CHEMISTRY OF NATURAL PRODUCTS

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

By
Mohammad Aqil

Under the Supervision of
Professor W. Rahman

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R E S U M E

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 techniques applied to their isolation and structure elucidation.

The work described in the thesis consists of the isolation and characterization of the biflavanoids from the leaves of:

1. Ochna squarrosa Linn (Ochnaceae).
2. Cephalotaxus harringtonia (Forbes)K.Koch. (Cephalotaxaceae).

Biflavones from the leaves of Ochna squarrosa Linn(Ochnaceae):

The isolation of some biflavanoids such as morelloflavone, fukugetin and Saharanflavone from Guttiferae plants, stimulated us to carefully investigate Ochnaceae plants for their biflavanoid contents as the family Ochnaceae is claimed to be closely related to guttiferae. The present work describes the results of our investigations on the phenolic extractives of the fresh leaves of Ochna squarrosa. The crude biflavone mixture obtained by solvent fractionation and column chromatography of the acetone extracts, has been shown to contain three biphenyl ether type biflavones of a new series. One of them has been isolated and characterized as parent biflavone and other two as its partial methyl ethers, the corresponding parent biflavone having the hitherto unknown interflavanoid linkage (I-3'-O-II-4') between two apigenin units has been named as 'Ochnaflavone'.(Ia). The structure of the complete methyl ether of the new biflavones has been established as

I-4',I-5,II-5,I-7,II-7-penta-O-methyl(I-3'-O-II-4') biflavone (Ib) by mass spectrometry,NMR studies including the lanthanide induced shifts and synthesis. The two other new biflavones have been identified as mono-(Ic) and dimethyl ethers (Id) of Ochnaflavone. Further the characterization of a tri-methyl ether (Ie) of Ochnaflavone after the diazomethylation of priviously isolated biflavones has also been carried out.

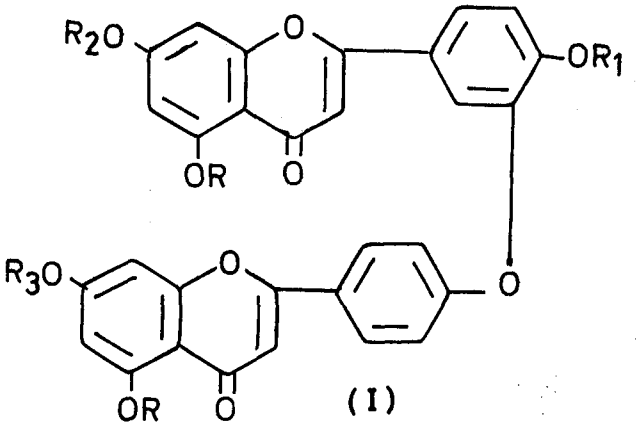
(a) $R=R_1=R_2=R_3=H$

(b) $R=R_1=R_2=R_3=CH_3$

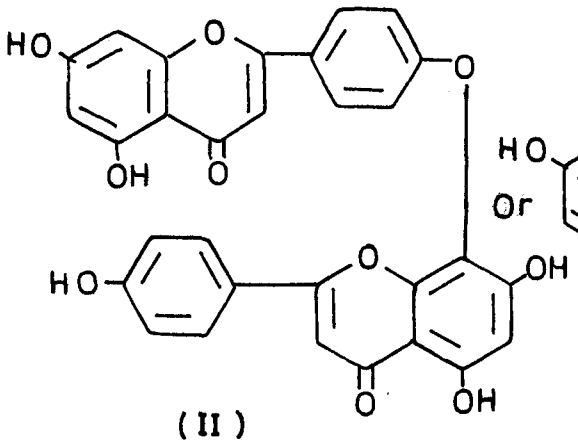
(c) $R=R_2=R_3=H;R_1=CH_3$

(d) $R=R_3=H;R_1=R_2=CH_3$

(e) $R=H;R_1=R_2=R_3=CH_3$



(I)



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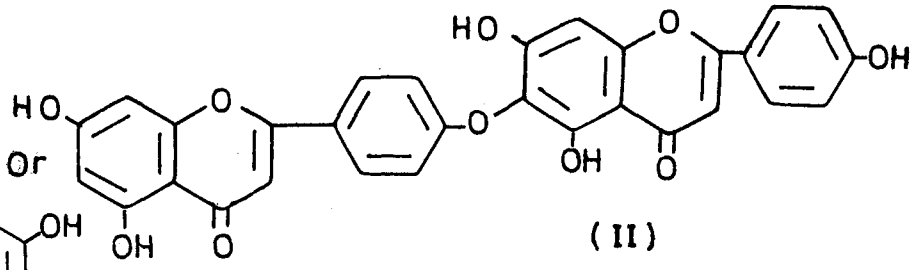


(II)

Ochnaflavone and its partial methyl ethers thus constitute a second example of naturally occurring biphenyl ether type biflavanoids the first one being hinokiflavone (II).

Biflavones from the leaves of Cephalotaxus harringtonia (Forbes) K.Koch (Cephalotaxaceae).

The phenolic extractives of the fresh leaves of C.harringtonia have been examined. The biflavones isolated and characterized are detailed below. Those marked with astrisk are only detected (TLC).

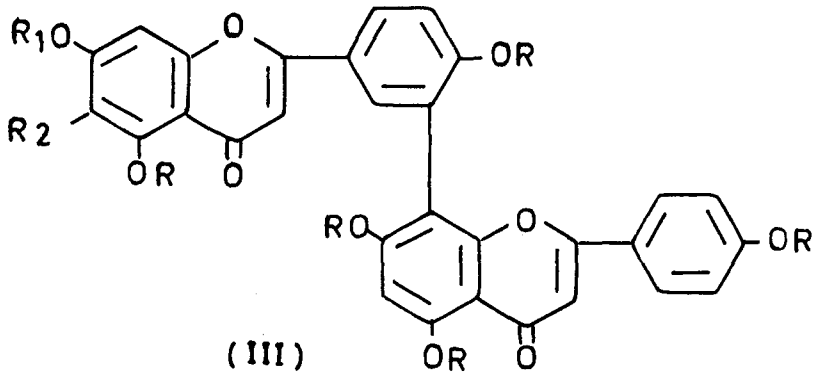
*i. Amentoflavone

ii. Amentoflavone monomethyl ether (Sequoiaflavone)(IIIa)

iii. I-6-C-methyl-I-7-O-methylamentoflavone (Cephalotaxoflavone) a new biflavone.(IIIb)

*iv. Amentoflavone dimethyl ether (Ginkgetin).

* v. Amentoflavone trimethyl ether (Sciadopitysin).



(III)

(a) $R_1=CH_3$; R= R_2=H

(b) $R_1=R_2=CH_3$; R=H

I-6-C-methyl-I-7-O-Methyl amentoflavone (Cephalotaxoflavone) constitutes the first report of the isolation and characterization of the naturally occurring C-methyl biflavone. Being a new biflavone, IIIb has been named as Cephalotaxoflavone.

A C K N O W L E D G E M E N T.

The author wishes to put on record his deep sense of gratitude to Professor W. Rahman who directed these researches, for his unstinted help and encouragement throughout and for providing the facilities necessary for execution of the work in these laboratories.

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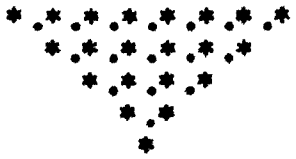
(MOHAMMAD AQIL)

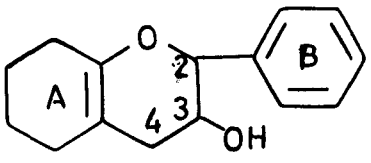
C_O_N_T_E_N_T_S.



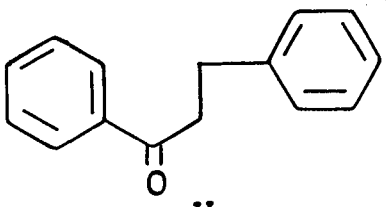
THEORETICAL

The term flavanoids is used to represent an important group of naturally occurring organic 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 chalcones (III), dihydrochalcones (II), aurones (X), flavanones (IV), flavones (VII), flavanonols (VI), flavanols (XI), isoflavanones (V), isoflavones (VIII), leucoantho-cyanidins (XII), anthocyanidins (IX), proanthoeyanins and catechins (I). All these compounds have different oxidation level of C_3 bridge. It is lowest in catechin (I) and highest in flavanol (XI).

The flavanoids are of commercial interest as antioxidants. The antioxidant property of a number of flavanoids has been studied[2]. Seshadri et al[3,4] screened twenty seven flavanoids as antioxidants for lard and Robinetin (3,7,3',4',5'-pentahydroxy flavone) and Gossypetin (3,5,7,8,3',4'-hexahydroxy flavone) were claimed as the most potent. 7,8-Dimethoxy 2',3,5'-trihydroxy flavone, 6-ethyl-2',3,5',7-tetrahydroxy flavone increased the keeping quality of milk powder[5]. The importance of flavanoid compounds in tanning of leather, the fermentation of tea, the manufacture of cocoa and in the flavour qualities of food stuffs is well established[6a,b]. Numerous physiological activities have been attributed to flavanoids[6b,1]. The potent uses of flavanoids may be listed as vitamin P activity (i.e. the property of reducing the capillary fragility and permeability), diuretic action, treatment of allergy, protection against X-rays and other radiation injuries,



I



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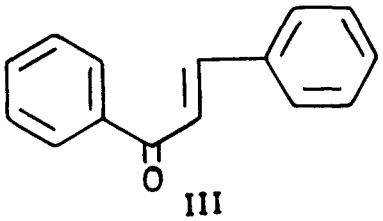

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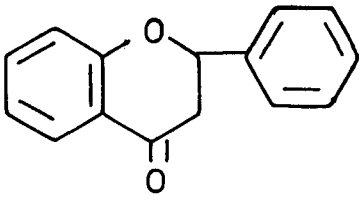

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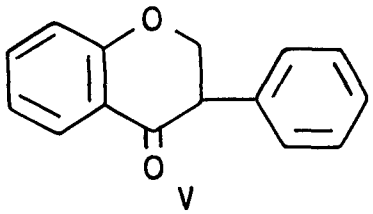

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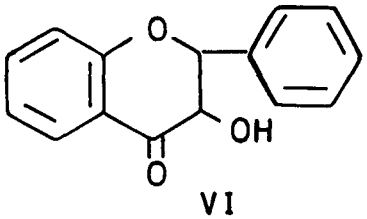

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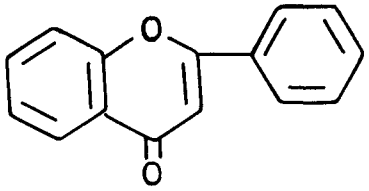

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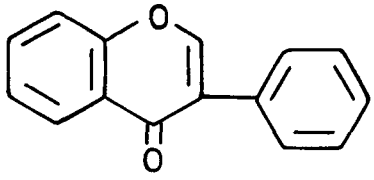
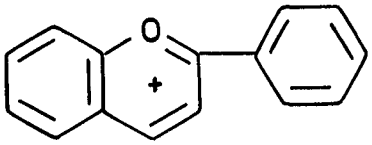
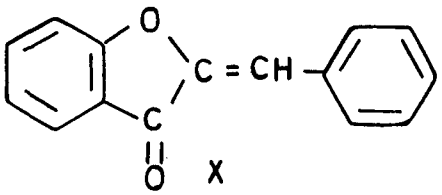


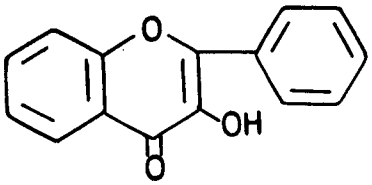
VIII



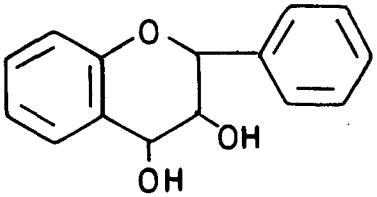
IX



X



XI



XII

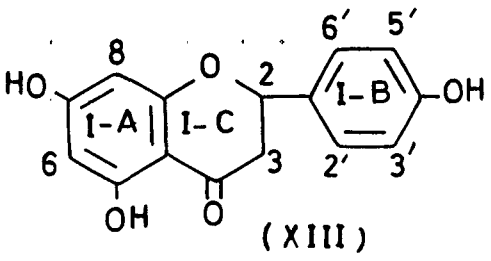
cure of frost-bite, antibacterial activity, prophylactic action, oestrogenic activity and antitumer effects[7]. The study of distribution of flavanoids in plants is of great chemotaxonomic value.[8,9]

Recent addition to this class is "biflavanoids", They are derived from two flavone or flavanone or flavanone-flavone units and have been mostly isolated from Gymnospermae. Among the angiosperms, some plants belonging to Guttiferae[10,11], Euphorbiaceae[12,13], Caprifoliaceae[14], Archegoniatae[15], Ochnaceae[16] and Anacardiaceae[17] and some ferns belonging to selaginellaceae[18] have been found to contain biflavanoids.

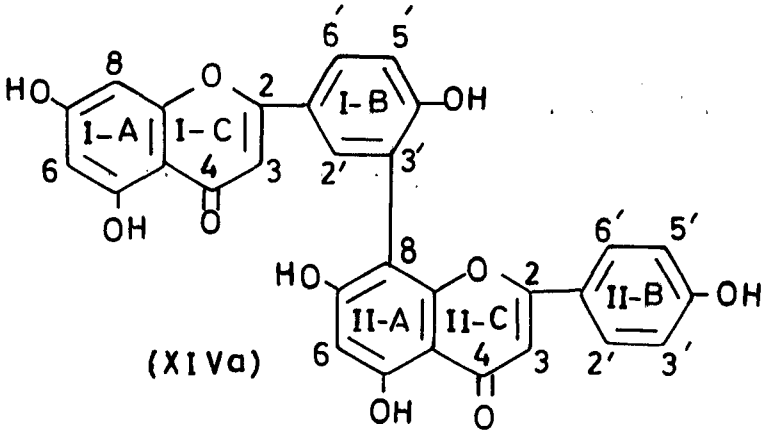
New Nomenclature for Biflavanoids[11]

After consideration of the views of Professor W.D.Ollis, N.Kawano, T.R. Seshadri and Drs. A. Pelter, R.S. Cahn and L.C. Cross, the following systematic nomenclature for flavanoid polymers has been evolved. In this nomenclature the generic term 'biflavanoid' has been adopted in preference to biflavanoid or biflavonyl since in general the saturated system is regarded as the parent for the nomenclature. The ending 'oid' may then be modified to cover specific types of flavanoid dimers such as biflavone, biflavanone, biflavan etc. and for mixed systems flavanone-flavone. This system follows general IUPAC policy; the unmodified nomenclature is utilized as a generic term in the naming of dimeric, trimeric, tetrameric etc.derivatives by insertion of the appropriate Greek prifices bi-, ter-, quater etc. giving biflavanoid, terflavanoid, quaterflavanoid etc.

To identify specific ring positions in flavanoids and their polymeric derivatives, the present long accepted system (examplified in formula XIII for narningenin) is retained extending it in the case of polymeric flavanoids by assigning to each monomer unit a Roman numeral I,II,III etc. running in the sequence from one end of the molecule to another.



(XIII)



(XIVa)

I-4′,II-4′; I-5,II-5,I-7,II-7-hexahydroxy [I-3′,II-8] biflavone(Amentoflavone)

The points of linkage between neighbouring flavanoid units are identified by a combination of a Roman numeral (to identify the flavanoid unit) and in Arabic numeral (to identify the position of the inter-flavanoid linkage), the two

numbers being coupled with a hyphen and enclosed within square brackets. This has been demonstrated in the case of amentoflavone (XI Va)

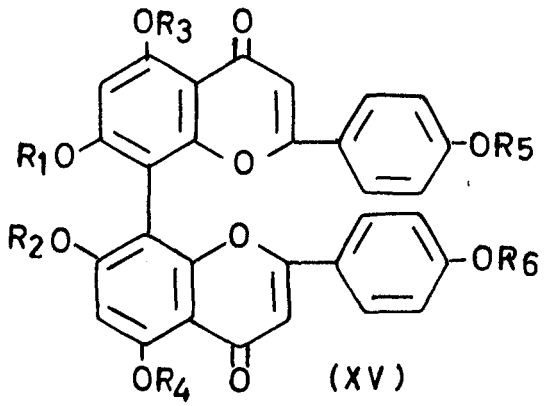
All the biflavanoids known to-date may be classified into two main groups-
(1) C-C linked biflavanoids, and (2) C-O-C linked biflavanoids.

C-C LINKED BIFLAVANOIDS

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

(A) Cupressuflavone Series

These are derived from two apigenim (4',5, 7-trihydroxyflavone) units with [1-8, II-8] linkage and are represented by seven members. Cupressuflavone (XVa)[19] is the parent compound while the other six are its partial methyl ethers.



(XV)

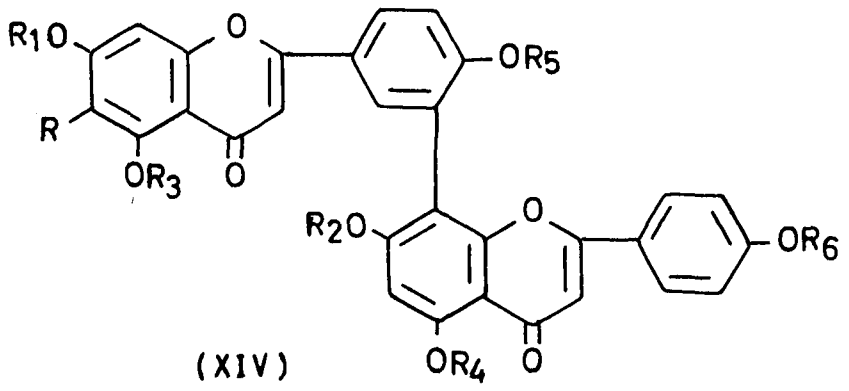
	R_1	R_2	R_3	R_4	R_5	R_6
(a) Cupressuflavone[19]	H	H	H	H	H	H
(b) I-7,O-Methyl[20, 38]	CH_3	H	H	H	H	H
(c) I-7-II-7-Di-O-methyl[20]	CH_3	CH_3	H	H	H	H
(d) I-4', I-7(or II-4',I-7)--Di-O-methyl[38]						
(d) I-4',I-7,II-7-Tri-O--methyl[21]	CH_3	CH_3	H	H	CH_3	H
(f) I-4', II-4', I-7-II-7-Tetra-O-methyl-[22a]	CH_3	CH_3	H	H	CH_3	CH_3
*(g) I-4',II-4',I-5,I-7.II-7-Penta-O-methyl-[23]	CH_3	CH_3	CH_3	H	CH_3	CH_3

*Synthetic.

The structure of I-4', II-4'- Di-O-methyl cupressuflavone, isolated from Araucaria cunninghamii and Araucaria Cokii[22a] has been revised to I-7, II-7-Di-O-methyl cupressuflavone.[22b]

(B) Amentoflavone Series

These are derived from two apigenin units with (I-3',II-8) linkage and are represented by seventeen members with amentoflavone (XIVa)[24,25] as the parent compound.



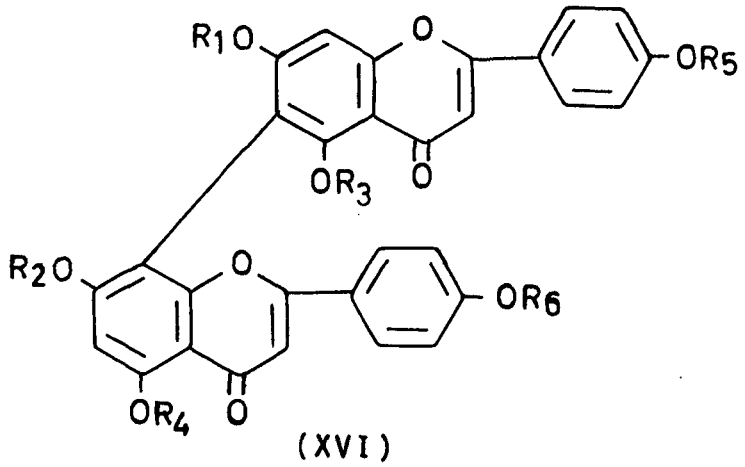
(XIV)

	R_1	R_2	R_3	R_4	R_5	R_6	R
(a) Amentoflavone [24,25]	H	H	H	H	H	H	H
(b) I-7-O-Methyl- (Sequoiaflavone)27	CH_3	H	H	H	H	H	H
(c) I-6-C-methyl,I-7-O- -methyl(Cephaloflavone)26a	CH_3	H	H	H	H	H	CH_3
(d) I-4'-O-Methyl- (Bilobetin) 28	H	H	H	H	CH_3	H	H
(e) II-7-O-Methyl- (Sotetsuflavone)26b,35-37	H	CH_3	H	H	H	H	H
(f) II-4'-O-Methyl- (Podocarpusflavone)A)[29]	H	H	H	H	H	CH_3	H
(g) I-4',I-7-Di-O-Methyl- (Ginkgetin)28,30	CH_3	H	H	H	CH_3	H	H
(h) I-4',II-4-Di-O-Methyl- (Isoginkgetin)28,30	H	H	H	H	CH_3	CH_3	H
(i) II-4',I-7-Di-O-methyl- (podocarpusflavone B)29	CH_3	H	H	H	H	CH_3	H
(j) I-4',II-7-Di-O-methyl-21	H	CH_3	H	H	CH_3	H	H
(k) I-7,II-7-Di-O-methyl-[26c]	CH_3	CH_3	H	H	CH_3	H	H
(l) II-4',I-7,II-7-Tri-O-methyl- (Heveaflavone) 13a,34	CH_3	CH_3	H	H	H	CH_3	H
(m) I-4',II-4',I-7-Tri-O-methyl- (Sciadopitysin)28,30	CH_3	H	H	H	CH_3	CH_3	H
(n) I-4',II-4',II-7-Tri-O- methyl(Kayaflavone) 31	H	CH_3	H	H	CH_3	CH_3	H
(o) I-4',I-7,II-7-Tri-O- methyl- 26c	CH_3	CH_3	H	H	CH_3	H	H
(p) I-4',II-4',I-7-II-7-Tetra- O-methyl 32	CH_3	CH_3	H	H	CH_3	CH_3	H
(q) I-4',II-4',I-5,II-5,I-7, II-7-Hexa-O-methyl- 33	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	H

Sotetsuflavone, reported as the sole biflavone constituent of cycas revoluta, was assigned the structure II-7-O-Methylamentoflavone (XIVe)[35]. But recently, it was found to be a mixture of amentoflavone(major) and its partial methyl ethers[36,37] II-7-O-Methylamentoflavone (XIVe) has, however, been recently isolated from Araucaria Cookii[26b].

