjugate with the electron withdrawing carbonyl group. This conjugation can lead to a decrease in π - electron density at oxygen atoms of methoxy groups in question, and so enhance an association with benzene at
these electron-deficient sites with a resultant increased shielding effect. The C-3 methoxy resonances are in contrast deshielded or only slightly shielded ($\Delta = -0.07$ to $+0.34$) in a benzene, suggesting that the C-3 methoxy group in general prefers conformation indicated in (XLVIII). Similarly a 5-methoxy group in presence of a 6-substituents shows small positive or negative solvent shift in benzene because a 6-substituent should lead to a higher population of the conformer (XLIX).

\begin{align*}
\text{(XLVIII)} & \quad \text{(XLIX)}
\end{align*}

In these conformations, the protons of the methoxy group in question lie in close proximity to the negative end of the carbonyl dipole which is a region of strong deshielding due to benzene association at the carbonyl group. The methoxy groups lacking one ortho hydrogen (i.e. flanked by two ortho methoxy functions or one ortho
hydroxy and ortho methoxy function) also shows small positive or negative $\Delta$ values ($\pm 0.13$ to $-0.12$ ppm) due to some combination of (i) steric inhibition of benzene solvation of the central methoxy group, (ii) reduction in solvation of the central methoxy group (relative to anisole case) due to the presence of two ortho electron-donating substituents, and (iii) solvation of outer methoxy group, the stereochemistry of benzene association being such as to place the central methoxy group in a region of deshielding. It is emphasised that the steric factor cannot be the major influence, since an electron-withdrawing substituent ortho to an methoxy function increases the upfield shift which is observed in benzene.27,128

In amentoflavone,60 cupressaflavone65 and $\text{I-4'-O-7-5',}^{37}$ biflavone methyl ethers all the methoxy groups move upfield ($\sim 50-60$ cps) on change of solvent from deuterochloroform to benzene showing that every methoxy group has at least one ortho proton, and therefore, a C-8 rather than a C-6 linkage is indicated. In agathisflavone hexamethyl ether, only five of the six methoxy groups show large upfield shifts. One methoxy group was unique that upto 50% dilution with benzene no shift was seen and then a strong downfield shift was evidenced.25
was reasonable to assume that the methoxy group in question was the one at C-5 flanked ring II-A on one side and a carbonyl group on the other. Similarly in the case of hinokiflavone only four methoxy groups move upfield.

Benzene induced shift have also been found useful in the flavonoids of BGE-series. All the methoxy signals in BGE-II and BGE-III methyl ethers move upfield indicating that the flavone substituents is at C-8 rather than at C-6 of flavone unit.

The benzene induced solvent shifts $\Delta (\delta_{CDCl_3/C_6H_6})$ are appreciably enhanced by the addition of small quantity (33% V/V) of trifluoroacetic acid (TFA) to the solution of the compound in benzene. Apparently protonation of certain groups enhances benzene association at these sites. This technique helps to distinguish between methoxy groups which can conjugate with the carbonyl group (L) and those which cannot conjugate ($L^I$) in the ground state.

\[
\text{Me-} ^3\text{C}=\text{C-O} \quad \rightarrow \quad \text{Me-} ^3\text{C}^\text{C}=\text{C-O}
\]

\[
(L)
\]

\[
\text{Me-} ^\text{C}^\text{C}=\text{C-O} \quad \rightarrow \quad \text{Me-} ^\text{C}-\text{C}=\text{C}
\]

\[
(L^I)
\]
Thus the basicity of the methoxy groups not conjugated (LI) with the carbonyl group is greater than those which are conjugated (L) and so the former will be expected to give more positive values of the TFA-addition shift \( \Delta \left( \text{C}_6\text{H}_6 / \text{C}_6\text{H}_6 - \text{TFA} \right) \). The TFA induced solvent shift \( \Delta \left( \text{CDCl}_3 / \text{TFA} \right) \) of a 5-methoxy group has a relatively large negative value (-0.36 to -0.44), which distinguishes it from other methoxy groups. A possible explanation is the formation of hydrogen bond between the protonated carbonyl group and the oxygen atom of the 5-methoxy group (LII). The carbonyl group will be protonated to a much larger extent in TFA relative to a solution in benzene containing only 3% TFA.

(LII)

\[ \text{Scheme 1} \]

![Scheme 1](image-url)
Limitation of solvent induced shift studies

The method of methoxy proton shift although very useful in structure determination, may lead to erroneous assignments if not used with caution. The following criteria have been laid down for an appropriate use of the method.