(C) Agathisflavone Series

These are derived from two apigenin units with [I-6,II-8] linkage, and are represented by five members with agathisflavone (XVIa)[26b,38] as the parent compound.

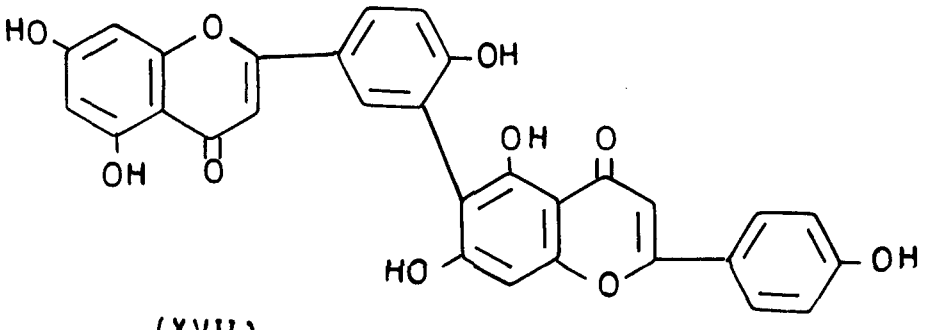


(XVI)

	R_1	R_2	R_3	R_4	R_5	R_6
(a) Agathisflavone[26b,38]	H	H	H	H	H	H
(b) I-7-O-Methyl-39	CH_3	H	H	H	H	H
(c) I-7,II-7-Di-O-methyl[38,40]	CH_3	CH_3	H	H	H	H
(d) II-4', I-7-Di-O-methyl[32]	CH_3	H	H	H	H	CH_3
(e) II-4', I-7,II-7-Tri-O-methyl[26c]	CH_3	CH_3	H	H	H	CH_3

(d) Robustaflavone Series

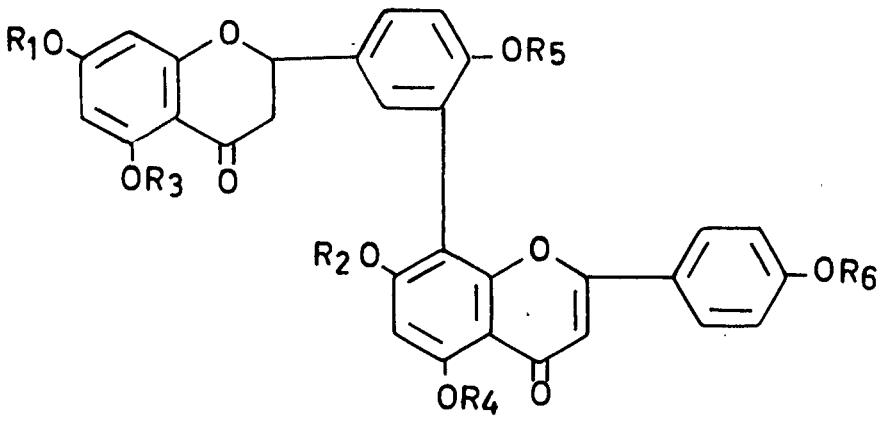
This class has been recognized very recently and is represented only by robustaflavone (XVII) as the parent compound[26d] and its mono-and dimethyl ethers, characterised only as their complete methyl ethers.[41] These are derived from two apigenin units with [I-3',II-6] linkage.



(XVII)

(E) 2,3-Dihydroamentoflavone Series

These are derived from a naringenin and an apigenin unit with flavanone (I-3,II-8) flavone linkage, and are represented by 2,3-dihydroamentoflavone[37,42a,b] (XVIIIa) as the parent compound and its two partial methyl ethers.

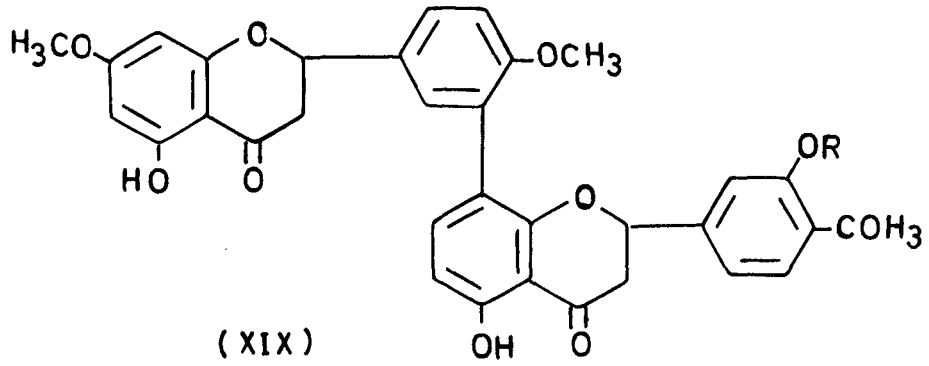


(XVIII)

	R_1	R_2	R_3	R_4	R_5	R_6
(a) 2,3-dihydroamentoflavone[37,42a,b]	H	H	H	H	H	H
(b) II-4',II-7-Di-O-methyl[42a,b]	H	CH_3	H	H	H	CH_3
(c) I-4',II-4',I-7-Tri-O-methyl[38a,b]	CH_3	H	H	H	CH_3	CH_3

(F) [I-3', II-8] Biflavanones.

Three new closely related biflavanones A, B and C have been recently isolated from defatted nuts of Semicarpus anacardium[17]. The first of these has been Characterised as its methyl ethers A_1 (XIXa) and A_2 (XIXb).



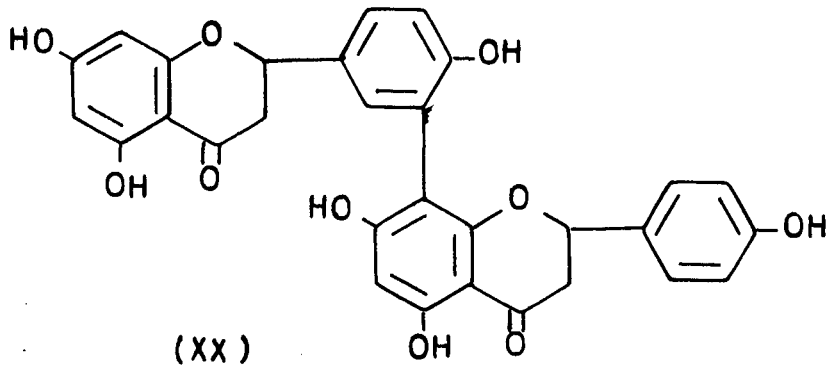
(XIX)

(a) A_1;R=H, I-4',II-4',I-7-Tri-O-methyl-II-3',I-5,II-5-trihydroxy [I-3',II-8] biflavanone.

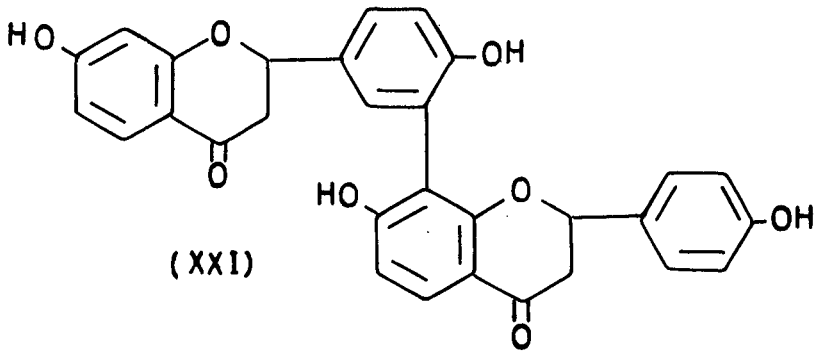
(b) A_2;R=CH_3,II-3',I-4',II-4',I-7-Tetra-O-methyl-I-5,II-5-dihydroxy [I-3',II-8] biflavanone.

The biflavanone B and C have also been characterised as their methyl ethers/corresponding chalcone methyl ethers.

Suggested structures are O-methyl derivatives of [I-3',II-8] binaringenin (XX) for the former and [I-3',II-8] biliquiritigenin (XXI) for the latter.



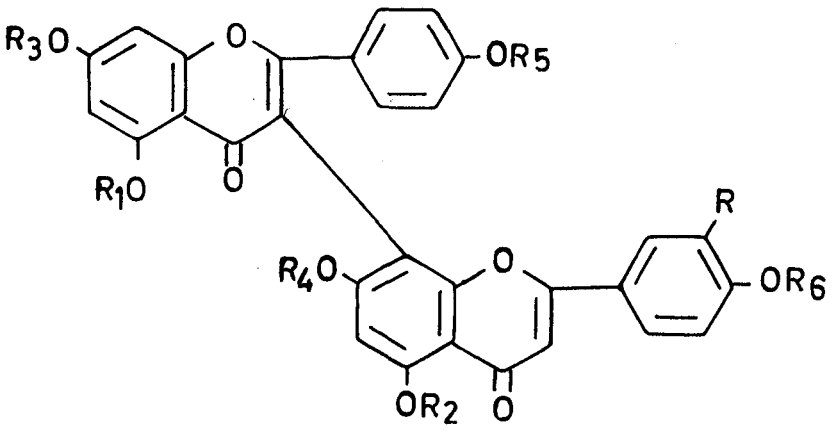
(XX)



(XXI)

(G) BGH Series

These are derived from a naringenin and an apigenin or luteolin unit with flavanone [I-3,II-8] flavone linkage and are represented by BGH-II (XXIIa) and BGH-III(XXIIg) as the parent compounds respectively.

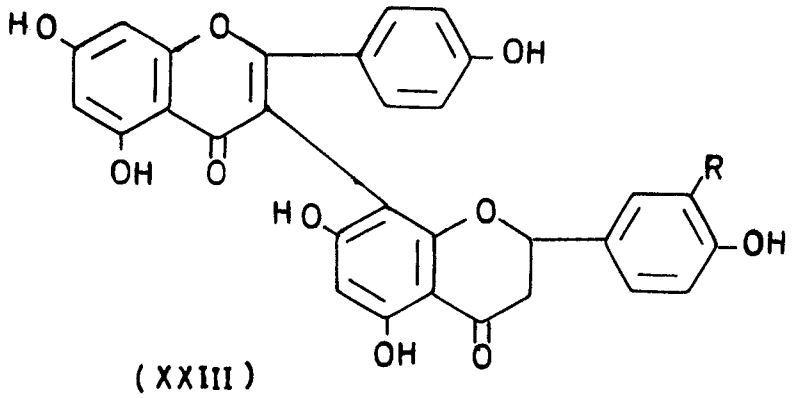


	R	R_1	R_2	R_3	R_4	R_5	R_6
(a) BGH-II(Morelloflavone/ fukugetin)10,43,44	OH	H	H	H	H	H	H
(b) II-3'-O-Methyl-44	OCH_3	H	H	H	H	H	H
*(c) I-4',II-4',I-5,I-7 II-7-Penta-O-methyl- II-3' methoxy 45	OCH_3	CH_3	H	CH_3	CH_3	CH_3	CH_3
*(d) I-4',II-4',II-5,I-7, II-7-Penta-O-methyl- II-3' methoxy 45-	OCH_3	H	CH_3	CH_3	CH_3	CH_3	CH_3
(e) I-4',II-4',I-7-II-7- Tetra-O-methyl II-3'-methoxy-45	OCH_3	H	H	CH_3	CH_3	CH_3	CH_3
*(f) II-4',I-7-,II-7-Tri-O- methyl-II-3' methoxy-46	OCH_3	H	H	CH_3	CH_3	H	CH_3
(g) BGH-III(Talbotaflavone/ Volkensiflavone) 10,47,48	H	H	H	H	H	H	H

*Synthetic.

(H)WGH-Series

Two biflavones, WGH-II(XXIIIa) and WGH-III(XXIIIb)[10], have been synthesised by dehydrogenation of BGH-II(XXIIa) and BGH-III(XXIIg), respectively.



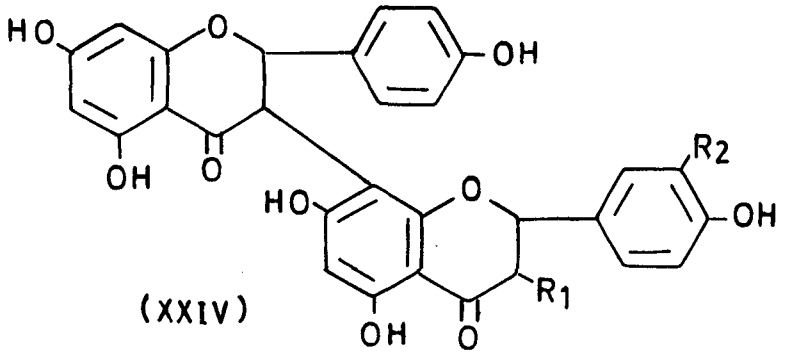
(XXIII)

(a) R=OH; II-3',I-4',II-4',I-5,II-5,I-7-II-7-Hepta-hydroxy I-3,II-8 biflavone (WGH-II or Saharanflavone)

(b) R=H; 1-4',II-4',I-5,II-5,I-7-II-7-Hexahydroxy I-3,II-8 biflavone (WGH-III)

(I) GB Series

These are derived from a naringenin linked with a naringenin or aromadendrin or taxifolin through I-3,II-8 linkage. Four members of this series are reported to occur in nature[11,49a,b].

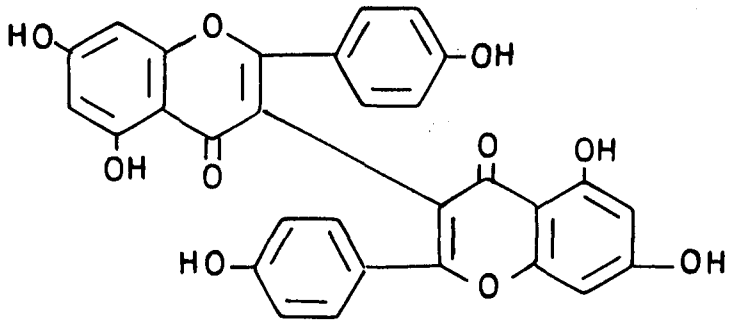


(XXIV)

	R_1	R_2
(a) GB-I	OH	H
(b) GB-Ia	H	H
(c) GB-II	OH	OH
(d) GB-IIa	H	OH

(J) I-3,II-3 Biflavone.

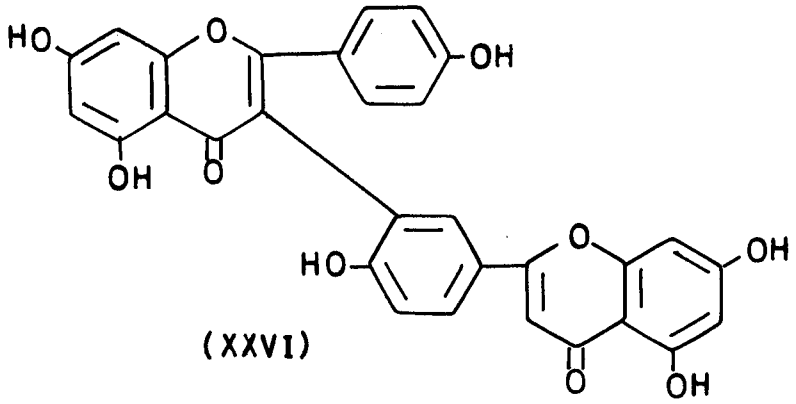
The sole member (XXV) of this series has been synthesised by oxidative coupling of apigenin[50]



(XXV)

(K) [I-3,II-3'] Biflavone

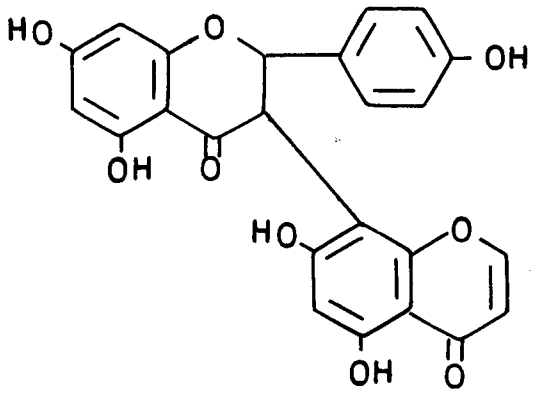
The sole member (XXVI) of this series has also been obtained during oxidative coupling of apigenin.[50]



(XXVI)

(L) I-4',I-5,II-5,I-7,II-7-Pentahydroxy flavanone [I-3,II-8] Chromone:

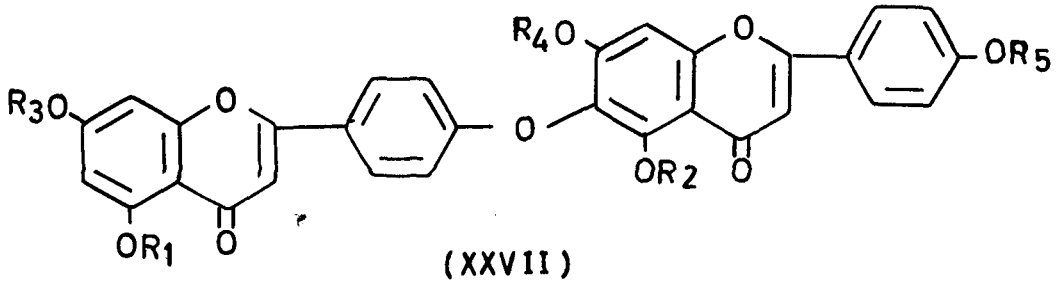
The compound has very recently been isolated from the leaves of Gercinia dulcis Kurz. It is dimer of naringenin and 5,7-dihydroxy Chromone linked through [I-3,II-8].[26d] Its isolation has introduced a new series comprising of flavanone-Chromone structure.



C-O-C linked biflavanoids

(A) Hinokiflavone Series

These are derived from two apigenin units with [I-4'-O-II-6] linkage. Hinokiflavone (XXVIIa) is the parent compound with six others as its partial methyl ethers.

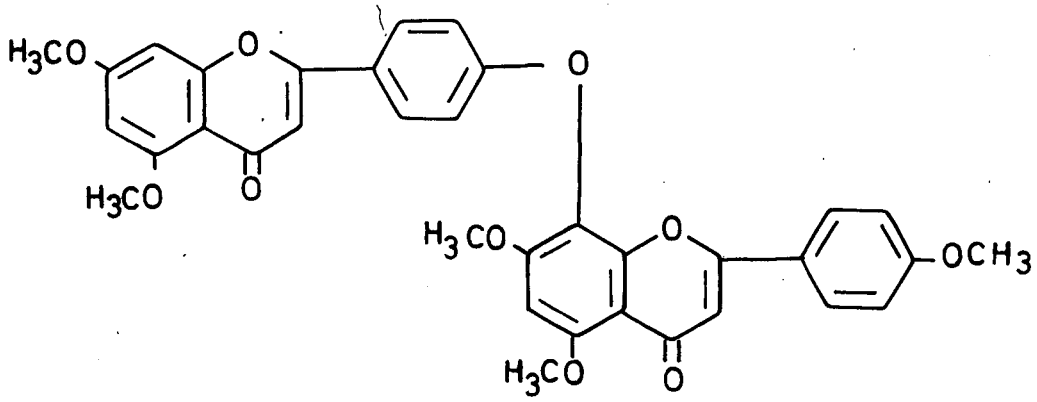


(XXVII)

	R_1	R_2	R_3	R_4	R_5
(a) Hinokiflavone[25,51,52,69]	H	H	H	H	H
(b) I-7-O-Methyl- (Neocryptomerin)[29]	H	H	CH_3	H	H
*(c) II-7-O-Methyl- (Isocryptomerin)[53]	H	H	H	CH_3	H
(d) II-4'-O-Methyl- (cryptomerin A)[54]	H	H	H	H	CH_3
(e) I-7,II-7-Di-O-methyl- (chamaecyparin)[29]	H	H	CH_3	CH_3	H
(f) II-4',II-7-Di-O-methyl- (cryptomerin B)[54]	H	H	H	CH_3	CH_3
*(g) II-4',I-7,II-7-Tri-O- methyl-[54]	H	H	CH_3	CH_3	CH_3

* Synthetic

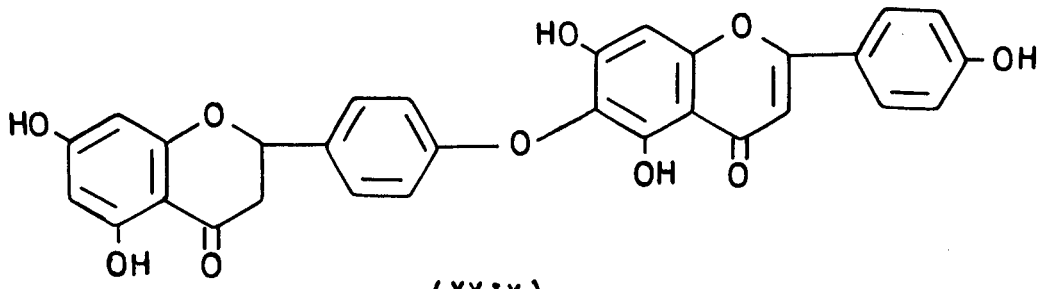
Previously naturally occurring hinokiflavone and its derivatives were assigned [I-4'-O-II-8] linkage on the basis of spectral and degradative evidence;[51] which has later been revised to [I-4'-O-II-6] [52;69] The [I-4-O-II-8] linked hinokiflavone penta methyl ether (XXVIII) has also synthesised.[52]



(XXVIII)

(B) 2,3-dihydrohinokiflavone Series

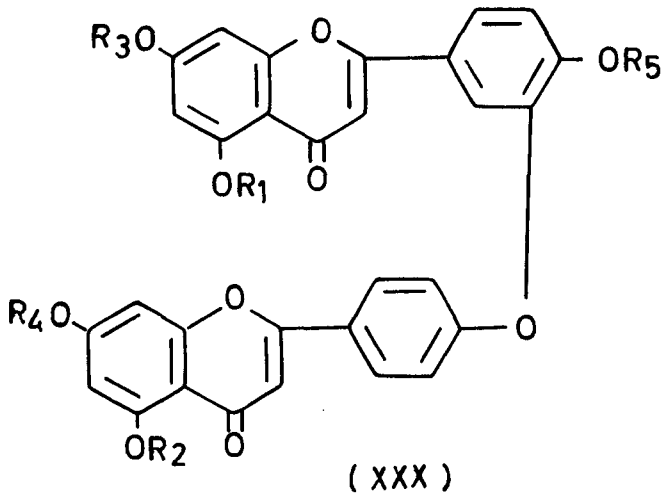
The sole member (XXIX) of this series has been isolated from Metasequoia glyptostroboides and cycas species.[37,42,b] The constituent monomeric units are a naringenin and an apigenin linked through [I-4'-O-II-6]



(XXIX)

(C) Ochnaflavone Series

This series has been recognized very recently and is represented by only four members with ochnaflavone (XXX) as the parent compound. Two apigenin units linked through I-3'-O-II-4' constitute the biflavone.[16,70]



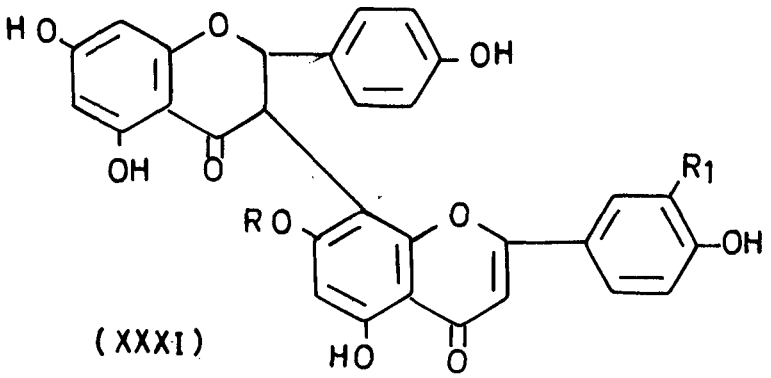
(XXX)

	R_1	R_2	R_3	R_4	R_5
(a) Ochnaflavone[16]	H	H	H	H	H
(b) I-4'-O-Methyl[16]	H	H	H	H	CH_3
(c) I-4',I-7-Di-O-methyl[16,70]	H	H	CH_3	H	CH_3
*(d) I-4',I-7-II-7-Tri-O--methyl[70]	H	H	CH_3	CH_3	CH_3

*Synthetic

Biflavanoid Glycosides

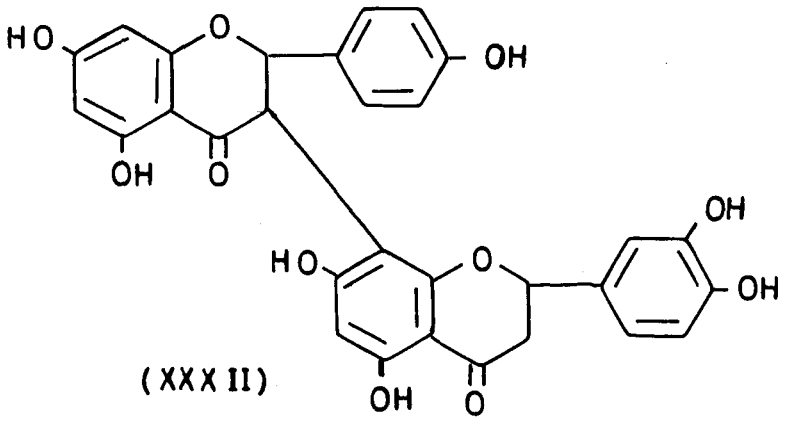
M.Konoshima et al[45,55] have isolated fukugiside (XXXIa) and spicatoside (XXXIb) from Garcinia spicata and xanthochymuside (XXXII) from G.xanthochymus.



(XXXI)

(a) Fukugiside, R_1=OH, R=β-D-glu.

(b) Spicdtoside R_1= H; R=β-D-glu



(XXXII)

Xanthochymuside; R=β-D-glu.

STRUCTURE DETERMINATION OF BIFLAVANOIDS

The problem of structure determination of biflavanoids is a complex one because of (a) Occurrence of more than one biflavanoid in chromatographically homogeneous fractions with consequent difficulty in their isolation in pure form, (b) insolubility in the usual organic solvents, (c) the intricate problem of establishing the interflavanoid linkage, and (d) difficulty in exact location of methoxy groups in partially methylated biflavanoids.

The various methods generally used for structure determination may be classified as below:-

1) Colour reactions
2) Physical methods
3) Degradation, and
4) Synthesis.

(1) Colour Reactions[6c,64]

A number of colour reactions have been reported in literature for detecting certain structural features among flavanoids. As the colour depends upon the pattern of hydroxylation and substitution, the diagnostic value of colours is only a broad indication. Biflavanoids are found to give more or less the same colour reactions as monomers. The reagents generally used for colour reactions are magesium-hydrochloric acid[56]; Sodium amalgum-hydrochloric acid;[57] Wilson boric acid[58a]

and Zinc-hydrochloric acid.[58b] It has however, been observed that unlike monomers all kinds of biflavanoids give positive test with zinc and hydrochloric acid, a test characteristic of flavanonols.[58b]

(2) Physical Methods

Chromatographic and spectral methods (i.e.IR,UV,NMR and Mass spectroscopy) have been most valuable in structure elucidation of flavanoids and biflavanoids. These will be described separately,

(a) Chromatographic Methods:

Paper chromatography in aqueous and alcoholic solvent systems has been extensively applied for the separation and identification of flavanoid pigments.[59,63] Gas chromatography has recently been used successfully for the quantitiative separation of flavanoids as their trimethylsilyl ethers.[60,61] Extensive thin layer chromatographic studies of biflavanoids, their partially and fully methylated derivatives have been carried out in our laboratories.[62] Benzene-Pyridine-formic acid (BPF,36:9:5), toluene-ethyl formate-formicacid (TEF,5:4:1) and Benzene-pyridine-ethyl formate-dioxan (5:1:2:2) have been found as the most satisfactory developing solvent systems both for qualitative as well as quantitative purposes. Homogeneous mixtures in one developing solvent have been successfully resolved in the other. Further, the relative differences in Rf values of the complete methyl ethers coupled with the characteristic fluorescence in U.V. light were found to be of some help in their identification.

Counter current distribution between ethyly methyl ketone and a borate or phosphate buffer of definite pH has been successfully used[18,40] for the separation of individual biflavanoids from isomeric mixtures as well as from mixtures of biflavanoids of different series.

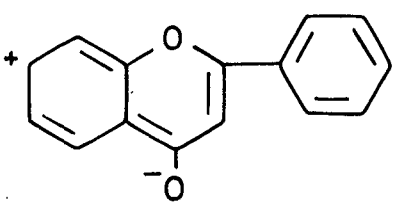
(b) Infrared Spectroscopy:

The ifrared spectra of 5-hydroxybiflavones show strong band at 1660 cm^{-1} as do those of mono-5-hydroxyflavanoids. The band is characteristic of 5-hydroxyflavones, and although this hydroxyl group is internally hydrogen bonded, the effect of 5-O-alkylation and 5-O-acylation is opposite to that shown in the case of simple ortho hydroxy ketones. Because of internal hydrogen bonding, the carbonyl bands of ortho hydroxy ketones show a shift to higher frequencies on either O-alkylation or O-acylation. However, a similar comparison of the infrared spectra of 5-hydroxyflavones and 5-hydroxychromones with the spectra of their 5-O-alkyl and 5-O-acyl-derivatives shows a shift in the opposite direction, that is to lower frequencies. The reason for this anomaly has been discussed by Looker and Hanneman.[28b] In practice, this effect is very useful in diagnosing the presence of 5-hydroxy flavone structure.[28a]

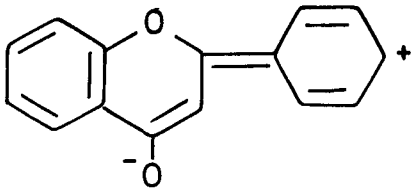
(c) Ultraviolet Spectroscopy:

The UV Spectra of flavanoids have been thoroughly studied and reviewed by L.Jurd[6d] and T.J.Mabry.[63] Flavones (VII) and flavanols (XI) generally exhibit high intensity absorption in the 300-380 m μ region (band I) and the 240-270 m μ region (band II).

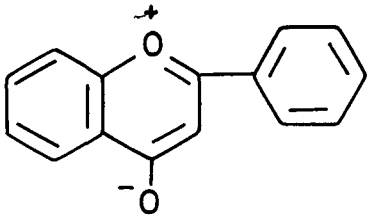
The position and intensity of the λ max of the absorption bands varies with the relative resonance contributions of the benzoyl (XXXIII), cinnamoyl (XXXIV) and Pyrone ring (XXXV) groupings to the total resonance of the flavone molecule.



(XXXIII)



(XXXIV)



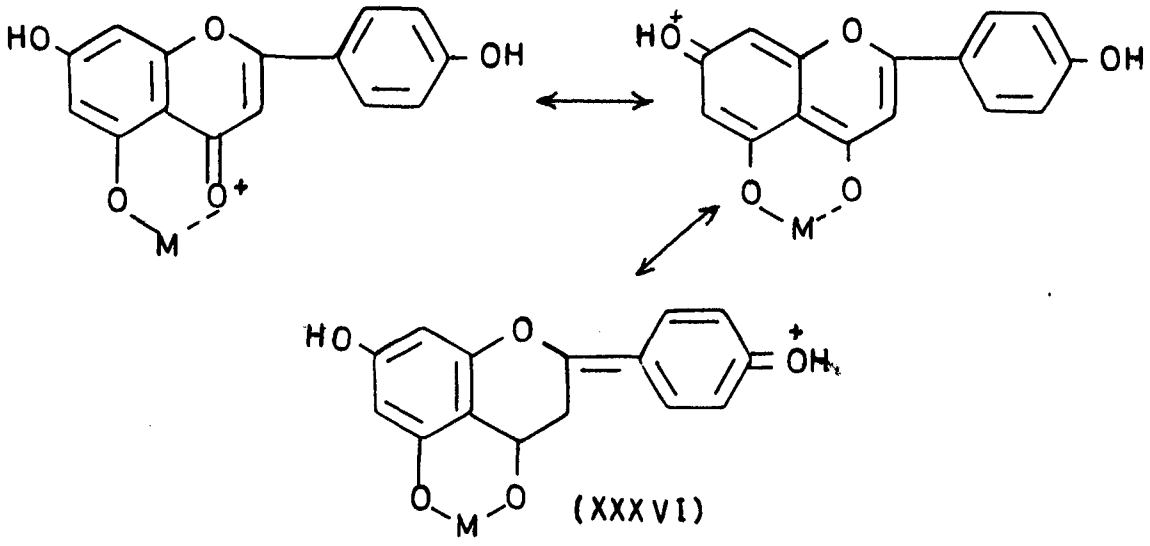
(XXXV)

Although these groupings interact, the spectra of substituted flavones and flavanols in the neutral and alkaline solutions suggest that band I is accociated chiefly with absorption in the connamoyl groupings (XXXIV) and band II with the absorption in the benzoyl groupings (XXXIII).

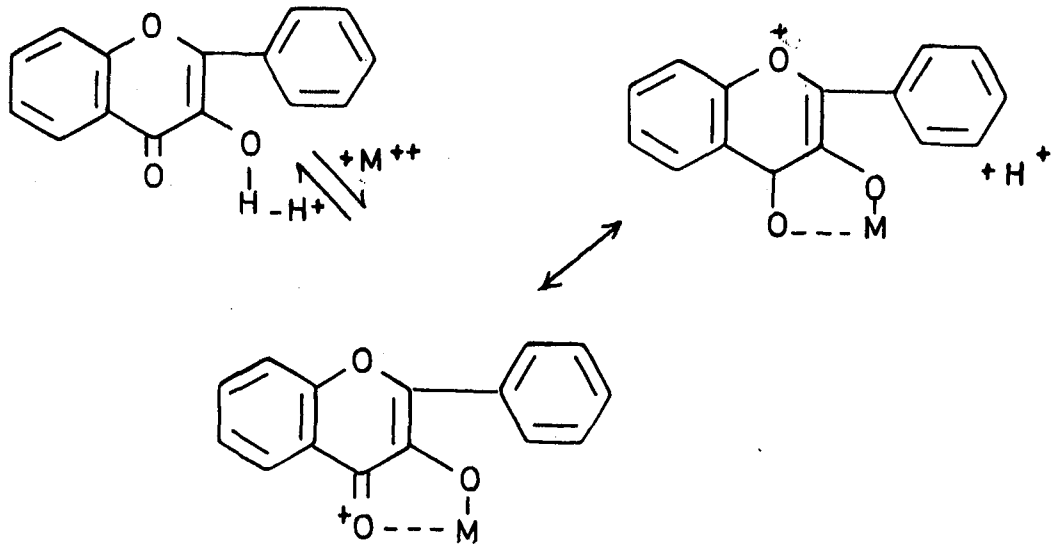
Spectra of Aluminium Complexes-Location of 5 & 3-hydroxy groups:

5-hydroxyflavones and 5-hydroxyflavanols in which the 5-hydroxyl group is protected, form stable yellow complexes of

the type (XXXVI),[65] which result in the considerable bathochromic shifts of band I and II. In flavones this shift is of the order of 20-45 mμ.



3-hydroxyflavones readily form aluminium complexes which are stable even in presence of dilute hydrochloric acid. As a result of complex formation, flavonols produce a flavylium structure(XXXVII). Which is greatly stabilized by its quasiaromatic character.



The bathochromic shift of the flavonol band I to the complex band I is consistently in the order of 60 m/u. A shift of this magnitude is, therefore, reliable evidence for the presence of a free 3-hydroxyl group.

Spectra in Alcoholic Sodium Acetate-Location of 7-Hydroxyl Group:

Sodium acetate is sufficiently basic to ionize hydroxyl groups located at positions 7,3 & 4' of the flavone nucleus. Hydroxyls at other positions are uneffected. Ionization of 3-and 4'- hydroxyls produces bato-chromic shifts of band II. Since band II is associated mainly with the absorption in A ring, ionization of 7-OH group results in a pronounced bathochromic shift of this band. Flavones and flavanols which contain a free 7-OH group may, therefore, be detected by the 8-20 m/u bathochromic shift of the low wavelength band on the addition of a little fused sodium acetate.[66]

Detection of a 3,4'- Dihydroxyl Grouping in Flavanols:

L. Jurd and Horowitz [66] found that flavanols in which the hydroxyl group at either C_3 or C_4, is protected by methylation or glycosidation are stable in sodium ethoxide and that their stability is not appreciably influenced by other hydroxyl group in the molecule. These compounds show normal spectral shifts i.e.the long wavelength band shift from 340-38 m/u in sodium ethoxide.

Detection of O-Dihydroxyl Groups:

Boric acid, in the presence of sodium acetate forms chelates with the phenolic compounds containing O-dihydroxyl

groups. Thus the max of band I in luteolin undergoes a bathochromic shift of 15-30 mμ on addition of a mixture of boric acid and sodium acetate.[67] The spectra of compounds which do not contain an O-dihydroxyl group are not appreciably affected.

The ultraviolet spectra of biapigenin or binaringenin type biflavanoids and their derivatives is very similar to those of consitutuent monomer units, with the only difference that the molecular extinction coefficients of the biflavanoids are approximately double as compared to the corresponding monomers. This demostrates the presence of two isolated chromophores of flavanoid per molecule of a biflavanoid. The ultraviolet spectra of mixed systems such as flavanone-flavone or chalcone-flavone combine the features of simple flavanone/chalcone and flavone chromophores and these features are virtually reproduced in the composite spectrum of an equimolar mixture of constituent monomer units.

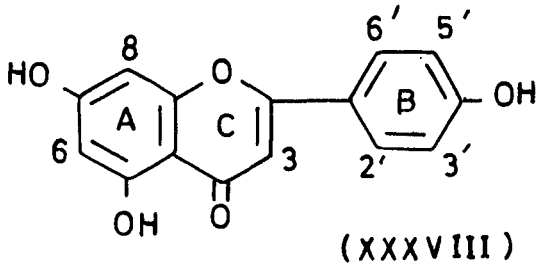
The effect of diagnostic reagents such as NaOEt, $AlCl_3$ etc., on the spectra of biflavanoids is similar to those in monomers. The difference in the activity of hydroxyl groups may arise due to steric factors. These differences have been well exploited by Baker et al[28a] in assignment of methoxy group in isoginkgetin and ginkgetin, kayaflavone and sciadopitysin etc.

(d) Nuclear Magnetic Resonance (NMR)Spectroscopy:

The application of NMR spectroscopy have proved to be the most powerful tool in the structure determination of flavanoids and biflavanoids. By the use of silyl derivatives,[68]

double irradiation technique,[71] solvent induced shift studies[39,72,69] and very recently introduced lanthanide induced shift studies,[73] one can come to the structure without tedious and time consuming chemical degradation and synthesis. The valuable contributions in this field have been made by Batterham and Highet;[74] Mabry;[63,75] Massicot;[76] Clark-Lewis,[77] Kawano[29,73,70] and Pelter and Rahman.[22,26d,39,71,16]

The most commonly occurring hydroxylation pattern in natural falvanoids is 4',5,7-trihydroxy system(XXXVIII). The chemical shifts of the protone of ring A and B prove to be independent of each other but are affected by the nature of the C ring.[74] In flavanones, the 6,8-protons give a single peak near τ4.05.



(XXXVIII)

With the introduction of a 3-hydroxy group (flavanonols), the chemical shifts of these protons are slightly altered and the pattern changes to a strongly coupled pair of doublets. The presence of the double bond in C ring of flavones and flavonols

causes a marked downfield shift of these paeks, again producing the two doublet pattern (τ3.4-4.0, J_{meta} = 2.5 cps). Out of 6- and 8-protons, the latter appears down field.

All B ring protons appear around τ, 2.3-3.3, a region separate from the usual A ring protons. The signals from the aromatic protons of an unsubstituted B ring in a flavanone appear as a broad peak centred at about τ2.55. In flavones, the presence of C ring double bond causes a downfield shift of 2',6'-protons and the spectrum shows two broad peaks, one centred at τ2.00 (2',6') and the other at τ2.4 (3',4',5').

With the introduction of a 4'-hydroxyl group, the B ring protons appear effectively as a four-peak pattern. This is called A_2 B_2 pattern. The hydroxyl group increases the shielding on the adjacent 3',5'-protons and their peaks move substantially upfield.

The 2',6'-protons of flavanones give signals centred at about τ2.65. Introduction of 2,3-double bond (flavones and flavanols) again causes these protons to resonate at much lower field. (τ2.00). Introduction of one more substituent to ring B gives the normal ABC pattern.

The olefinic protons (ring C) of flavones and isoflavones of normal structure give rise to signals near τ3.2 and τ1.7, respectively. Their position, however, is affected by the substitution in A or B ring, the electron donating groups causing upfield shift and electron withdrawing groups causing downfield shift. The spectra of flavanones

contain typical ABX multiplets arising from a 2-proton and two 3-protons. The 2-proton is generally a double doublet near τ 4.5(J_{cis}=5cps, J_{trans}= 11cps), the precise position depending upon the substitution in ring B. The two 3-protons give rise to multiplets of eight lines near τ7.00 ($J_{H-3a,H-3b}$=17cps). However, they appear as two doublets since two signals of each quartet are of low intensity.

3-Hydroxyflavanones give rise to a doublet (J=11 cps) near τ5.1 for C-2 proton and another doublet at about τ5.8 for C-3 proton. The relative stereochemistry of 3-substituted flavanones can usually be established from a consideration of vicinal coupling constants and the "KARPLUS equation". In all cases, the heterocyclic ring appears to adopt the chair or half chair conformation in which 2-aryl substituent is quasi-equatorial. Massicot and Marthe,[76] analysing the ABX spectrum of heterocyclic ring protons of 6,7-dimethoxyflavanone, have shown the two vicinal coupling constants to be 13.5 and 3.2 cps. The former is clearly a diaxial interaction, thus establishing the equatorial character of 2-aryl group in flavanones. All 3-hydroxy and 3-acetoxy flavanones, which have been examined, exhibit vicinal coupling constants 12 cps and were therefore, assigned the trans (diequatorial) configuration, although in the case of naturally occurring compounds the possibility of epimeriztion can not be excluded.

The proton of 5-hydroxyl group next to C-4 carbonyl group of a flavanoid gives rise to a sharp signal at a very

low field (τ3.00) consistent with the strong hydrogen bonding between the two groups. Methylation of a hydroxyl group commonly produces an upfield shift ($\sim$0.2 ppm)of the signals of ortho protons with a somewhat smaller effect on those of para protons and little or no effect on the meta protons. Acetylation of a hydroxyl group, as expected causes downfield shift of ring protons.

In the structure elucidation of biflavanoids certain useful information can be obtained by comparison of their NMR spectra with those of their corresponding monomers. Such a choice, however, is compelling but by no means infallible. Comparison of the NMR spectra of methyl and acetyl derivatives of a biflavanoid with those of biflavanoids of the same series as well as with those of biflavanoids of other series in which at least one monoflavanoid unit is similarly constituted, is very helpful in assigning each and individual proton and the position of the methoxy groups. The problem of interflavanoid linkage has been successfully solved by solvent induced shift studies of methoxy resonances [39,41,69] and lanthanide induced shift studies.[41,73]

In biphenyl type biflavones such as amentoflavone, cupressuflavone, agathisflavone etc., the peaks of ring protons involved in interflavanoid linkage appear at somewhat lower field ($\sim$0.5 ppm) as compared with the peaks of the same proton in monomer due to extended conjugation.

It has been observed[39] both in biphenyl as well as in

biphenyl ether type biflavanoids that the 5-methoxy group of an 8-linked monoflavanoid unit in a biflavanoid shows up below τ6.00 in deuterochloroform in all the cases examined so far (Table-I). This observation may be explained in the basis of extended conjugation. 5-methoxy group of an 8-linked monoflavanoid unit in biflavanoids of BGH-series, WGH-series and GB-series does not show up below τ6.00 as the linkage is through heterocyclic ring.

T_A_B_L_E - I

Methoxy proton shifts (τ values) of fully methylated biflavanoids.

Biflavanoid		I-5-OMe	II-5-OMe
Cuperessuflavone	[I-8,II-8]	5.85	5.85
Amentoflavone	[I-3',II-8]	6.13	5.94
Agathis flavone	[I-6,II-8]	6.41	5.95
*Hinokiflavone	[I-4'-O-II-8]	6.00	5.92
2,3-Dihydroamento-flavone	[I-3',II-8]	-	5.95

* Synthetic

By examining the methoxy and acetoxy shifts certain useful correlations emerge but they should be used only as supproting evidence. It is only by looking at the full series (parent, fully methylated and acetylated products) and comparing multiplicities and positions 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-B appear consistently lower than those of ring II-B.[22,39,71]

The protons at II-8 in hinokiflavone [I-4'-O-II-6] methyl ether and at I-8 in agathisflavone [I-6,II-8] methyl ether appear at exceptionally low positions, τ2.95 and τ3.09, respectively. This may be diagnostic of H-8 of a 6-sibstituted ring in biflavanoid methyl ether both of biphenyl and biphenyl ether type.