1. The method should not be used directly for compounds containing phenolic groups. Even acetylation of the phenolic function does not completely overcome the difficulty. Only the fully methylated compounds are safest to use but even then the results may be misleading if solvation of a separate site close to the methoxy groups being examined occurs.

2. In WGH series, the C-3\(^*\) methoxy group of WGH-II methyl ether appears at an exceptionally high position (\(7.58\)) in CDCl\(_3\). This is suggestive of its being entirely internally solvated. A model of this biflavone shows that there are in fact certain positions in which that particular methoxy group can be solvated by a benzene ring of the other flavanoid unit, thus rendering it unique in being resistant to external solvation. On change of solvent from CDCl\(_3\) to C\(_6\)H\(_6\) all the methoxy groups
are expected to move upfield by more than 30 cps as each methoxy group has an ortho proton. The methoxy group in question, however moves very little.
Lanthanide induced shift studies in NMR spectroscopy

During the last twelve years lanthanide shift reagents (LSR) have been extensively used for the structural and conformational studies of organic natural products. The introduction of these reagents (paramagnetic compounds) has greatly enhanced the power and versatility of PMR spectroscopy. The addition of certain lanthanide complexes (shift reagents) to a $^1$H-NMR solution of a compound which possesses an appropriate lone pair of electron causes the proton resonances to become spread out with the effect being greatest on the resonances of hydrogens nearest the site of co-ordination. The shift reagent coordinates with electronegative atom in the substrate and thus modifies the magnetic field experienced by neighbouring protons (and therefore simplify the spectra). Since the strength of this field varies with the distance from the paramagnetic source, the chemical shift of each proton is modified by a different amount. Coupling constants appear to be virtually unaffected. The effect is to spread out the absorptions which previously overlapped and this frequently allows a first order analysis of the spectrum.
These lanthanide induced shifts (LIS or \( \Delta \gamma_i \)) are thought to be due primarily to pseudocontact interaction, and for any particular molecule at a given temperature are inversely proportional to the cube of the internuclear distance \( (r_i)^3 \) between the lanthanide metal ion and the proton under consideration (eqn. 1):\(^{135,147}\)

\[
\Delta \gamma_i = \frac{K}{r_i^3} \tag{1}
\]

A more complete form of equation (1) is eqn. (2) in which \( \Delta \gamma_i \) is the pseudocontact shift for the \( i \) th proton, \( \theta \) is the angle describing the position of the proton relative to the assumed symmetry axis of the europium complex, \( r_i \) is the Su-H internuclear distance and \( K \) is a constant. The angle term \( (3 \cos^2 \theta - 1) \) is positive for \( \theta \) values from 0 to 54° and from 126 to 180° and a positive \( \Delta \gamma_i \) (shift to lower field) is observed, however when \( \theta \) has a value from 55 to 155° the angle term and \( \Delta \gamma_i \) becomes negative (i.e. shift to higher field) are observed.\(^{148}\)

\[
\Delta \gamma_i = \frac{K(3 \cos^2 \theta - 1)}{r_i^3} \tag{2}
\]
The most commonly used shift reagents are tris-chelates of lanthanide ions with β-diketones, 2,2,6,6-tetramethylheptane-3,5-dione (dipivaloylmethane) and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,8-dione. Typical reagents are tris-(dipivaloylmethanato) europium and tris-(dipivaloylmethanato) praseodymium. The names of which are normally abbreviated to Eu(dpm)_3 and Eu(fod)_3.

Rondeau and Sievers have reported that europium and praseodymium complexes of 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-6-octanediol (fod) are superior shift reagents for weak Lewis bases such as ethers and esters. Eu(fod)_3 and Pr(fod)_3 are also superior in terms of solubility (which can be problematic with the dpm analogs) but require greater care in handling since they are extremely moisture sensitive.

The extent of the lanthanide induced shift is dependent on the basicity of the functional group and on the nature, purity and concentration of the shift reagent. Alcohols and amines generally exhibit the largest shift, but many other compounds such as ethers, carbonyl compounds, nitriles, sulfoxides, oximes, etc., exhibit useful shifts. Of the commonly used reagents Eu(fod)_3 normally causes the greatest shifts as it is a stronger Lewis acid.
The two major applications of lanthanide shift reagents are: firstly the simplification of the spectrum, and secondly the confirmation of assignment of the signals by relating the extent of the shift to the concentration of the shift reagent.