The methoxy group at C-5 (ring I-A) of agathisflavone methyl ether (τ6.41) and one methoxy in chalcone-flavone corresponding to BGH-III methyl ether (τ6.80) and WGH-II methyl ether (τ6.56) showed up at exceptionally high field than the other methoxy groups. This internal shielding effect is also evident in the case of chalcones BGH-III heptaacetate and BGH-II octaacetate in which the protons of one acetoxy group appear at τ8.08 whereas those of others at τ7.26-7.80.

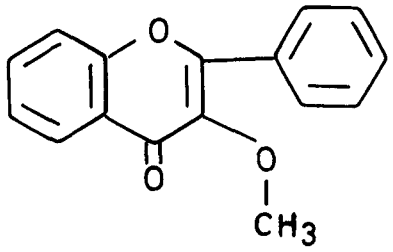
The dependency of H-6 of ring II-A upon its mode of bonding with the other half of the biflavanoid has been observed;-

Biflavanoid methyl ether	H-6(Ring II-A)	C_8(Ring II-A) bonded to
BGH-III	τ3.82	Reduced heterocyclic ring
BGH-II	τ3.74	"
WGH-III	τ3.55	heterocyclic ring
WGH-II	τ3.49	"
Cupressuflavone	τ3.41,3.42	aromatic ring I-A
Amentoflavone	τ3.38	aromatic ring I-B
Agathisflavone	τ3.36	aromatic ring I-A

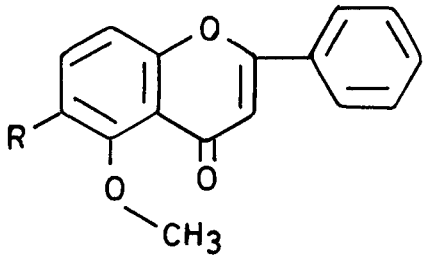
Solvent induced shift studies in NMR spectroscopy:

Williams and Co-workers[78] have observed that methoxy groups at C-5, C-7, C-2' and C-4' exhibit large positive Δ values ($\Delta = \delta CDCl_3 - \delta C_6H_6 \sim$ 0.5-0.8 ppm) in the absence of methoxyl 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 deshilded or only slightly shielded (Δ =-0.07 to +0.34) in benzene, suggesting that the C-3 methoxy group in general prefers conformation indicated in (XXXIX). Similarly a 5-methoxy group in presence of a 6-substituent shows small positive or negative solvent shift in

benzene because a 6-substituent should lead to a higher population of the conformer (XL).



(XXXIX)



(XL)

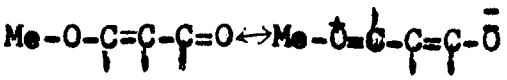
In these conformations, the protons of the methoxy group in question lie in close proximity to the negative end of the carbonyl dipole which is a region of strong deshielding due to benzene association at the carbonyl group.[78] The methoxy groups lacking one ortho hydrogen (i.e.flanked by two ortho methoxy functions or one ortho hydroxy and one ortho methoxy function) also show small positive or negative Δ values. (+ 0.13 to - 0.12 ppm) due to some combination of (i) steric inhibition of benzene solvation of the central methoxy group, (ii) electron donating nature of ortho substituents, and (iii) solvation of the other methoxy groups, the stereochemistry of benzene association being such as to place central methoxy group in a region of deshielding. It is emphasised that the steric factors can not be the major influence, since an electron withdrawing substituent ortho to methoxy function increases the upfield shift which is observed in benzene.[78,79]

In amentoflavone[22], cupressuflavone[71] and hinokiflavone (I-4' -O-II-8)[69] methyl ethers, all the methoxy group moved

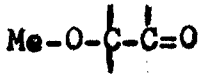
upfield (50-60 cps) on change of solvent from deuterochloroform to benzene showing that every methoxy group has at least oe ortho proton and, therefore, a C-8 rather than a C-6 linkage is indicated. In agathisflavone hexamethyl ether, however, only five of the six methoxy groups showed large upfield shifts. One methoxy group was unique in that upto 50% dilution with benzene no shift was seen and then a strong dowfield shift was evidenced. It was reasonable to assume that the methoxy group in question was the one at C-5 flanked by ring II-A on one side and a carbonyl group on the other.[39] Similarly in the case of hinokiflavone ([I-4' - O-II-6]) only four methoxy groups moved upfield.[69]

Benzene induced shift studies were also found useful in the biflavanoids of BGH-Series. All the methoxy signals (6.08-6.36) in BGH and BGH-III methyl ethers moved upfield indicating that the flavanone substituent is at C-8 rather at C-6 of the flavone unit.[10]

The benzene induced solvent shifts $\triangle(\delta CDCl_3/C_6D_6)$ are appreciably enhanced by the addition of small quantity (3% 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 (XLI) and those which can not conjugate (XLII) in the ground state.



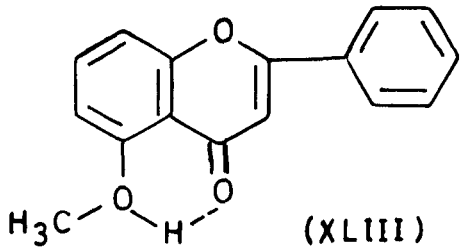
(XLI)



(XLII)

Thus the basicity of the mthoxy groups not conjugated (XLII) with the carbonyl group is greater than those which are conjugated (XLI) and so the former will be expected to give more positive values of the TFA-addition shift $[\Delta(C_6H_6/C_6H_6\text{-}TFA)]$.

The TFA induced solvent shift $[\Delta(CDCl_3/TFA)]$ of a 5-mthoxy group has a relatively large negative value (-0.36 to -0.44 ppm), 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 (XLIII). The carbonyl group will be protected to a much larger extent in TFA relative to a solution in benzene containing only 3% TFA.[80]



(XLIII)

Limitations of the solvent induced shift studies:

The method of methoxy proton shifts, although very useful in structure determination, amy lead to erroneous assignments if not used with caution.[81] 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.[81]

(2) In the biflavanoid series, the II-3' methoxy group of WGH-II methyl ether appears at an exceptionally high position (τ6.56) 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 of the other flavanoid unit, thus rendering it unique in being resistant to external solvation. On change of solvent from $CDCl_3$ to C_6H_6 all the methoxy groups were expected to move upfield by more than 30 cps as each methoxy group has an ortho proton. The methoxy group in question, however, moved very little.[10]

Mabry et al[72] have recently reported that trimethylsilyl ethers of flavanoids are still better derivatives for locating certain methoxyl groups in all flavone and flavanol aglycones and glycosides utilizing benzene induced shifts. In addition, when a O-trimethylsilyl group is at C-5, it also exhibits a diagnostic benzene induced shift (-0.14 to -0.20 ppm as compared to -0.05 to + 0.12 ppm for other trimethylsilyl groups) and furthermore in benzene all the signals for the O-trimethylsilyl groups are well separated thus permitting the determination of the number of hydroxyl groups present in flavanoid before trimethylsilylation.

Paramagnetic induced shift studies in NMR Spectroscopy:

During the last five years lanthanide shift reagents (LSR) have been extensively used for the structural and conformational studies of organic natural products.[91-94]. The introduction of these reagents has greatly enhanced the power and versatlity of NMR spectroscopy. The addition of a LSR to an NMR solution of a compound which possesses an appropriate lone pair of electrons[94] causes the proton resonances to become "Spread out", often into a first order pattern, making possible safe assignment of formally non-equivalent, but usually coincident resonances and enabling decoupling experiments to be carried out. Several hundred papers have appeared describing the use of these reagents since the first was reported by Hinckley.[95]

The lanthanide induced shifts (LIS or $\Delta\nu_i$) are due primarily to pseudocontact interactions,[95] resulting from the association of lanthanide complex and lone pair functionality of the substrate, 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)[94,111]

$$\Delta\nu_i = \frac{K}{r_i^3} \quad \text{---} \quad (1)$$

Equation(1) shows that the principal factor influencing the shift of a particular resonance in NMR spectrum, is the distance, either bondwise or spatially separating the metal ion from the proton which is responsible for that peak. Thus the closer the proton to the metal ion in the shift reagent-substract complex, the greater the shift observed.

A more complete form of the equation (1) is equation (2) where θ is the angle describing the position of the proton i relative to the principal magnetic axis of the lanthamide substrate complex: ri is the (Eu-H) internuclear distance, $\Delta\nu i$ is the pseudocontact shift for the ith proton and K is a constant. The angle term ($3\cos^2\theta-1$) is positive for θ values from 0^o-54^o and from 126^o-180^o and a positive $\Delta\nu i$ (shift to lower field) is observed; however, when θ has a value from 55^o to 125^o, the angle term and become negative (i.e. shifts to higher field are observed).[112]

$$\Delta\nu i = \frac{K(3\cos^2\theta-1)}{ri^3} \quad \text{----- ----(2)}$$

The most commonly used complexes are tris (dipivalomethanato) europium (III), $Eu(DPM)_3$[113] and tris (dipivalomethanato) praseodymium (III), $Pr(DPM)_3$[114]; where HDPM represents dipivalomethane which is 2,2,6,6-tetramethylheptane-3,5-dione. The two are complementary in that $Eu(DPM)_3$ shifts proton resonances to lower field while $Pr(DPM)_3$ shifts resonances to higher field. $Eu(DPM)_3$, however, is generally most useful because the t-butyl resonance of the complex appears above TMS and thus does not interfere. The t-butyl resonance of $Pr(DPM)_3$ in the presence of substrates, occurs in the 7-5τ range and can mask resonances of interest in some cases. It is specially useful for the observations of methyl groups in steroids.[114]

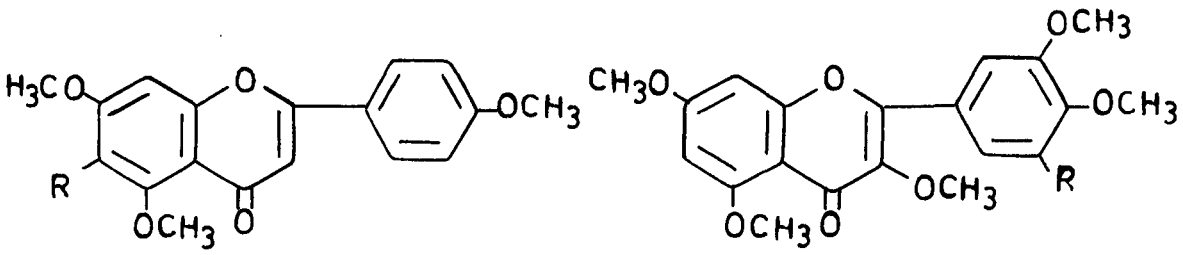
The magnitude of induced shift for a proton is usually expressed in terms of "S-Value" proposed by Cockerill and Rackham,[96] as the slope of straight line obtained by plotting the shift value ($\Delta\nu$) against the molar ratio of $Eu(DPM)_3$ to a substrate. Usually spectra are determined at 8-10 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[97] that the shift reagent exhibits its effect by establishment of a rapid (on the NMR time scale) equilibrium between a labile complex of $Eu(DPM)_3$ with a lewis base and unassociated solutes. This labile complex contributes very significantly to the observed shift through at least two mechanisms, through bonds 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 induced shifts are sums of contributions due to magnetic interaction from metal association at each site.

Kawano et al,[37,16] have recently reported paramagnetic induced shift studies in the NMR spectra of flavones and biflavones using $Eu(FOD)_3$. These studies provide an excellent method to distinguish between a proton attached to either C_6 or C_8 of a flavone nucleus because H-6 shows much larger shift than H-8.

The results on four monoflavanoid methyl ethers, namely, apigenin trimethyl ether (XLIV), 6-hydroxyapigenin tetramethyl ether (XLV), quercetin pentamethyl ether (XLVI) and myricetin hexamethyl ether (XLVII) are recorded in Table-II. It follows that (a) OCH_3-5 shows the largest shift (12.34 ~ 18.88 ppm) meaning that complexation occurs mostly at neighbouring carbonyl group, (b) H-6 shows considerable shift (5.70 ~ 7.16 ppm),



XLIV, R = H
XLV, R = OCH_3

XLVI, R=H
XLVII, R= OCH_3

(c) H or OCH_3 attached to side phenyl groups show the least shifts, and (d) H-3 (-1.54, 0.08 ppm) and OCH_3-3 (0.08, 0.92 ppm) show rather small shifts in comparison with those of OCH_3-6 (5.16 ppm) and OCH_3-7 (1.02 ~ 1.28 ppm) whose positions are at a distance from carbonyl group. It is noteworthy that the H-3 of compound XLV shows an upfield shift (—1.54).

TABLE - II

S-values of flavone compounds by Eu (FOD)$_3$

Positions	XLIV	Compounds XLV	XLVI	XLVII
3.	0.08	—1.54	(0.80)	(0.92)*
5.	(13.34)	(12.34)	(14.08)	(18.88)
6.	6.32	(5.16)	5.70	7.16
7.	(1.12)	(1.02)	(1.28)	(1.14)*
8	1.56	1.18	1.12	1.30
2',6'	0	— 0.50	0.46	0.46
3',5'	—0.02	— 0.26	0.14,(0.12)	(0.12)
4'	(0)	(—0.18)	(0.04)	(0.32)

* Assignment is tentative. Parentheses show methoxy proton shifts. Spectra were taken in $CDCl_3$ solution using internal TMS

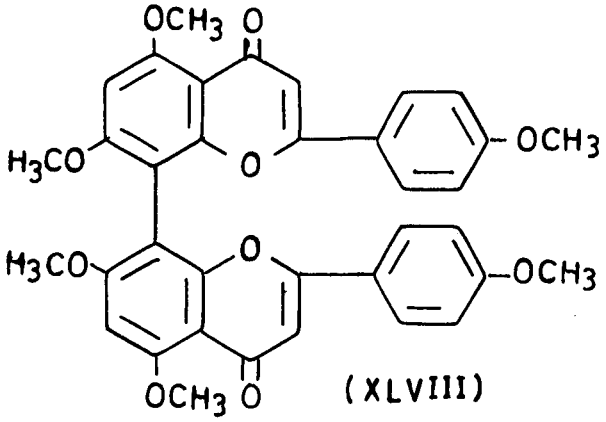
Six fully methylated biflavones, namely, hexa-O-methyl cupressuflavone (XLVIII), hexa-O-methylagathisflavone (XLIX), hexa-O-methyl amentoflavone (XIVq), hexa-O-methylrobustaflavone(L), penta-O-methylhinokiflavone (LI) and penta-O-methyl (I-4'-O-II-8) biapigenin (LII)[73] have been studied using $Eu(FOD)_3$ and their S-values are recorded in Table-III.

T_A_B_L_E - III

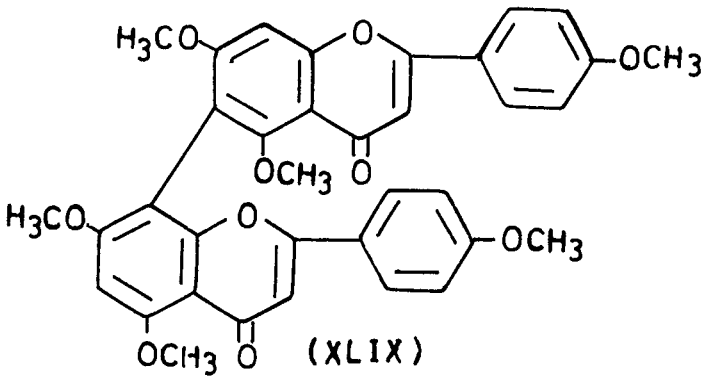
S-values of fully methylated biflavones by $Eu(FOD)_3$

Protons	XLVIII	XLIX	XLVq	L	LI	LII
OCH_3-I-5	7.34	2.14	6.12	2.64	10.02	6.88
II-5	-	11.16	8.78	10.58	4.38	6.60
I-7	0.72	0.44	0.36	0.74	0.80	0.82
II-7	-	0.04	1.06	0.56	0.58	0.52
I-4'	- 0.06	0.02	0.12	0.32	-	-
II-4'	-	- 0.08	—0.08	- 0.06	- 0.06	—0.10
H-I-3	0.18	0.28	0.02	0.30	—0.06	0.14
II-3	-	0.06	- 0.16	0.16	0.36*	0.26
I-6	3.66	-	2.76	4.84	4.80	3.36
II-6	-	5.80	4.24	-	-	3.52
I-8	-	0.64	0.50	1.20	1.14	0.72
II-8	-	-	-	0.50	0.74*	-
I-2',I-6'	0.56	0.08	--0.36,0	14.2, 0.36	0.04	- 0.02
II-2'-,II-6'	-	0.52	- 0.12	— 0.08	—0.14	0
I-3',I-5'	0.24	0.02	— 0.10	—,-0.52	2.00	—0.10
II-3',II-5'	-	0.06	— 0.08	—0.06	—0.08	0.18

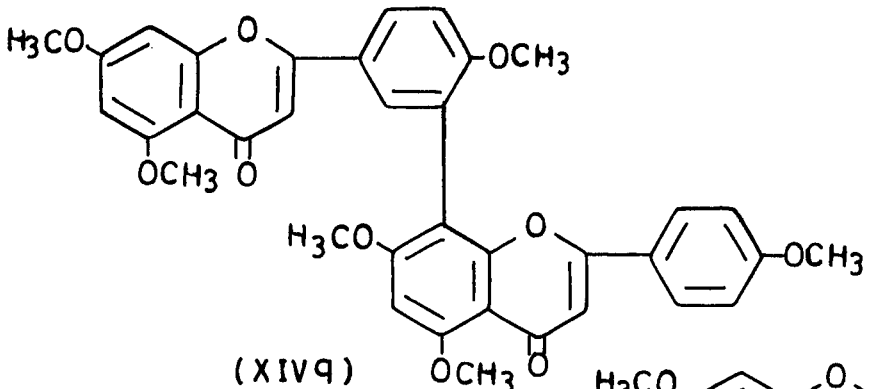
* Assignment is tentative.



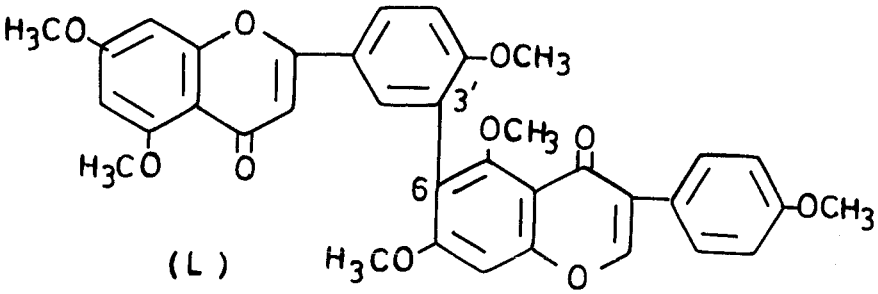
(XLVIII)



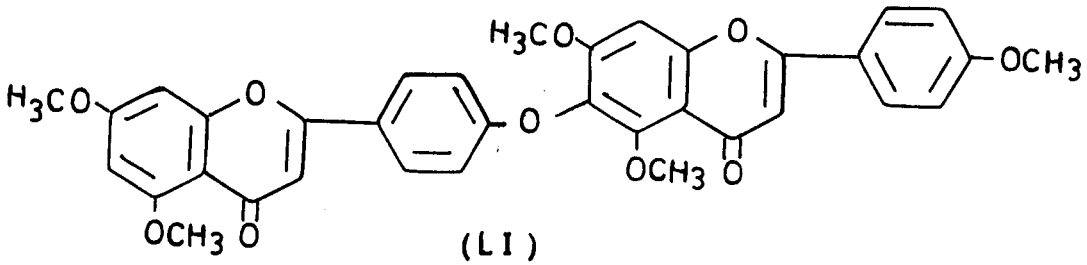
(XLIX)



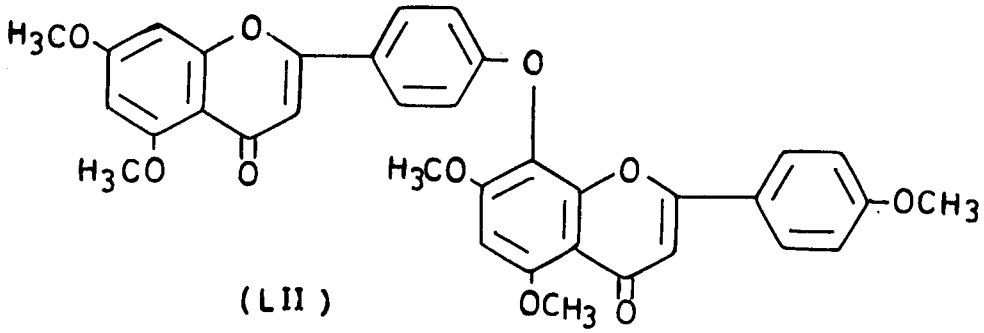
(XIVQ)



(L)



(LI)



(LII)

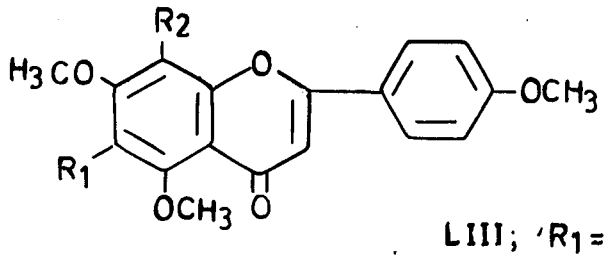
The induced shifts show the same tendency as observed for the four monoflavones. In biflavones, an half amout of used reagent is effective to each flavone nucleus when the same molar ratio of the reagent is added. However, OCH_3-I-5 and II-5 show different shift values from each other except for a symmetrical compound XLVIII. This means that complexation of $Eu(FOD)_3$ to both flavone nuclei is not even but characterstic to each compound due to their chemical structures.

It is interesting that H-I-3' and I-5' of compound (LI) show a much larger shift value (2.00 ppm) than those (0.10~0.24 ppm) of the other compounds perhaps because the side phenyl group is attached to 6-position of the other flavone nucleus.

The largest shift value among those of H-I-8 and II-8 is 1.14 ppm (Compound 'LI'), which is still much smaller value than those of H-I-6 and II-6 (2.76~5.80 ppm). This fact makes it possible to distinguish between 6 and 8 protons in a flavone and accordingly to decide the interflavanoid linkage through either C-6 or C-8 in biflavones.