The magnitude of induced shift for a proton is usually expressed in terms of "S value" proposed by Cockerill and Rackam, as the slope of straight line obtained by plotting the shift value ($\Delta Y$) against the molar ratio of Eu(dpdm)$_3$ to a substrate. Usually spectra are determined at 8-10 different molar ratios to obtain each slope. The larger the S value, the greater the particular proton is shifted downfield by the shift reagent. It is suggested that the shift reagent exhibits its effect by establishment of a rapid (on the NMR time scale) equilibrium between a labile complex of Eu(dpdm)$_3$ with a Lewis base and unassociated solutes. This labile complex contribute very significantly to the observed shift through at least two mechanisms, through bond and through space. The former is important when only two or three bonds separate hydrogen and europium. The latter effect becomes dominant when four or more bonds are involved if close approach of europium and hydrogen is likely. In the case of polyfunctional
molecules, the observed paramagnetic shifts are sums of contribution due to magnetic interaction from metal association at each site.

Kawano et al.\textsuperscript{121} have recently reported the application of lanthanide shift reagent, Eu(fod)\textsubscript{3} for the structure elucidation of flavones and biflavones.

\textbf{Monoflavones}

For tri-O-methyl apigenin (LIII), tetra-O-methylisocutellarein (LIV), tetra-O-methylisocutellarein (LV) and ponkanetin (LVI). The magnitude of lanthanide\textsuperscript{121,141} induced shifts (\(\Delta\ Eu\)) have been estimated (Table II).

\begin{center}
\begin{tabular}{c}
(LIII) \(R_1=R_2=\bar{R}\) \\
(LIV) \(R_1=\text{H}; R_2=\text{OMe}\) \\
(LV) \(R_1=\text{OMe}; R_2=\text{H}\) \\
(LVI) \(R_1=R_2=\text{OMe}\)
\end{tabular}
\end{center}
Out of three reagents shown in Table III, Eu(fod)$_3$ is most effective for methoxy protons at the 3-position (MeO-3) and the best reagent for distinguishing H-3, -6 and -8 from each other by means of $\Delta$Eu values. The $\Delta$Eu values of H-6 or MeO-6 are much larger than those of H-3 or MeO-3, providing a method for distinguishing a proton attached to either C-6 or C-8. Eu(dpm)$_3$ is less effective for MeO-3 and comparatively similar values for other protons except those on the phenyl ring. The shift effects of Eu(fod)$_3$ seem to be smaller than those of Eu(fod)$_3$.

The effect of Eu(fod)$_3$ on H-3 is markedly different from that of Eu(dpm)$_3$, especially in the case of tetra-O-methylscutellarein (LV) and ponkanetin (LVI), which have a 3- as well as a 5-methoxy group. For compounds (LV) and (LVI), the H-3 signal shows an upfield shift on addition of Eu(fod)$_3$ and a downfield shift with Eu(dpm)$_3$. This constitutes the first example of proton signal shifting in opposite directions due to the different reagents Eu(dpm)$_3$ and Eu(fod)$_3$ (2.04 ppm and 1.54 ppm respectively). The presence of OCH$_3$-6 in the 5,7-dimethoxyflavone derivatives seems to be an important factor in the phenomenon. The other two
### Table III

<table>
<thead>
<tr>
<th>Compound</th>
<th>Protons</th>
<th>6 (ppm)</th>
<th>ΔEu (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Eu(fod)</td>
</tr>
<tr>
<td>LIII</td>
<td>MzO-5</td>
<td>3.92</td>
<td>13.37</td>
</tr>
<tr>
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<td>-7</td>
<td>3.37</td>
<td>1.03</td>
</tr>
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<td>-4'</td>
<td>3.84</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>H-3</td>
<td>6.55(s)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>-6</td>
<td>6.34 (d, J=2 Hz)</td>
<td>5.94</td>
</tr>
<tr>
<td></td>
<td>-8</td>
<td>6.53 (d, J=2 Hz)</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>-2',6'</td>
<td>7.79 (d, J=9 Hz)</td>
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</tr>
<tr>
<td></td>
<td>-3',5'</td>
<td>6.97 (d, J=9 Hz)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