As described above, H-3 of the 6-hydroxyapigenin tetramethyl ether (LV) shows an upfield shift (−1.54 ppm in S-value, Table -II) on addition of $Eu(FOD)_3$.[73] However, this proton shows downfield shift (Δ Eu=2.04 ppm) when $Eu(DPM)_3$ is used as a shift reagent (Table IV) as reported recently by Kawano et al.[98] This constitutes the first example of a proton signal shifting in opposite directions due to different reagents,

$Eu(DPM)_3$ and $Eu(FOD)_3$ (2.04 ppm and —1.50, respectively). The presence of OCH_3-6 in the 5,7-dimethoxyflavone derivatives seems to be an important factor in the phenomenon because the H-3 of 5,6,7,8,4' -pentamethoxyflavone (LVI) also shows similar shifts values (1.79 ppm and —1.99, Table IV) to those of compound (LIV) when $Eu(DPM)_3$ or $Eu(FOD)_3$ are added. 8-Hydroxy-apigenin tetramethyl ether (LV), however, shows similar shifts (little shift of —0.30 ppm) by $Eu(FOD)_3$ and relatively large downfield shift of 4.35 ppm by $Eu(DPM)_3$ to that of tri -O-methyl apigenin (LIII).



LIII; $R_1 = R_2 = H$

LIV; $R_1 = OCH_3$, $R_2 = H$

LV ; $R_1 = H$, $R_2 = OCH_3$

LVI; $R_1 = R_2 = OCH_3$

T_A_B_L_E - IV

Values of H-3 from TMS and Δ Eu values by shift reagents

Compound	(ppm)	$Eu(FOD)_3$	$Eu(DPM)_3$
4',5,7-Trimethoxyflavone (LIII)	6.56	0.04	3.68
4',5, ,7,8-Tetramethoxyflavone (LV)	6.60	—0.30	4.35
4',5,6,7,-Tetramethoxyflavone (LIV)	6.59	— 1.50	2.04
5,6,7,8,4'-pentamethoxyflavone (LVI)	6.60	— 1.99	1.79

(e) Mass spectroscopy

The mass spectra of a wide variety of organic natural products have been studied only during the last few years. The inlet system suitable for volatilization of high molecular weight (M^+, 300-1200) organic materials has increased the utility of mass spectroscopy. Generally fragmentation pattern 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 biflavanoid have appeared.[82-85]

Flavones:

In a recent paper Kingston[86] has discussed the mass spectra of a large number of flavones, flavonole and their ether derivatives. He has summarised the manner in which monoflavones fragment as follows:

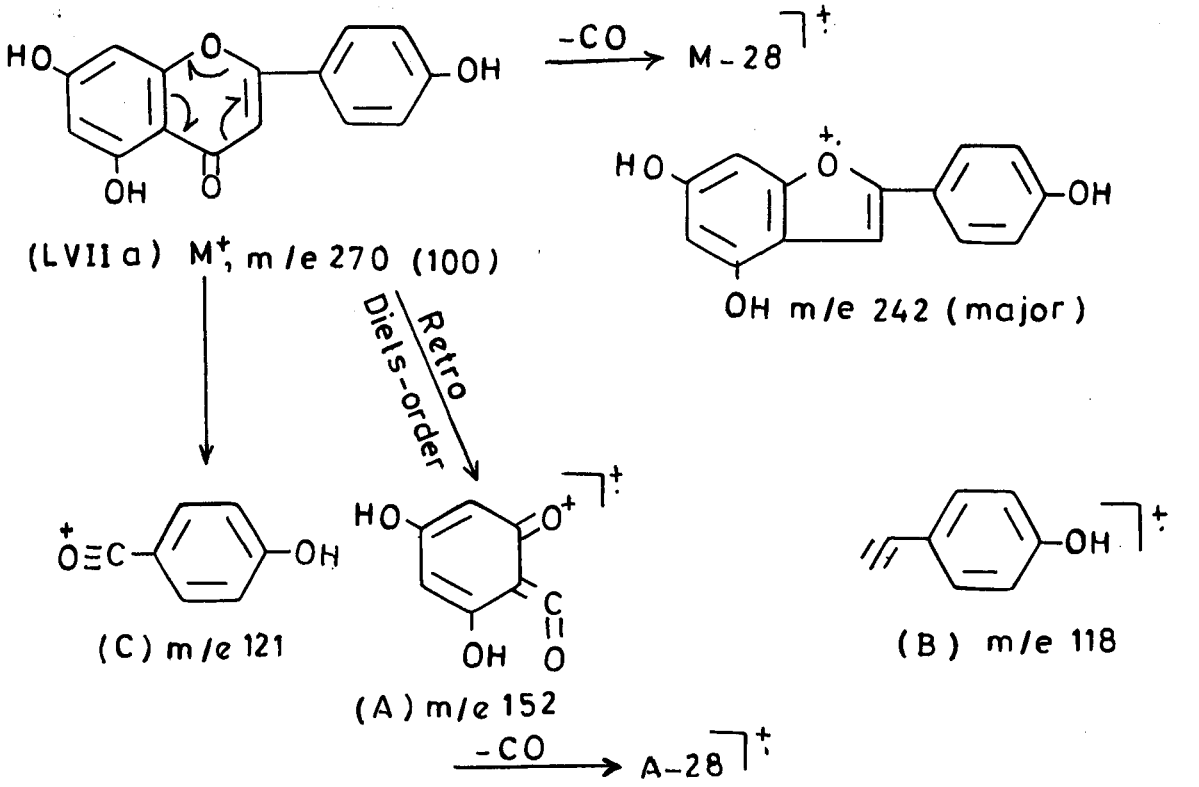
(a) Flavones with fewer than four hydroxy groups do not readily fragment, a consequence of the stability of their molecular ion.

(b) Flavones with fewer than four hydroxy groups tend to undergo decomposition predominantly by way of the retro Diels-Alder process.[115,116] This and other common fragmentation processes are shown in Chart-1a using apigenin (LVIIa)[115] as a typical example.

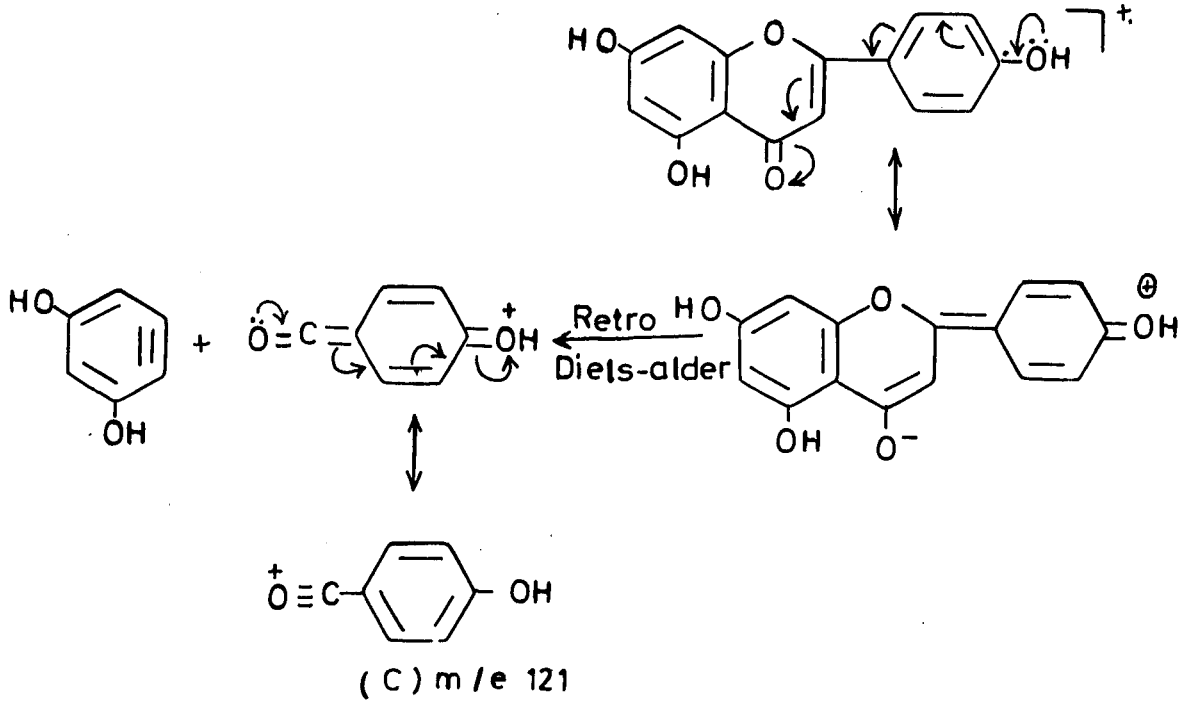
(c) An $M-1^+$ ion is often found in the mass spectra of flavones, its origin is, however, obscure.

(d) The presence of ion (C) (Chart-1a), 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-1a.

(e) Doubly charged ions are frequently present.



Alternative retro Diels-Alder process to give fragment (C)



C H A R T - 1a

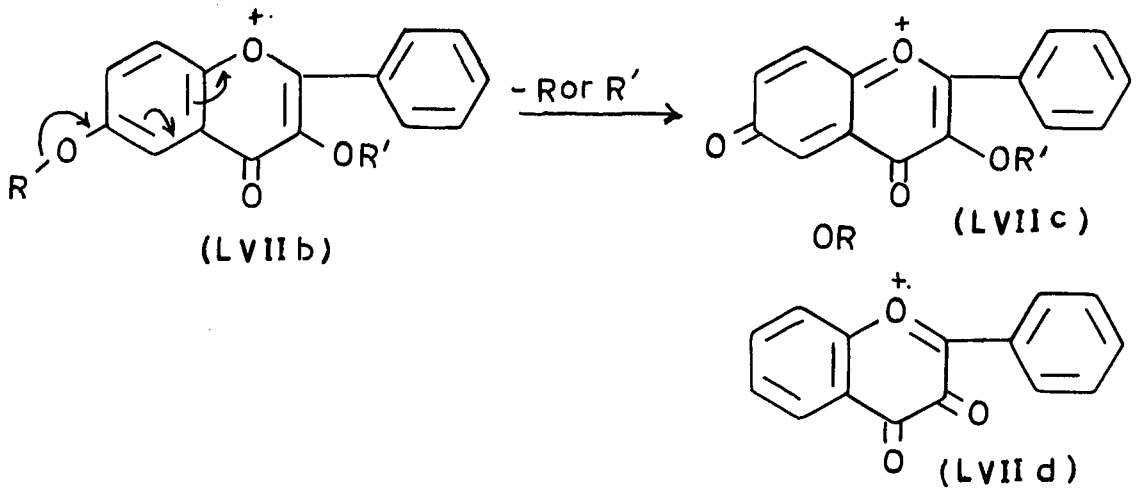
(f) When heavily substituted with hydroxyls and methoxyls, the flavone tends to fragment in a less predictable manner, retro Diels-Alder process becomes insignificant and the spectrum is deminated by the molecular ion and ions at $(M-15)^+$, $(M-28)^+$ and $(M-43)^+$.[116,117]

The principal peaks in the mass spectra of flavones (1-19) are given in Table-V. The major conclusions deduced from analysis of these spectra and other spectra in the literature are discussed below:

<u>$(M-H)^+$</u>:

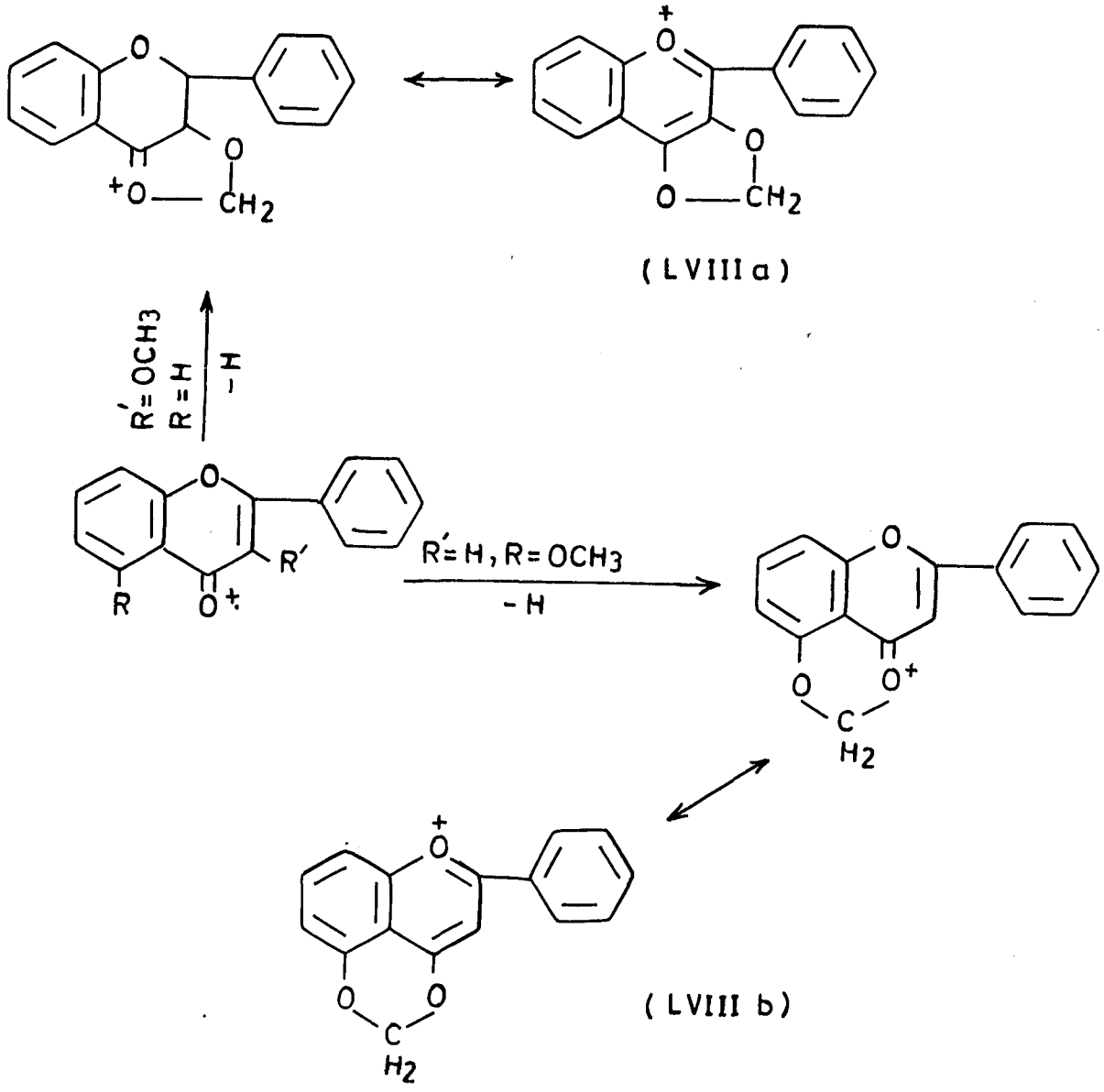
Loss of hydrogen from the molecular ion is a significant process in two distinct situations.

(i) Loss of hydrogen is observed from all the samples examined which contain a free 3-or 6-hydroxy group (e.g.compounds 11,13,15) with the sole exception of the 2', 3-dihydroxy flavone(17) where fragmentation is dominated by the intense $(M-CH)^+$ion. This observation may be rationalized by the assumption that loss of hydrogen occurs from the 3-or the 6-OH group to give the stable quinonoid ions (LVIIc) or(LVIId) (Chart-1b,R=R'=H).



(CHART- 1b)

(ii) Hydrogen atom is lost from all the flavone methyl ethers examined which have an OCH_3 group at either the 3-position (e.g. compounds 12,14,16,18,19) or the 5-position (e.g. compounds 2, 5,12,16,18,19), with the exception of compound (10) where another more facile fragmentation takes place. The H atom is probably lost from the 3- or the 5-OCH_3 group, and it is proposed that the ionized CO group displaces an H atom from one of these groups with the formation of the stabilized intermediate (LVIIIa or LVIIIb) (Chart -II).



(LVIII a)

(LVIII b)

Relative Intensities of the principal ions in the mass sp

Flavones	M^+	$(M-1)^+$	$(M-CH_3)^+$	$(M-OH)^+$	$(M-OH_2)^+$	$(M-OH_3)^+$	$(M-CO)^+$	$(M-CHO)^+$	$(M-CH_3O)$
1. 5,7-OH	100	-	-	-	-	-	12	-	-
2. 5,7-OME	100	60	-	22	-	-	8	42	22
3. 5-OH-4'-OMe-7Me	100	5	-	-	-	-	-	-	-
4. 5-OH-4',7-OMe	100	8	-	-	-	-	-	8	-
5. 4',5,7-OMe	100	62	-	8	-	-	7	32	21
6. 7-OH-3',4'-OMe	68	-	6	-	-	-	6	11	-
7. 3',4',7-OMe	100	-	11	-	-	-	6	-	-
8. 4',5,7-OH-6-OMe	96	-	63	-	-	-	-	-	-
9. 4',5-OH-6,7-OMe	100	-	100	-	-	-	-	43	15
10. 4',5,6,7-OMe	25	-	100	-	-	-	-	-	5
11. 3,5,7-OH	100	45	-	-	-	-	34	20	-
12. 3,5,7-OMe	47	100	10	7	7	39	-	11	21
13. 3,3',4',7-OH	100	38	-	32	-	-	14	10	-
14. 3,3',4',7-OMe	100	85	70	6	6	9	-	8	28
15. 3,4',5,7-OH	100	24	-	-	-	-	28	18	-
16. 3,4',5,7-OMe	78	100	12	-	5	25	-	7	9
17. 2',3,4',5,7-OH	67	-	-	100	-	-	5	13	-
18. 2',3,4',5,7-OMe	74	50	19	-	-	12	-	10	100
19. 3,3',4',5,5',7-OMe	100	39	98	-	-	7	-	7	28

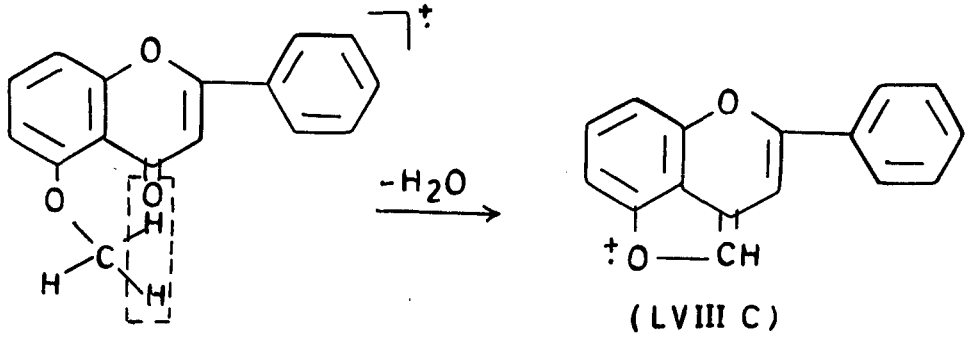
Only peaks of intensity greater than 5% of base peak are

$(M-CH_3)^+$:

This ion is abundant in the mass spectra of all the 6-and 8-methoxyflavones examined (e.g.compounds 8-10). It is almost certain that formation of the stable quinonoid cation LVIIC (or the isomeric cation derived from an 8-methoxyflavone)from the precursor (LVIIb) (Chart-1b, $R=CH_3$) provides the driving force for this fragmentation, which is so facile that competing fragmentations such as the formation of the $(M-H)^+$ion are greatly reduced (e.g.compound 10). The 3-methoxyflavones examined (e.g.compounds 12,14,16,18,19) also display a moderately intense or intense peak at $(M-CH_3)$, presumably due to formation of the stabilized cation corresponding to LVIId from (LVIIb) ($R=H, R'=CH_3$; Chart-1b). In this case, however, loss of a CH_3 radical appears not to compete so effectively with other processes such as loss of an H atom (compounds 12,16 and 18) and the intensity of the $(M-CH_3)^+$ion is correspondingly reduced. The observation of an intense $(M-CH_3)$ peak is thus diagnostic for a 3-, 6-or 8-methoxyflavone, but failure to observe this peak does not necessarily exclude the presence of a 3-methoxy group.

$(M-CH)^+$ and $(M-CH_2)^+$:

Bowie and White[117,118] have reported that the spectra of all those flavones with a $5-OCH_3$ group exhibit $(M-17^+)$ and $(M-18^+)$ peaks. This could be explained on the basis of "Ortho effect" observed in the case of aromatic carbonyl compounds containing an ortho methoxy substituent, resulting in the formation of benzofuran type ion (LVIIIc).



The M-18 ion is generally produced by two pathways, M-CH-H and M-H_2O (metastable ions substantiate all these processes). Isotopic labelling studies in O-methoxy benzaldehydes show that oxygen of the carbonyl group is specifically involved in the elimination process.[118]

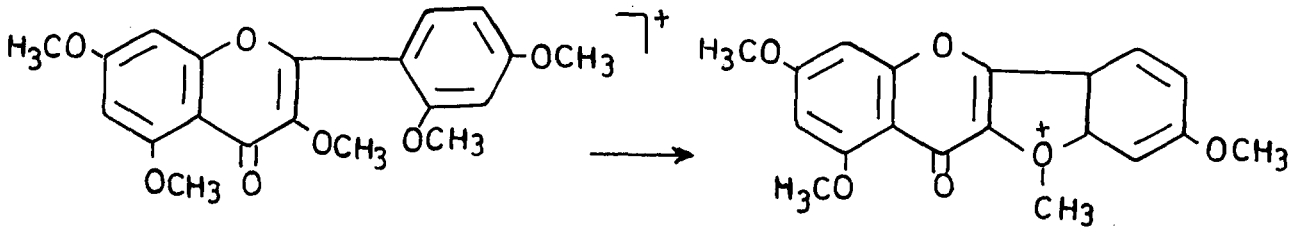
However, the spectrum of 3-OD, 4',5,7-trimethoxyflavone[118] showed that the phenolic hydrogen is specifically involved in $(M-18)^+$ process. Furthermore, a large number of flavanoid compounds with no 5-methoxy substituent also exhibit significant loss of 17 and 18 mass units, thus vitiating a simple "ortho effect" explanation of the phenomenon.

$(M-OH_3)^+$:

A number of flavone methyl ethers examined yielded intense or moderately intense ions corresponding to the loss of 19 mass units, or H_3O, from the molecular ion. It was noted that only those compounds (12,14,18,19) which had OCH_3 groups in both the 3-and the 5-positions gave this ion, and although its origin is obscure it could thus be of diagnostic importance in the structural analysis of flavanoid methyl ethers.