| LIV      | MzO-5   | 3.95    | 15.29    | 9.34    | 6.34    |
|          | -7      | 3.98    | 1.93     | 1.39    | 1.48    |
|          | -8      | 3.92    | 0.58     | 0.53    | 2.12    |
|          | -4'     | 3.85    | -0.07    | 0.03    | 0.13    |
|          | H-3     | 6.60 (s) | -0.30   | 0.38*   | 4.35    |
|          | -6      | 6.44 (s) | 7.48    | 5.08    | 5.36    |
|          | -2',6'  | 7.90 (d, J=9 Hz) | -0.12  | 0.28    | 0.74    |
|          | -3',5'  | 7.02 (d, J=9 Hz) | -0.09  | 0.02    | 0.02    |
TABLE - III (Contd.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Protons</th>
<th>δ</th>
<th>Δ Eu(ppm)</th>
<th>Eu(fod)</th>
<th>Eu(fhd)</th>
<th>Eu(dpm)</th>
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<td>4.47</td>
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<tr>
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<td>6.79 (s)</td>
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<td>-0.25</td>
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<td>1.58</td>
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<td></td>
<td>R-3</td>
<td>6.60 (s)</td>
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<td>-1.30</td>
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<td></td>
<td>-2',6'</td>
<td>7.97 (d, J=9.5 Hz)</td>
<td>-0.66</td>
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<td>-0.25</td>
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<tr>
<td></td>
<td>-3',5'</td>
<td>7.01 (d, J=9.5 Hz)</td>
<td>-0.30</td>
<td>-0.30</td>
<td>-0.24</td>
<td></td>
</tr>
</tbody>
</table>

* Difficult to estimate owing to signal broadening. Minus signs indicate upfield shifts:

$$\Delta \delta = \delta_{Eu}^{n=1} - \delta_{Eu}^{n=0} (CDCl_3)$$

where n is the molar ratio of shift reagent to solute.
compounds (LIII) and (LIV) show little shifts with Eu(fod)₂ and relatively large downfield shifts with Eu(dpm)₂.

Biflavones

Eight fully methylated biflavones, namely, hexa-0-methylamentoflavone (XIIq), hexa-0-methyl-cupressuflavone (XVIIh), hexa-0-methylagathisflavone (XXg), hexa-0-methylrobusflavone (XXIIIb), hepta-0-methylsaharanflavone (XXVIIIb),²³ penta-0-methyl-hinokiflavone (XXX), penta-0-methylxochanflavone (XXXIIa) and penta-0-methyl 1-4'-0-II-9' bipigenin (XXXVI)²² have been studied using Eu(fod)₂ as shift reagent and their S-values are recorded in Table IV.
<table>
<thead>
<tr>
<th>Protons</th>
<th>(XIIIq)</th>
<th>(XXIIIb)</th>
<th>(XVIIb)</th>
<th>(XXg)</th>
<th>(XXVIIIb)</th>
<th>(XXI)</th>
<th>(XXXI)</th>
<th>(XXXII)</th>
<th>(XXXIII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeO-I-3</td>
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<td>10.59</td>
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<td>10.02</td>
<td>6.88</td>
<td>10.26</td>
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<tr>
<td>-II-5</td>
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<td>-</td>
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<td>6.60</td>
<td>11.36</td>
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<tr>
<td>-I-7</td>
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<td>0.58</td>
<td>0.52</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>I-4'</td>
<td>0.12</td>
<td>0.32</td>
<td>-0.06</td>
<td>0.02</td>
<td>-0.50</td>
<td>-</td>
<td>-</td>
<td>-0.14</td>
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<td>-</td>
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<td>0^*</td>
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<tr>
<td>H-1-3</td>
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<td>0.28</td>
<td>-</td>
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<td>0.14</td>
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<tr>
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<td>II-8&quot;</td>
<td>-</td>
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<td>-0.08</td>
<td>-</td>
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<td>0.00,</td>
<td>-0.10</td>
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<tr>
<td>-I-3',5'</td>
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<td>0.24</td>
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<tr>
<td>-II-3',5'</td>
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<td>-0.06</td>
<td>-</td>
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<td>0.34*Me, -0.03</td>
<td>-0.03</td>
<td>0.18</td>
<td>-0.12</td>
<td></td>
</tr>
</tbody>
</table>

*Tentative assignment
Table IV shows the S-values of every proton signal of these compounds obtained on addition of Eu(fod)$_3$, which gives a straight line up to a molar ratio of reagent to substrate. Therefore in these compounds S values are the same as $\Delta$Eu values. The same induced shift bends were observed as in the case of the four compounds in Table III, although the effectiveness of the added Eu(fod)$_3$ is halved owing to the presence of two flavone nuclei per molecule. However, MeO-I-5 and OCH$_3$-II-5 showed different shift values except in the case of symmetrical compound (XVIIb), because the co-ordination of Eu(fod)$_3$ to the two flavone nuclei is not even but is characteristic of the structure of each compound.