$(M-CH_3O)^+$:

The ion of postulated structure (LVIIIe) forms the base peak in the spectrum of compound 18 (LVIIId). A number of other methylated flavones also yielded $(M-OCH_3)$ peaks of moderate intensity, but the intensity of this peak in the spectrum of 18

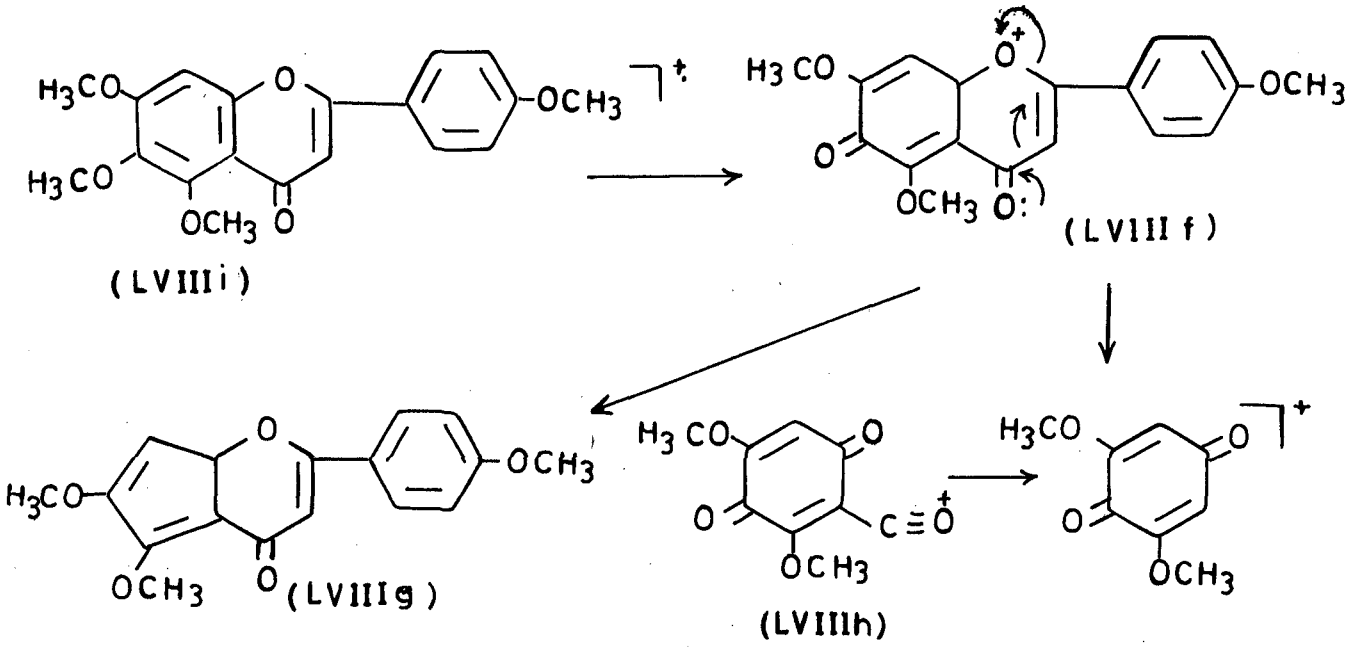


(LVIIId) (LVIIIe)

suggests that it can be used as a diagnostic tool for 2,3-dimethoxyflavones.

$(M-CH_3CO)^+$:

These ions are significant in the spectra of 3-6-, and 8-methoxyflavones investigated (compounds 8-10,12, 14, 16, 19). Metastable and high resolution evidences[117] indicate that the ions are formed by loss of CO from the $(M-CH_3)^+$ ion. Since compounds 8-10 do not contain a 3-methoxy group, loss of CO must occur from the quinonoid ion (LVIIIf) (Chart-III) to give an ion of possible structure (LVIIIg). Loss of CO from (LVIIIf) would be expected to occur readily by analogy with the facile loss of CO from benzoquinones and napthoquinones. A few compounds with no OCH_3 groups in the 3-,6- or 8-positions (5-10) also gave moderate or week peaks at $(M-43)^+$. It may thus be concluded that the observation of an intense peak at $(M-43)^+$ is diagnostic for a 3-, 6- or 8-substituted methoxy flavones, but the observation of a weak peak at this mass is inconclusive.



(CHART- III)

RDA fragments $(A+H)^+$, A^+ and $(A-H)^+$:

These ions are in general significant only for flavones bearing fewer than four oxygen substituents, although the $(A+H)^+$ peak can be intense in the spectra of a 3-hydroxyflavones with as many as five oxygen substituents (compound 17). On the other hand, apigenin trimethylether (5) shows only weak peaks due to this fragmentation, so it is a somewhat unreliable indicator of molecular structure and substitution pattern.

$(A-CH_3)^+$:

This ion gives intense to moderate peak in the spectra of compounds 8-11 and also of some other 6- and 8-methoxyflavones.[120-122] Metastable measurements confirm that the $(A-CH_3)^+$ ion arises by fragmentation of the $(M-CH_3)^+$ ion as indicated in Chart-III (LVIIIf),(LVIIIg). The $(A-CH_3)^+$ ions should thus be useful diagnostic ions for the structural elucidation of 6-and 8-methoxyflavones, with the low intensity in the spectra of

3,6-dimethoxyflavones.[118]

RDA fragments B^+ and $(B-15)^+$:

These ions are most abundant in the spectra of flavones with upto three oxygen substituents, and provide useful diagnostic peaks. The more fully oxygenated flavones do not give abundant ions of this composition.

C^+ and $(C-CO)^+$:

These ions are moderately abundant in the spectra of nearly all the flavones examined, and their abundance is roughly inversely proportional to the abundance of the RDA fragments A^+, $(A-15)^+$ and B^+. They are thus important diagnostic ions inasmuch as they yield information on the compostition of ring C and hence. by difference, on the composition of ring A also. In particular , if a fully methylated flavone which does not show intense RDA fragment is examined, the possible mass numbers at which fragments can occur are restricted to a small number of possibilities, namely the ions at m/o 105, 135, 165 and 196 for flavones unsubstituted and none, di, and trisubstituted, respectively, in ring C. In such a case, if attention is limited to ions with these m/e values, the most intense ion will be one corresponding to ion C, even when all the fragments examined are of relatively low intensity. This "rule" has been found to hold in most of the cases examined. Partially methylated flavones can be dealt with by a simple extension of the m/e values considered. The observation of a second ion with m/e (C-28) is confirmatory evidence for the fragmentation proposed.

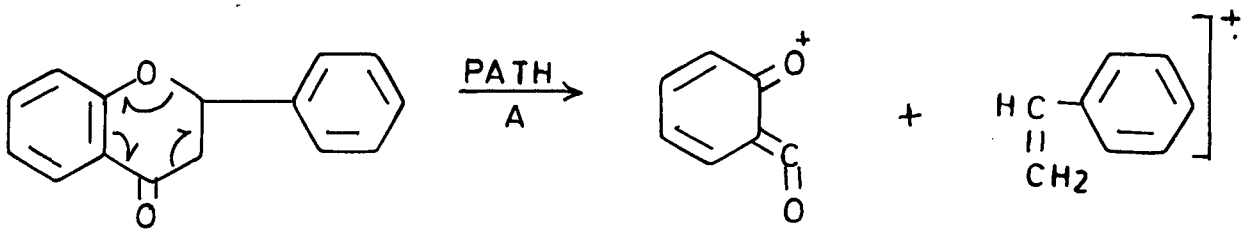
Doubly Charged ions:

Peaks due to doubly charged ions are moderately intense in the spectra of all the flavones examined, and can be distinguished

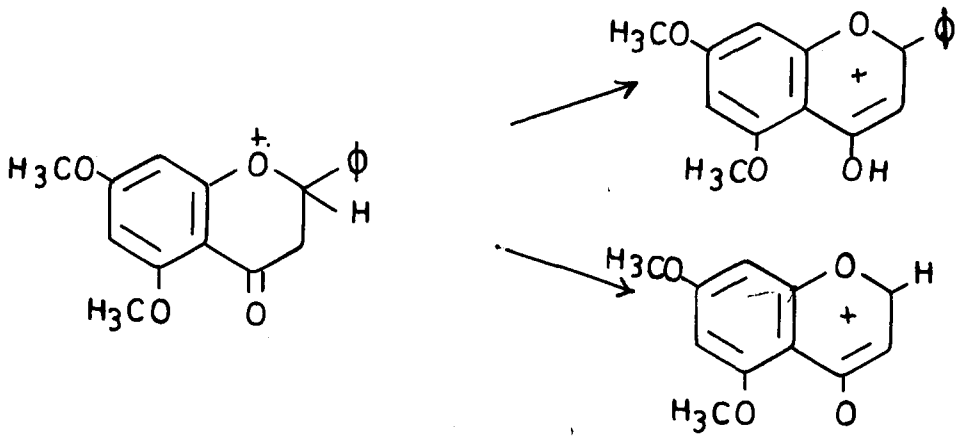
from other significant peaks due to fragments A,B and C, by the presence of isotope peaks at non-integral masses.

FLAVANONES:

In the case of flavanones, fragmentation by path-A (RDA-fission of the heterocyclic ring) and path-B are of great importance as they lead to clean cut, charactersitic spectra.[85]



Another method of breakdown, that helps to characterise the flavanone is the loss of either a hydrogen atom or any aryl radical at C-2 from the molecular ion to give even electron fragments.



These fragmentation processes are illustrated in the case of 4'-methoxy flavanone (chart -IV).

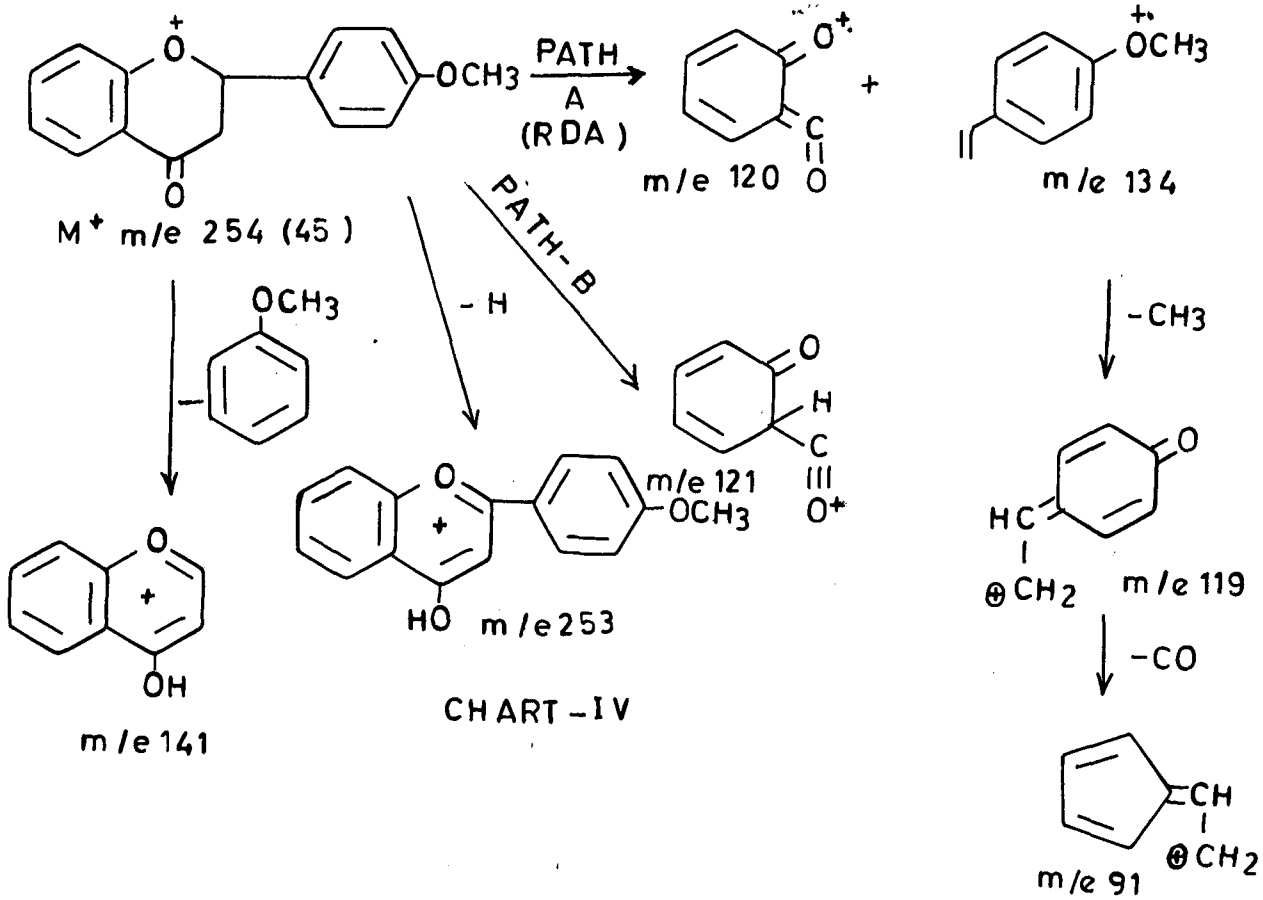
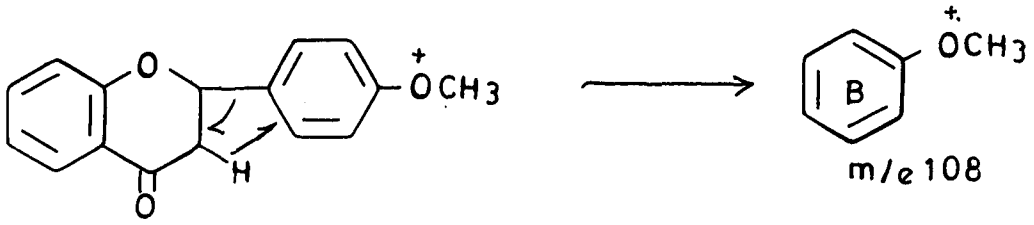


CHART - IV

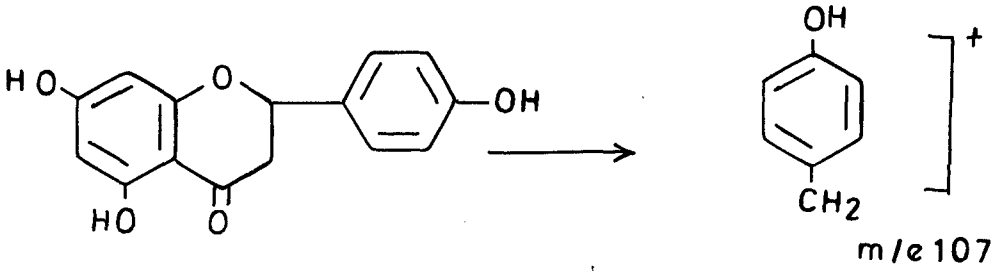
The fragment with methoxyl group takes nearly all the charge. A further peak is at m/e 108 arising from a hydrogen transfer reaction.



The presence of a 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-methoxy benzyl ion respectively (or their equivalent tropolium ions).

These ions appear as peaks of significant intensity in the mass spectrum of naringenin/ its trimethyl ether.[85,88]



The mass spectrum of 3,5,7-trihydroxy-4'-methoxy flavanone,[88] is of particular interest, as the base peak is neither the molecular ion nor a fragment arising from breakdown via path-A. (Chart-V).

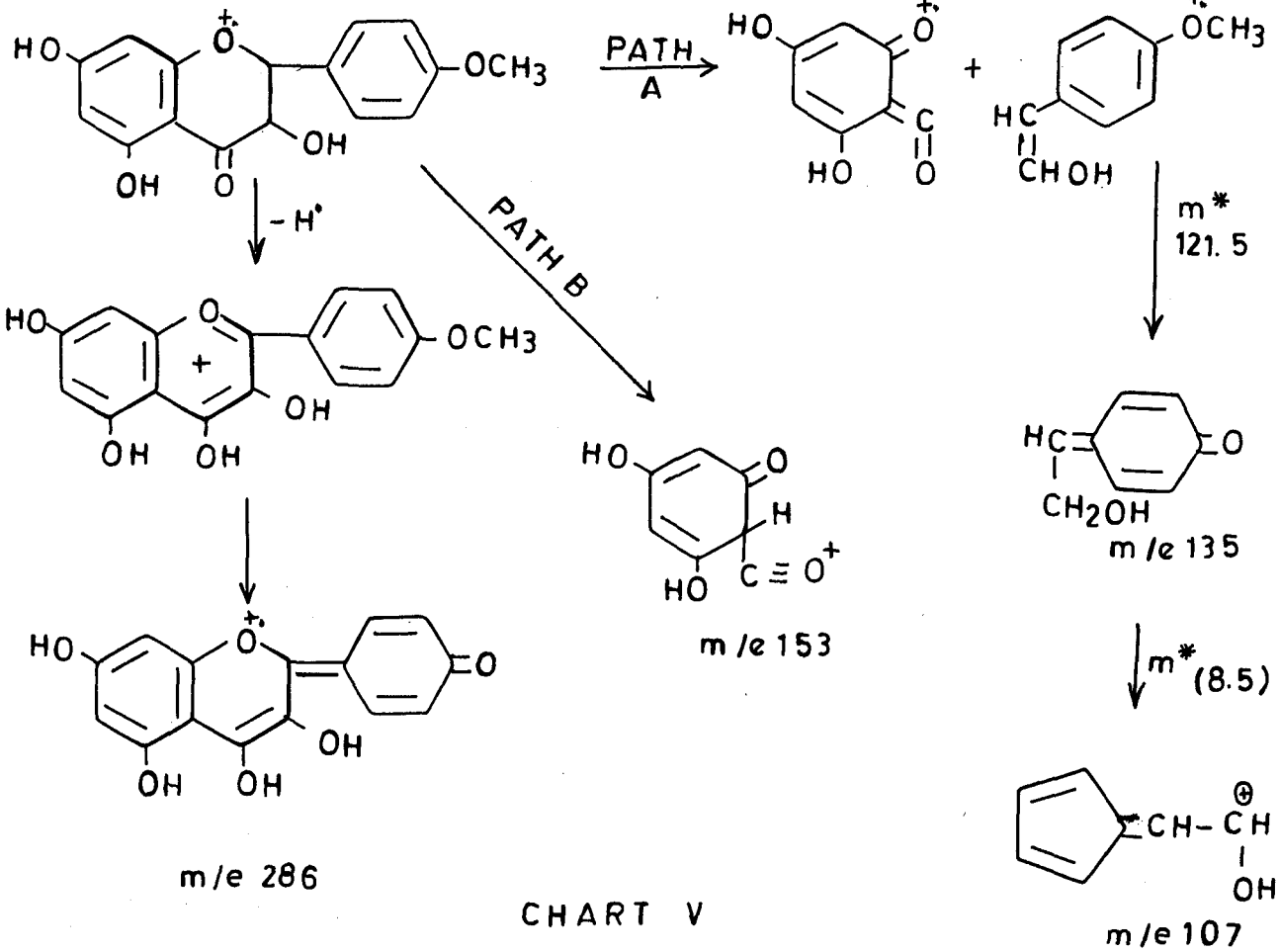
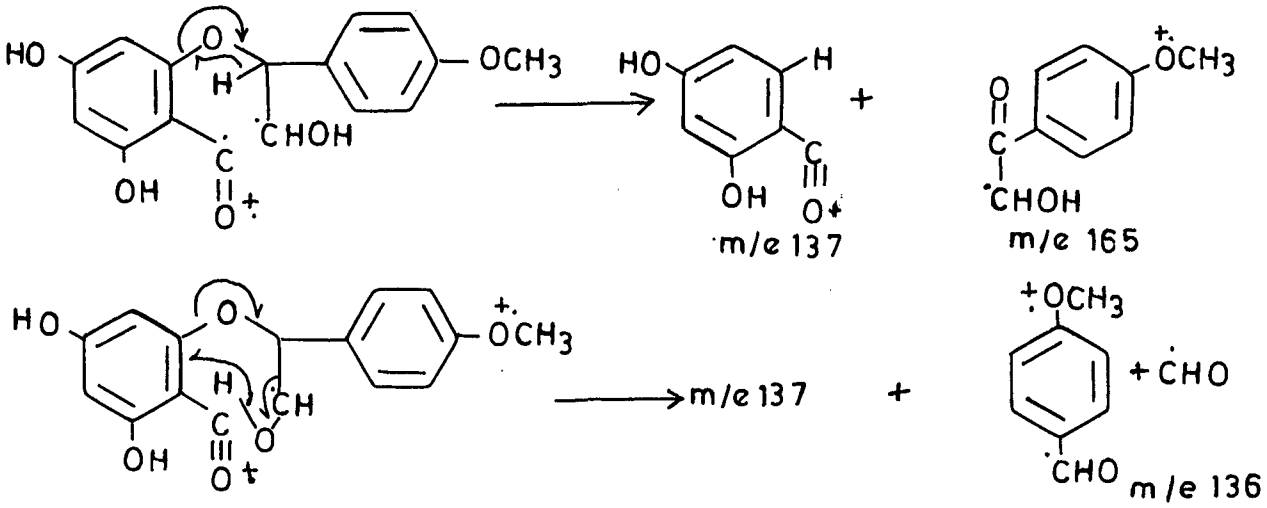


CHART V

The loss of a hydrogen atom followed by the loss of a methyl radical is important, but the base peak is found at m/e 137. The metastable peak at m/e 62.2 indicates that this fragment is formed directly from the molecular ion. Several processes can give rise to this species.



In the case of 2'-hydroxy flavanoids strong intra-molecular occur and the breakdown pattern becomes so profoundly modified that it is frequently difficult to classify the substance by reference to standard breakdown patterns.[87]

2'-Hydroxy flavanone (LX)[87] showed breakdown patterns A&B as well as the loss of phenyl or hydrogen radical from C-2 to give even electron species, but the base peak was at $M^{+}-18$ and the third largest peak at $M^{+}17-18$. It has been proposed that these peaks arise by ring opening of the molecular ion followed by ring closure on to the 2'-hydroxy group as shown in chart -VI.