For example, a large difference is observed between MeO-I-5 and MeO-II-5 in the case of 3- or 6-linked compounds $\left(\text{IXa}, \text{XXIIIb}, \text{XXVIIb}, \text{XXX}\right)$ in comparison with compounds $\left(\text{XIIa}, \text{XXXIf} \right)$ and $\left(\text{XXVI}\right)$. The result in Table IV may be summarized as follows:

(a) The smallest S value for H-I-6 or II-6 is still larger than the largest S value for H-I-8 or II-8. Thus it is not difficult to distinguish signals due to H-I-6 or II-6 from those of H-I-8 or II-8 and accordingly to decide the interflavanoid linkage through either C-6 or C-3 in biflavones.
(b) $S$ values of H-I-3 or II-3 are so small that these protons are usually distinguishable from H-I-8 or II-8 in the same flavone nucleus.

(c) The smallest $S$ values are observed for phenyl protons, although H-I-3' and I-5' of compound (XXXI) show a significantly larger shift (2.00 ppm) than those of the other compounds because the phenyl group is attached to C-6 of the other flavone nucleus.

This method is potentially very useful for determination of the structures of new flavones. In the light of these results some of the reported assignments of hexa-0-methylagathisflavone (XXg) and hepta-o-methyl-saharanflavone (XXVIII) are revised as shown in Table V.

The corrected assignments of hexa-0-methylagathisflavone (XXg) were confirmed by n.o.e. studies as follows: On irradiation at the frequency of MeO-II-5 (δ 4.08, the lowest methoxy signal) enhancement was observed at the H-II-6 signal, and on irradiation at the frequencies of MeO-I-7 (δ 3.90), MeO-II-7 (3.39), and MeO-II-4' (3.75), enhancements were observed at the H-I-8, H-II-6 and H-II-3' and II-5' signals, respectively. No effect was observed at H-I-3 and II-3 signals on irradiation of any methoxy signal.
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<td></td>
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<td>6.68 (dJ=9 Hz)</td>
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C-NMR Spectroscopy of Biflavonoids

PMR spectroscopy involving shifts of the methoxyl signals in the spectrum of the permethyl ether, upon progressive addition of deuterobenzene, has been used for the determination of interflavanoid linkage. The shift of the signal occurs if one position ortho- to a given methoxyl group is unsubstituted. Though applied successfully in many cases, this method is restricted in its applicability. Thus in the case of hepta-O-methylsaharanflavone, one methoxy signal does not shift at all on the addition of C\textsubscript{6}D\textsubscript{6}, supporting a \( \square \text{I-3',II-8} \) linked structure, in spite of the fact that a \( \square \text{I-3,II-8} \) linkage was later confirmed by synthesis. The use of the paramagnetic shift reagent Eu(tod)\textsubscript{3} helped to differentiate the signals due to H-2, H-6 and H-8 in 5,7-dimethoxyflavanoids and this has been extended to the biflavonoids permethyl ethers. However, since both flavanoid moieties are complexed, different shifts may result from the same substituents on each nucleus. Hence a method of wider applicability is necessary for an unambiguous determination of the interflavanoid linkage in such compounds. The assignment of the signals
in the $^{13}$C-NMR spectra of ten oxygenated biflavonoids was achieved on the basis of off-resonance and proton coupled spectra and by analogy with published values for the monomeric compounds. This method obviates the necessity of preparing the permethyl ethers which are obligatory for the $^1$H-NMR solvent induced shift studies. As a consequence, therefore, this method has potential also for the location of methoxyl substitution directly in a naturally occurring methylated biflavonoid.