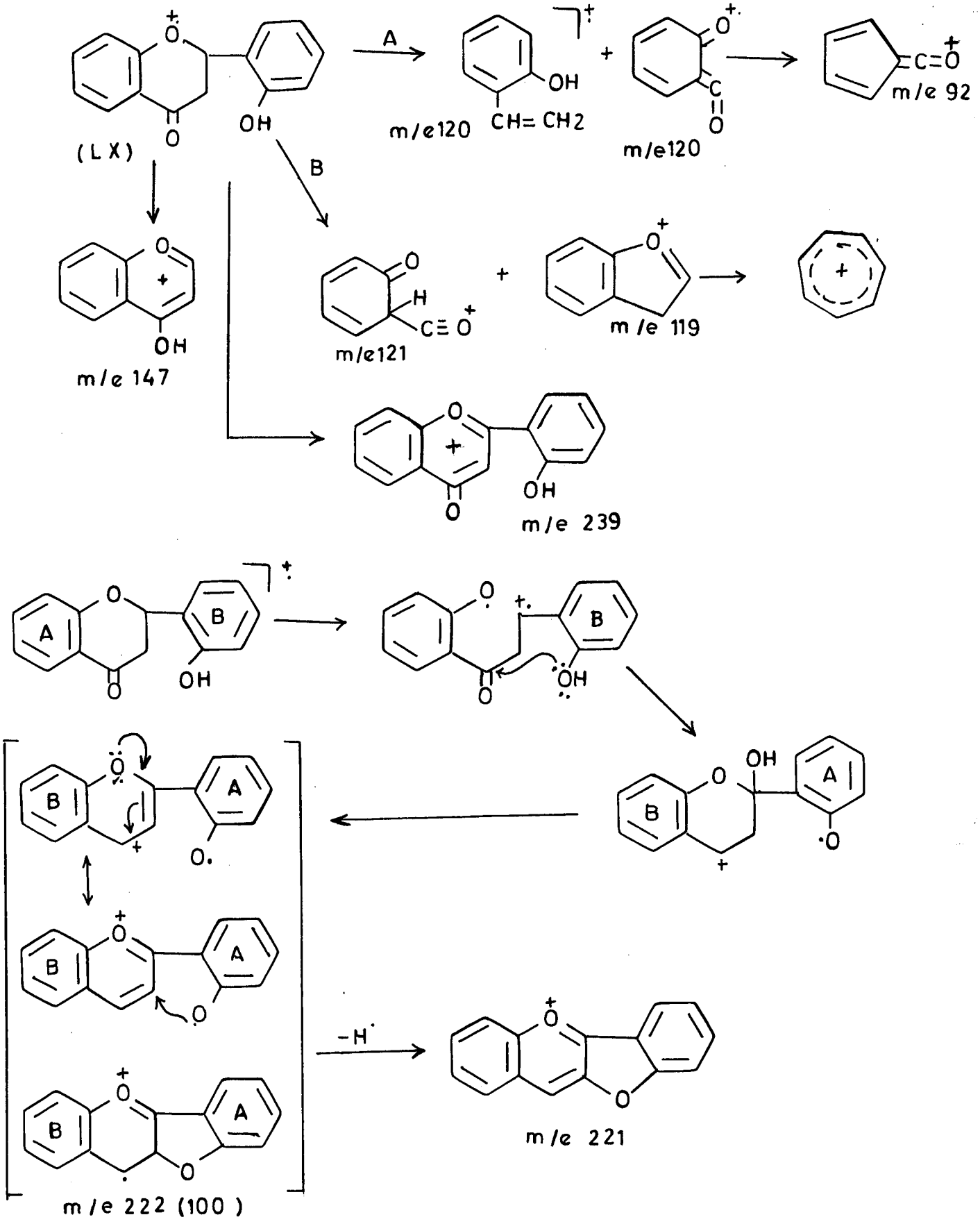

(LX)
A
B
m/e120
CH= CH2
m/e120
m/e 92
m/e 147
m/e121
m/e 119
m/e 239
m/e 222 (100)
-H·
m/e 221

CHART - VI

Biflavones:

Seshadri et al[89] have reported the fragmentation pattern in biphenyl and biphenyl ether type biflavones. Molecular ion is usually the base peak. Apart from the processes mentioned earlier for apigenin/ its trimethyl ehter, these compounds also undergo (i) fission of the C-C or the C-O-C linkages between the aromatic residues, (ii) elimination of CO and CHO from the biphenyl ethers and (iii) rearrangements involving condensation between the phenyl rings. Steric factor seem to play an important role in influencing the breakdown mode and internal condensations. Formation of doubly charged ions is frequently observed.

The mass spectra of amentoflavone hexamethyl ether and cupressuflavone 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 (XIVa):

622(100);621(33);592(8);576(10);312(2); 311(5); 245(5); 181(2); 180(3); 135(16); and 132(3) (chart -VII).

Cupressuflavone hexamethyl ether (XLVIII):

622(100); 621(38); 607(8); 592(18); 576(7); 312(7); 311(4); 245(11); 135(26); and 132(14); (Chart -VIII)

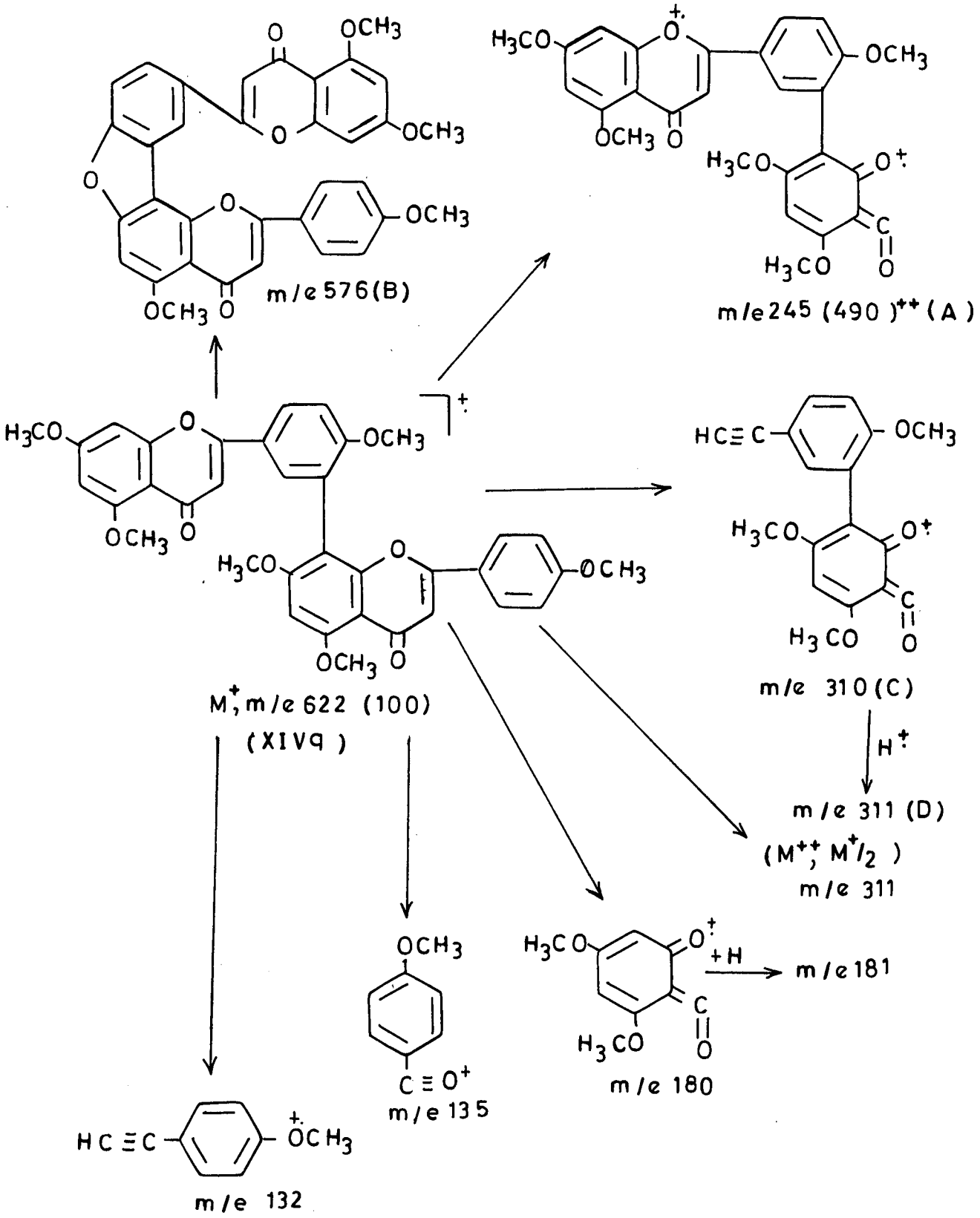

m/e 576(B)
m/e 245 (490)++ (A)
M+, m/e 622 (100)
(XIVq)
m/e 310 (C)
H+
m/e 311 (D)
(M++, M+/2)
m/e 311
m/e 181
m/e 180
m/e 135
m/e 132

CHART-VII

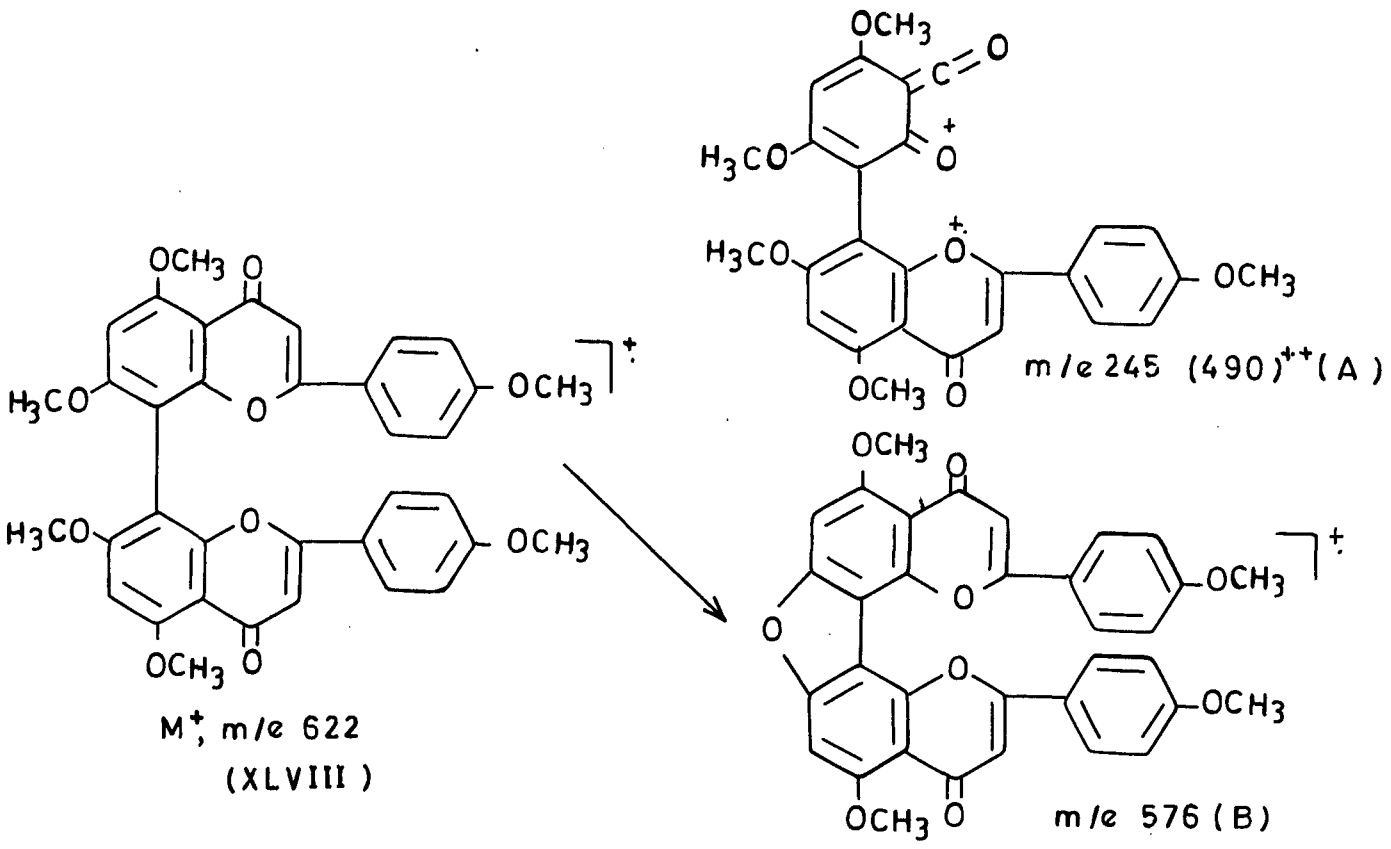


CHART-VIII

The peaks at m/e 607 and 592 obviously arise by the loss of methyl groups. The peak at m/e 576 has been assigned to structure (B) (chart-VII and VIII), a condensation product. Such a condensation product has been reported to be formed when amentoflavone is heated with zinc dust.[90] The difference in the intensity of this ion in the above biflavone methyl ethers points to the difference in the steric disposition of one flavone unit relative to the other, thus hindering or favouring condensation between the phenyl rings.

The ion at m/e 311 is due to both the doubly changed ion(M^{++}) and the apigenin trimethyl ether fragment ($M^{+}/2$). The difference in the intensities (XIVq,5% and XLVIII,14%) is due to variation in the oxygenation pattern of the biphenyl residues in the two

compounds, which is responsible for the differences in the labile nature of the inter-apigeninyl bond. Another explanation would be that the removal of another electron from M^+ is difficult in the case of (XIVq). Thirdly, double RDA fission of molecular ion)XIVq) may yield the fragment C which, after accepting an hydrogen atom gives an ion D having m/e 311.

A perplexing observation is the complete absence of the ketene ion (E) (m/e 180) in the spectrum of (XLVIII) and its feeble intensity (4%) in amentoflavone hexamethyl ether.

If the biphenyl linkage in both the cases breaks easily to give apigenin trimethyl ether units, it should be expected that the latter would give the ketene in considerable intensity. The observation that the ketene fragment (E) is either absent or of only a feeble abundance may indicate that the breaking of biphenyl linkage is not a favourable process. It may, therefore, be sumrised that the ions 180, 135 and 132 originate directly from the molecular ions M^+ Or M^{++} by RDA fission.

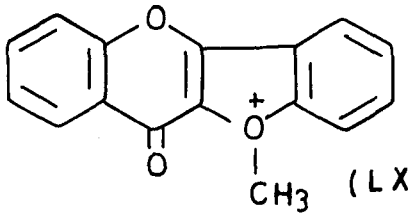
Steric factors become so much dominant in agathisflavone hexamethyl ether[26b] that the ion at m/e 311 appears as base peak instead of the molecular ion, m/e 622(90). The main peaks in its spectrum are : 622(90); 607(54); 591(98); 573(24); 561(15); 521(12); 497(24); 325(20); 311(100); 281(12); 245(22); and 135(65);

The mass spectrum of Robustaflavone hexamethyl ether (L)[41] showed all the salient features of a biapigenin type biflavone methyl ether when compared to the mass spectra of the methyl ethers of amentoflavone,[89] cupressuflavone[89] and agathisflavone.[26b] The main

peaks of interest are: 622(M^+, 35); 621; 607 (28); 592(68); 591 (100); 576 (10); 573 (12); 561(9); 311(18); 296(13); 181(10); 135(12.5) and 132(10). The fragmentation pattern is shown in chart- IX.

The base peak appeared at m/e 591 (C_{35} H_{27} O_9)corresponding to the loss of OCH_3 from the molecular ion (m/e 622) which amounted to 39% of this peak. The peak at 591 was also very intense(90%) in the case of agathisflavone hexamethyl ether (XLIX). The resonance stabilized structure B could be assigned to this peak in analogy with the structure (LXI) proposed for ($M-OCH_3^+$) ion in 2', 3-dimethoxyflavones.

The peak at m/e 311 arising as a result of the C-C bond fission was more intense (18%) in the present case than amentoflavone hexamethyl ether (5%) and cupressuflavone hexamethyl methyl ether (14%)[89]. This was suggestive of relatively facile C-C bond cleavage in robustaflavone due to steric factors as the linkage involved 6-position of one flavanoid unit. Steric factors become so much dominant in agathisflvone hexamethyl ether (XLIX) that the ion 311 appears as the base peak.



(LXI)

The ions 132,135 and 181 obviously arise via retro-Diels-Alder reaction in I-C or II-C rings. Loss of CH_3 from the molecular ion

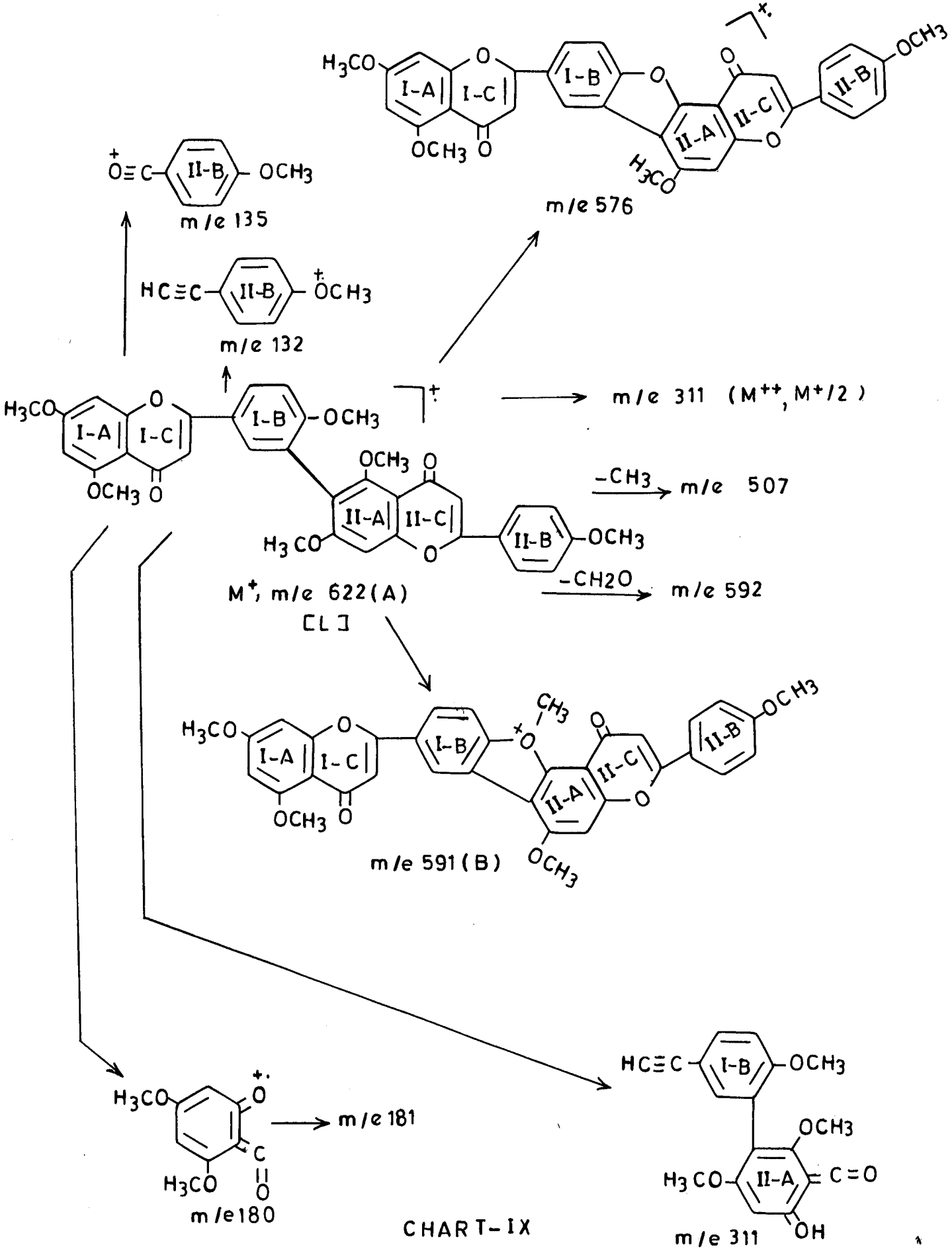

m/e 576
m/e 135
m/e 132
m/e 311 (M++, M+/2)
-CH3
m/e 507
M+, m/e 622(A)
-CH2O
m/e 592
m/e 591(B)
m/e 181
m/e 180
m/e 311

CHART-IX

leads to 607 and from 311 ion to 296. The ion 576 is the condensation ion (C) observed in all other biflavone methyl ethers.

The mode of fragmentation of hinokiflavone penta methyl ether (LI)[89], which contains a biphenyl ether system, is considerably different from those of biphenyl type biflavones described above. The main peaks in its spectrum are: 608(39); 607(12); 593(36); 580(4); 579(11); 578(11); 576(6); 431(7); 327(23); 313(100); 312(22); 311(22); 304(2); 297(29); 296(75); 281(22); 181(3); 135(11); and 132(18), (Chart-X).

The base peak in this case appears at m/e 313 and the molecular ion (608) amounts to 39% of this peak. This could be attributed to the fact that the biphenyl ether bridge suffers easy rupture; hydrogen transfer then leads to the 313(100) fragment. The fission of the ether bridge in (LI) can take place in two ways: (1) by route-1 giving the ions 297 (29) (A) and 311(22) (B) and (2) by route-2 yielding the ions 281(22) (C) and 327(23) (D)(Chart-X). However, the observation that the 313 ion is most intense suggests that route -1 is favoured i.e. the bond between the oxygen bridge and the highly oxygenated phenyl ring breaks preferably.

The ion at m/e 304 is obviously $M_{;}^{++}$ since the molecular ion in this case can not split into two equal fragments having this m/e value. Further evidence for it being doubly charged is provided by the appearance of the isotope peak at half a mass unit higher (304.5). Ions at m/e 593 and 578 arise by the loss of methyl groups, m/e 380 and 579 by the loss of CO and CHO respectively, and m/e 576 (E) (Chart-X) by internal condensation.