**Linkage involving ring A only**

The signals for C-6 and C-8 in the $^{13}$C-NMR spectra of monomeric flavanones, flavones and flavonols with a 5,7-dihydroxy substitution can be unambiguously differentiated by a consideration of their multiplicities in proton coupled spectra and by specific proton decoupling. For a large number of such compounds the resonances for these carbon atom were found between 90.0 ppm to 100 ppm. The signal for C-6 is always found to be at lowerfields than C-8 in a variety of 5,7-dihydroxy compounds. This difference is small (ca 0.9 ppm) in the flavanones and larger (ca 4.8 ppm) in flavones and flavonols. In the case of permethylepicatechin the corresponding difference was 1.7 ppm. The signal for
Fig. 1. $^{13}$C noise decoupled and off-resonance spectra of cupressulflavone in DMSO-d$_6$ 90.
C-9 appeared downfield relative to that of C-3 and the assignment was confirmed by specific deuteration at C-9. On the basis of well established results alkyl or aryl substitution on an aromatic nucleus should not essentially alter (± 0.3 ppm) the chemical shift of the metacarbon atoms. This is well exemplified by a comparison of the spectrum of pinocembrin (5,7-dihydroxyflavone) with that of its 6-C-methyl and 8-C-methyl derivatives, as well as that of luteolin (5,7,3',4'-tetrahydroxyflavone) and its 8-C-benzyl derivatives. In all these compounds, the signals for the quaternary C-substituted carbon atom shifts by 6.0 to 9.6 ppm downfield whereas the signal for the unsubstituted carbon is not markedly altered. Even a C-6 hydroxy substitution, as in 6-hydroxyluteolin only slightly alters the position of the signal for C-8 compared with that of luteolin.

### TABLE VI

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<td>6-Hydroxyluteolin</td>
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In the spectrum of cupressuflavone \( I-3, II-8 \) diapigenin, there are only 13 resonances present due to the high symmetry of the molecule. The signal for \( I-6 \) and \( II-6 \) appears at 99.0 ppm whereas the signal for \( I-8 \) and \( II-8 \) shifts downfield (relative to apigenin) to 99.7 ppm, due to substitution effect of the interflavonic linkage. These assignments were confirmed by the proton coupled \(^1^3\)C-NMR spectrum. Methylation of both \( I-7 \) and \( II-7 \) hydroxyl groups in cupressuflavone shifts the signal for \( I-6, II-6 \) upfield to 95.6 ppm whereas the \( I-3 \) and \( II-8 \) signal moves downfield to 99.1 ppm. Again confirmation was achieved by taking the proton coupled spectrum in which the signal at 99.1 ppm exhibited a \( ^3\)JCH interaction with \( I-6 \) hydrogen atom.

In the hexa-O-methyl ether of cupressuflavone, the signal for both \( I-8 \) and \( II-8 \) appears downfield relative to that of apigenin-tri-O-methyl ether, at 101.2 ppm, whereas \( I-6 \) and \( II-6 \) are not appreciably shifted. The spectrum of agathisflavone \( I-6, II-8 \) diapigenin shows eight distinct resonances in the region 93.0 ppm to 104.0 ppm. The signals for the unsubstituted carbon atom \( I-9 \) and \( II-6 \) at the expected value 93.7 ppm and 98.9 ppm respectively while \( I-6 \) and \( II-8 \) has their resonances at 103.6 ppm and 99.4 ppm. The downfield shifts experienced
by the latter two carbon atoms of 4.7 ppm to 5.7 ppm are due to the substitution effect of the interflavanoid linkage. The other four signals between 102.8 ppm and 104.0 ppm can be assigned to the carbon atoms I-3, II-3, I-10 and II-10 respectively.

There are seven signals in the region 94.0 ppm to 104.0 ppm in the spectrum of \text{I} naringenin I-8, II-8 epigenin. They can be assigned by analogy with agathis itself and the published values for naringenin. Thus the carbon atoms II-6 and II-3 of rhusflavone and agathis-flavone had almost identical chemical shift values 98.8 ppm and 99.3 ppm for the respective carbon atoms. The carbon atom I-6 resonated at 100.3 ppm. The difference in the level of the two C rings in rhusflavone is clearly reflected in two well separated carbonyl resonances for I-4 (196.5 ppm) and II-4 (192.3 ppm). This thus demonstrated the potential of \text{\textsuperscript{13}}C-NMR spectroscopy when one is dealing with biflavanoids with differing oxidation levels in the C rings.

The spectral region 93.0 ppm to 103.0 ppm in the spectrum of rhusflavone \text{[I-8, II-8 binaringenin]} comprises six resonances. The methine carbon lines for I-8 and II-8 as expected at 94.8 ppm and 95.7 ppm respectively. The signals at 101.2 ppm and 100.3 ppm
Fig. 2 Correlation of I-6, II-6, I-8 and II-8 carbon atom signals in $^1$C-NMR spectra of A-ring linked flavanoids.
can be assigned to I-6 and II-9 and those at 101.8 ppm and 102.2 ppm to I-10 and II-10 carbon atoms.

As in the case of cupressuflavone, the spectrum of succedaneaflavanone $\{I-6,II-6\}$ binaringenin showed only twelve signals due to high symmetry of the molecule and the coincidence of the signals for I-5,II-5,I-9 and II-9 carbon atoms. The signal for I-6 and II-6 appears at 101.1 ppm whereas the unsubstituted I-8 and II-8 carbon atom resonate at 94.7 ppm. The signal at 101.9 ppm for two quaternary carbon atoms can be assigned only to the I-10 and II-10 carbon atoms in succedaneaflavanone.

The signals at 101.1 and 101.9 ppm were differentiated by the fact that the signal intensity of the latter was much greater than that of former, reflecting different relaxation behaviour. The assignment of the signals for I-2,II-2, I-3,II-3 and the carbon atom of the ring I-5 and II-6 in all the compounds were made by analogy with epigenin, its methyl ethers and maringenin.

**Linkages involving ring A and B**

The three methine carbon atoms II-8, I-6 and I-8 of robustaflavone $\{I-3',II-6\}$ epigenin can be easily assigned to the signals at 93.0 ppm, 99.0 ppm and 94.0 ppm respectively. The signals for II-6 carbon atom appear
Fig. 3. Correlation of carbon atom signals in the $^{13}$C-NMR spectra of amentoflavone and robustaflavone in the region 90 ppm to 110 ppm.
at 103.5 ppm. The spectral intervals 116.0 ppm to 131.0 ppm consist of eight resonances of the carbon atom. The signal for II-6 carbon atom appears at 103.5 ppm. The spectral interval 116.0 ppm and to 131.0 ppm consist of eight resonances of the carbon atoms of the two B rings. This is almost identical with the same spectral region of amentoflavone \( \text{amentoflavone} \) and the signals can be easily assigned on the basis of shifts expected for aryl substitution at I-3' and the off resonance spectrum. The signals for three carbon atoms I-6, I-8 and II-6 in the spectrum of amentoflavone can also be readily identified to be at 89.9 ppm, 94.2 ppm and 99.1 ppm respectively. The II-8 resonance appears at 104.1 ppm and is 5.6 ppm more downfield as compared with the position of the same signal in cupressuflavone.

**Linkages involving I-3**

The spectrum of volkensiflavone \( \text{volkensiflavone} \) at 100°C showed 22 resonances with coincidence of the signals for I-5, I-9 and II-2 (at 163.7 ppm), I-1', I-2', II-2', I-6' and II-6' (at 128.1 ppm), II-3' and II-5' (at 115.9 ppm) and I-3' and I-5 (at 114.5 ppm). The resonances I-2 and I-3 appeared at 81.4 ppm and 89.2 ppm respectively and are 3.0 ppm and
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*Note: Certain elements may be reversed.*
6.2 ppm downfield of the corresponding ones of naringenin. These shifts are due to the α- and β-substituent effect of the 8-apigenyl moiety at the carbon atom 1-3. The resonances for I-6 and I-8 appear at 96.4 ppm and 95.3 ppm whereas those of II-6 and II-8 appear at 98.5 ppm and 100.6 ppm respectively. As is to be expected the two carbonyl resonances at 186.0 ppm (I-4) and 181.6 ppm (II-4) reflect the respective levels of oxidation of two C rings. The presence of conformational equilibrium, due to inhibition of free rotation about the C-C bond in the interflavanoid linkage, in volkensiflavone was demonstrated by running the spectrum at 35°. Two signal of differing intensity for each of the carbon atom 1-2 (91.3 ppm and 92.4 ppm) and 1-3 (48.7 ppm and 47.9 ppm) are present in the spectrum. In addition, several carbon resonances were considerably broadened.

**Location of methoxy groups**

The position of a methoxy substituent in a biflavonoid can not be determined on the basis of chemical shift of the methoxy carbon as the variation is too small to be of diagnostic value. However, steric crowding as in the case of 5,6,7-tri-C-methyl compounds does cause a downfield shift of the methoxy carbon by 3.6 ppm,
whereas in a 5-hydroxy-6,7-dimethoxy derivative the shift is only of the order of 3.0 ppm. The downfield shift of the signal for the carbon bearing the hydroxyl group, on methylation is also variable. However, the upfield shift of the signal for the ortho carbon atom is more reliable diagnostically, thus enabling an indirect determination of the site of O-methylation. In conclusion, it is apparent that more inspection of chemical shift values in the spectral region 90.0 ppm to 105.0 ppm, in $^{13}$C-NMR spectrum of a biflavanoid, can give a good indication of the linkage position if ring A is involved in a C-C linkage. This may be additionally confirmed by a normal off resonance spectrum. In the event of any new C-C linked biflavanoid being isolated, involving the ring A in the linkage, this method will be ideal for determining the linkage position.
MASS SPECTROSCOPY

Mass spectrometry has become an integral part of organic chemistry. Along with infrared, ultraviolet and nuclear magnetic resonance spectroscopy, it is an indispensable tool for structure elucidation of organic natural products. It finds much of its importance in increasing, not replacing, the effectiveness of other techniques. The link up with gas chromatography and the use of computers to analyse mass spectral data have added new dimensions to mass spectrometry. Generally, fragmentation is related to the structures of the intact molecule. Recently, a number of papers on the evaluation of structure fragmentation pattern relationship in mono and biflavonoids have appeared.

Flavones

Kingson[49] has discussed the mass spectra of large number of flavone, flavonoids and their other derivatives. He has summarized the manner in which monoflavones fragment as follows:
(a) Flavones with fewer than four hydroxy groups do not readily fragment, a consequence of the stability of their molecular ion.

(b) Flavones with fewer than four hydroxy groups tend to undergo decomposition predominantly by way of the retro Diels-Alder process. This and other common fragmentation processes are shown in (Chart - 1) using apigenin as a typical example.

(c) An M-1 ion is often found in the mass spectra of flavones, its origin however, obscure.

(d) The presence of ion (c) (Chart-I), frequently more intense when a 3-hydroxy group is present, is attributed to the alternative mode of retro Diels-Alder fragmentation also depicted in Chart I.

(e) Doubly charged ions are frequently present.

(f) When heavily substituted with hydroxyls and methoxyls, the flavone tends to fragment in a less predictable manner. Retro-Diels-Alder process becomes insignificant and the spectrum is dominated by the molecular ion and ions at M-15, M-23 and M-43.
Alternative Retro-Diels-Alder Process to give fragment (C)

$\text{m/e } 270 (100)$

$\text{m/e } 242$ (major)

$\text{(C) m/e } 121$

$\text{(A) m/e } 152$

$\text{(B) m/e } 118$

$\text{(C) m/e } 121$
Flavonones

In the case of flavonoids with reduced heterocyclic ring, fragmentation by path A and B are of great importance as they lead to clear-cut characteristic spectra [Chart - 1]. Another method for breakdown that helps to characterise the flavonones is the loss of either hydrogen atom or any aryl radical from the molecular ion to give even electron fragments. These fragmentation processes are illustrated in the case of 4'-methoxyflavone [Chart - 2]. The presence of hydroxyl or methoxyl group at C-4 position of ring B facilitates, by enhanced resonance stabilization of the resulting fragment ion, the formation of p-hydroxy benzyl or p-methoxybenzyl ion (or their equivalent tropolium ions). p-Hydroxy/p-methoxy benzyl ion appears as base peak of significant intensity in the mass spectrum of naringenin/its methyl ether [LVIII].

Nigflavones

Schneider and his coworkers have made a more specific study of the mass spectral fragmentation of the permethyl ether derivatives of amentoflavone, cupressusflavone and hinosflavone. The following fragmentation
schemes (Chart 3,4 and 5) have been proposed to explain the appearance of some of the ions observed. Molecular ion is usually the base peak. Apart from the processes mentioned earlier for apigenin/its methyl ether, these compounds also undergo (i) fission of the C-C or the C-O-C linkage between the aromatic residues, (ii) elimination of CO and CH\textsubscript{2}O from the biphenyl ethers and (iii) rearrangement involving condensation between the phenyl rings. Steric factors seem to play an important role in influencing the breakdown mode and internal condensations. Formations of doubly charged ion is frequently observed.

The mass spectra of amentoflavone hexamethyl ether and cupressusflavone hexamethyl ether are similar, molecular ion being the base peak in each case. Difference lies in the intensities of the corresponding peaks due to variation in substitution patterns and steric factors. The main peaks together with their intensities in the mass spectra of these compounds are given below.
**Amentoflavone hexamethyl ether (XIIIq)**

The mode of fragmentation is shown in Chart - 3.

Main peaks: 622(100); 621(33); 592(8); 576(10); 312(2); 311(5); 245(5); 181(2); 180(3); 135(18) and 132(3).

![Diagram of fragmentation](chart_3.png)