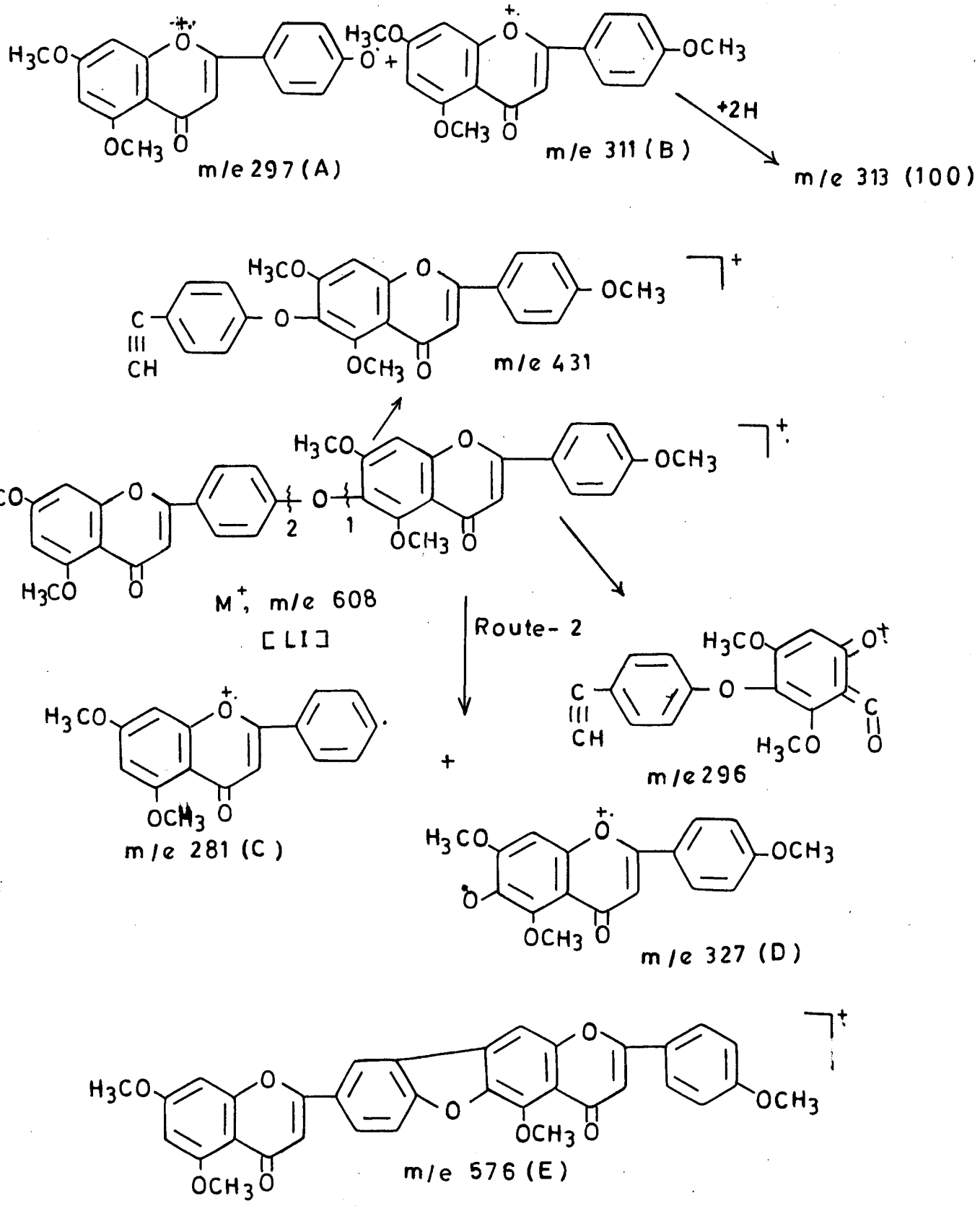

m/e 297 (A)
m/e 311 (B)
+2H
m/e 313 (100)
m/e 431
M+, m/e 608
[LI]
Route- 2
m/e 281 (C)
m/e 296
m/e 327 (D)
m/e 576 (E)

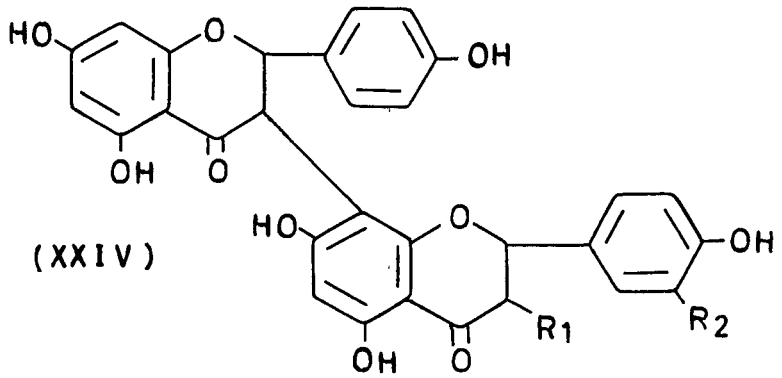
CHART - X

The ions due to loss of CO and CHO are not found in the spectra of biphynyl type biflavones. The ions at m/e 431 and 296 arise via various modes of RDA fission.

Biflavanones:

Jackson et al[11,49] have successfully applied mass spectroscopy to elucidate the structure of bivlavanoids of GB-Series (XXIV) containing two biflavanone units linked through (I-3,II-8).

(XXIV)



(a) GB-I; R_I=OH R_2=H

(b) GB-Ia; R_I=R_2=H

(c) GB-II R_1=R_2=OH

(d) GB-IIa R_1=H R_2=OH

All the features observed in the massspectra of biflavanoids of GB-Series and their methyl ethers have amalogies with similarly substituted monoflavanones.

The mass spectrum of GB-I heptamethyl ether (LXII)[49a] showed the presence of ions at m/e 121, 154, 181 and 476. The presence of ions at m/e 154 and 181 consistent with the fragments $[C_6H_3.(OMe)_2.OH]^+$ and $[C_6H_2.(OMe)_2OH.CO]^+$, respectively, supported the presence of phloroglucinol ring system derived from a 5,7-dihydroxyflavanone system. An idea about the nature of linkage

could also be derived since the fragmentation of the molecular ion that 656 can be rationalized by RDA reaction of a flavanone, first at ring I-C to give a fragment ion at m/e 476, followed by a similar fragmentation at II-C to give an ion at m/e 312. (This two stage breakdown pattern is fully substantiated by the presence of metastable peaks). These results can only be accommodated by a linkage from the oxygen heterocyclic ring I-C to the phloroglucinol ring II-A(Chart-XI).

The production of phloroglucinol dimethyl ether at m/e 154, a process which is not observed in simple analogous flavanones, is probably of thermal origin. In fact phloroglucinol is so readily lost from GB biflavanones that if the temperature of the chamber in the mass spectrometer much exceeds the minimum ($\sim 220^{\circ}$) for evaporation of the sample, there is difficulty in detecting the molecular ion. The thermal instability of GB-I was established by heating it in a tube at 280° and from the pyrolysis products, phloroglucinol was isolated and characterised.

As suggested by Petter[49c], the ion at m/e 312 can not be due to the formation of apigenin trimethyl ether. The ions, m/e 180 and m/e 132, which could arise by RDA reaction of apigenin trimethyl ether, are entirely absent from the spectra of GB-biflavanone methyl ethers. Further, the alternative isoflavanone-flavanone structure (LXIII) proposed [49c] for GB-biflavanones was ruled out on the basis of the appearance of the ion at m/e 121 in the spectrum of GB-II octamethyl ether, which could only arise from C_2 of a flavanone.

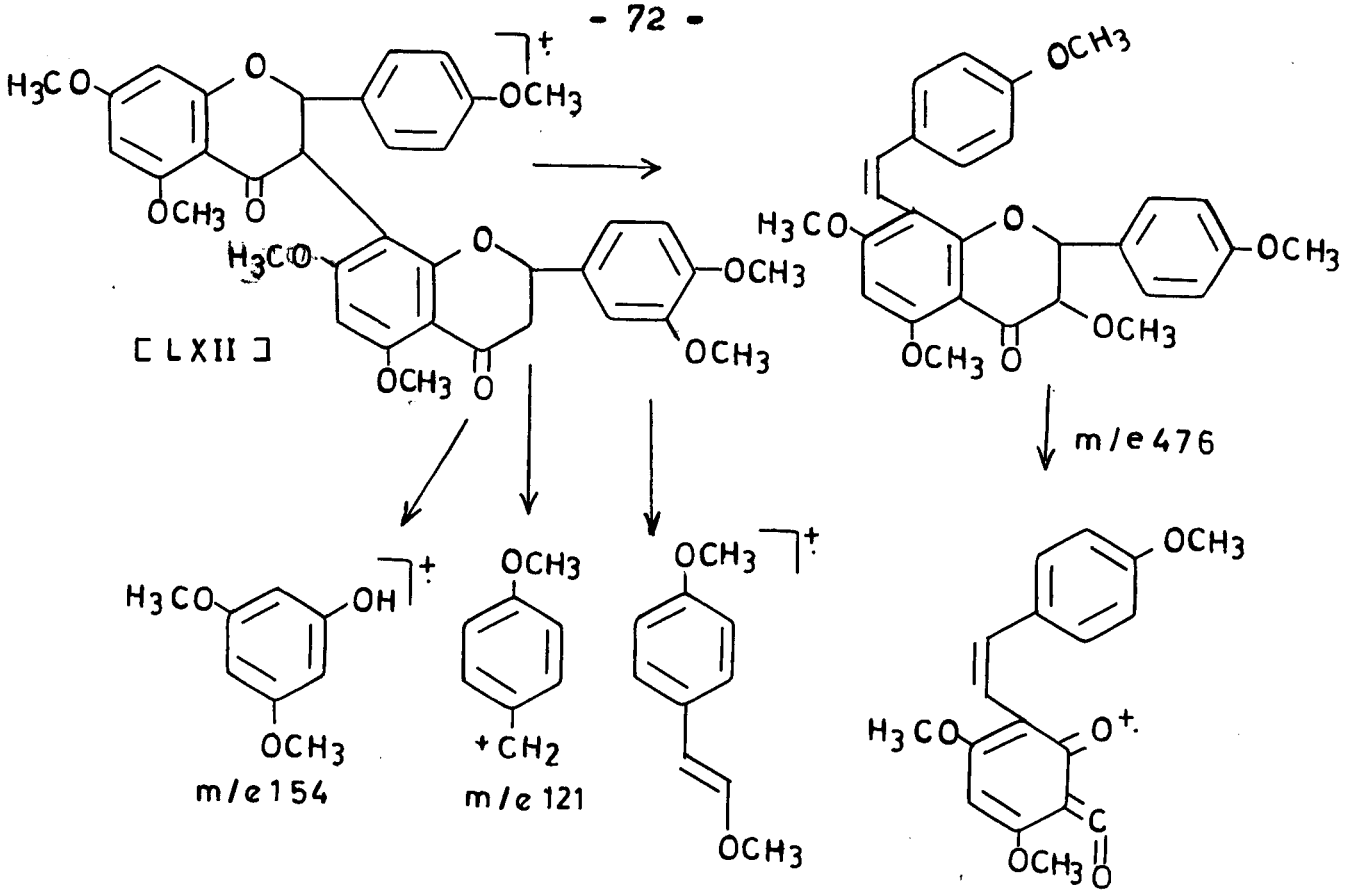
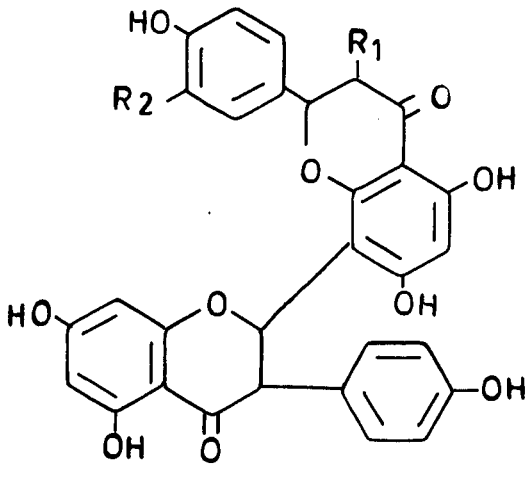


CHART XI



LXIII

(a) GB-I ; R_1=OH; R_2=H

(b) GB-Ia; R_1=R_2=H

(c) GB-II; R_1=R_2=OH

(d) GB-IIa; RI=H; R_2=OH

Further the massspectra of the parent compounds GB-II and GB-IIa (Chart-XII) showed clearly the presence of ions at m/e 107 and 123 consistent with the fragments obtained from aromatic rings I-B and II-B respectively. GB-II initially loses the elements of phloroglucinol, and then, by RDA process, ring II-C fragments to give an ion with m/e 296 (and not m/e 312). Aditionally, two successive RDA fragmentations around rings I-C and II-C of GB-IIa give an ion at m/e 270 (and not at m/e 286). This evidence clearly established that the 3,4-dihydroxyphenyl system constitutes ring II-B and not ring I-B in GB-II (XXIVC) and GB-IIa (XXIVd)[11].

Flavanone-flavone type biflavanoids:

Venkataraman et al,[43] Jackson et al[47] and Vishwanathan et al[48] have reported the mass spectral studies of (I-3, II-8) linked flavanone-flavone type biflavanoids. These combine the characteristic fragmentation patterns of a flavanone and flavone. The mass spectrum of talbotaflavone hexamethyl ether (LXIV)[48]. Shows fragmentations as shown in Chart-XIII.

The mass spectral fragmentations of morelloflavone heptamethyl ether[43] are similar to those shown for talbotaflavone hexamethyl ether(LXIV) except for the increase of 30 mass units in the ions (Chart-XIII) C(3%), D(10%), E(2%), F(46%), and G(22%) due to an extra methoxyl group in the II-B ring. The intensities of other ions are A(32%), B(32%), H(13%) and I(100%).

The mass spectrum of I-2, I-3-dihydroamentoflavone[37,42] hexamethyl ether(LXV) and its corresponding chalcone isomer(LXVI) which is formed during the methylation[37]

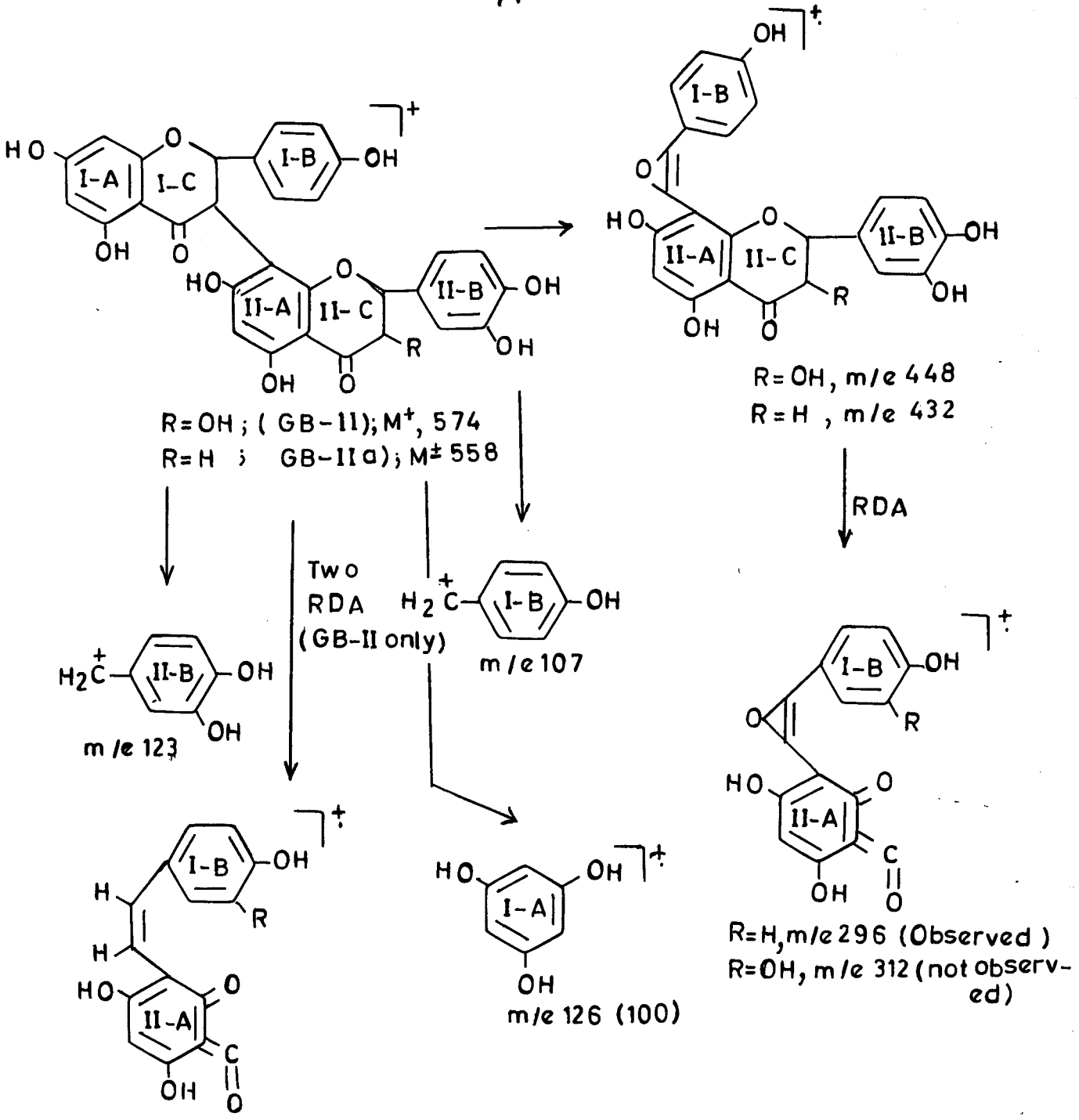

R=OH ; (GB-11); M+, 574
R=H ; GB-IIa); M± 558
R=OH, m/e 448
R=H , m/e 432
RDA
Two RDA (GB-II only)
m/e 107
m/e 123
m/e 126 (100)
R=H, m/e 296 (Observed)
R=OH, m/e 312 (not observed)
R=H, m/e 270 (Observed)
R=OH, m/e 286 (not observed)

CHART-XII

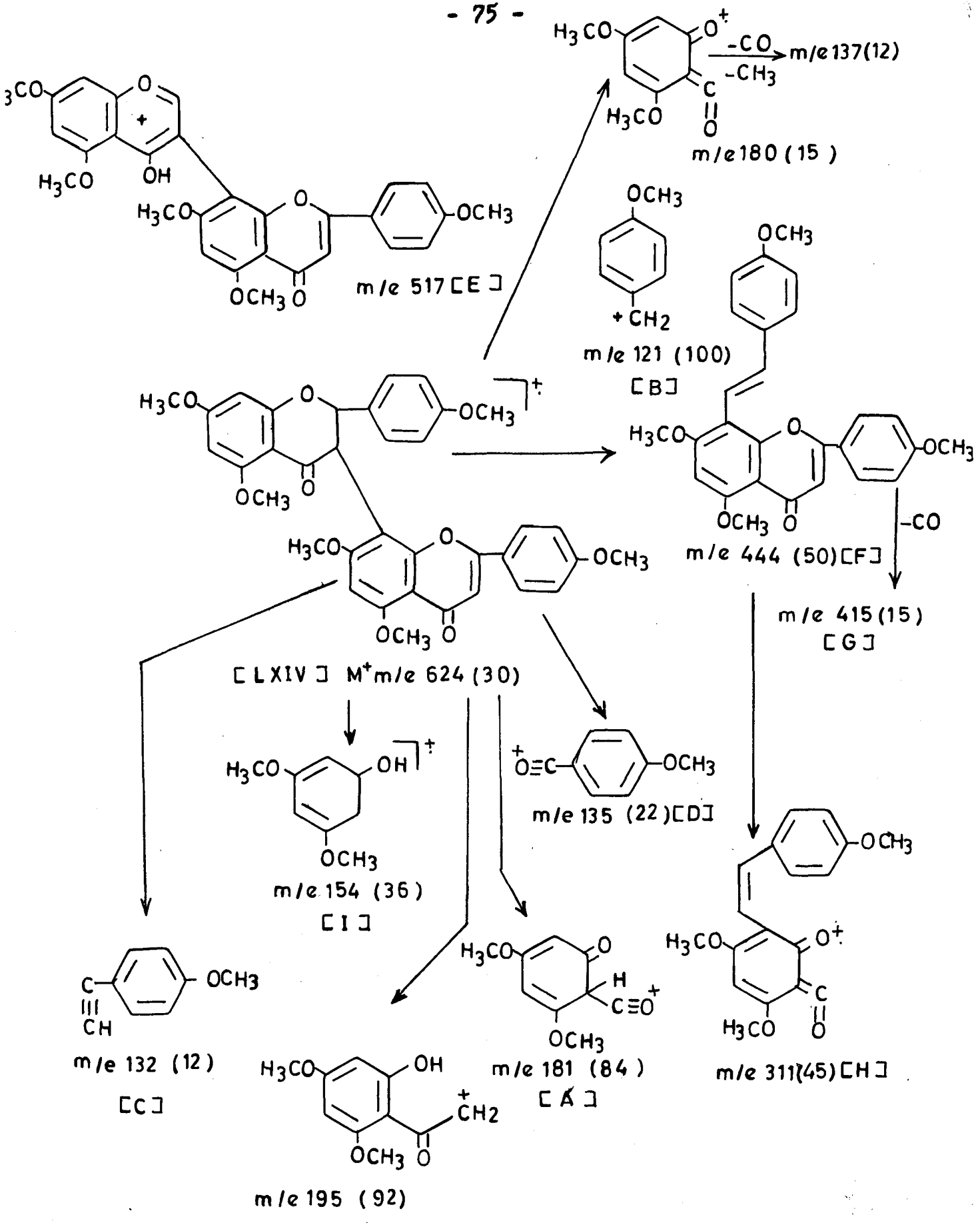
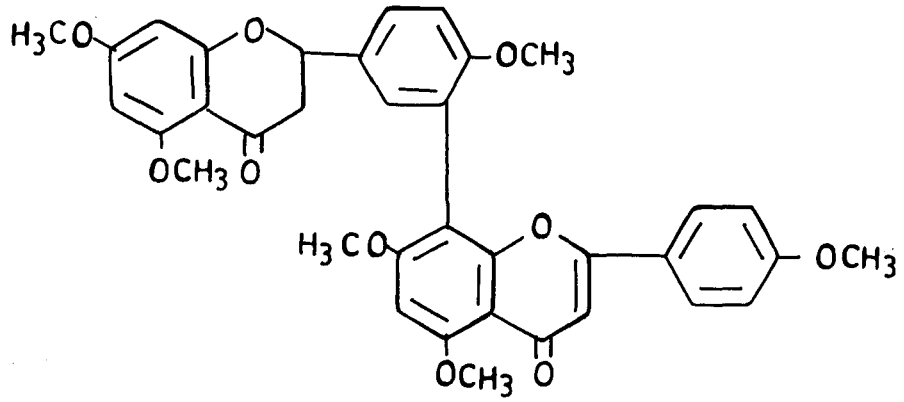
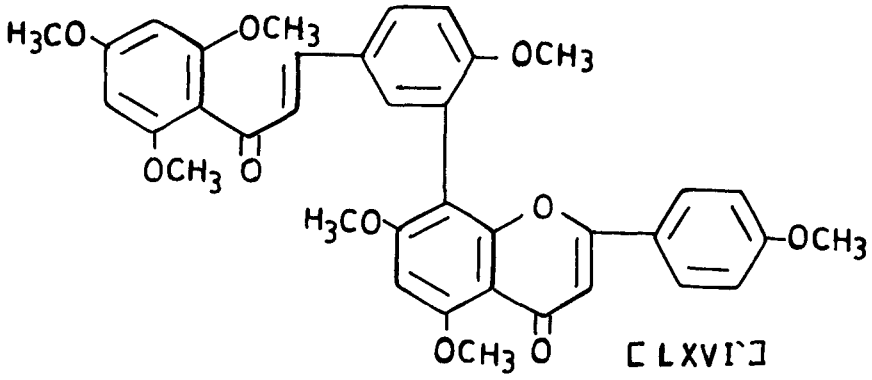

m/e 180 (15)
m/e 137(12)
m/e 517 [E]
m/e 121 (100)
[B]
m/e 444 (50)[F]
m/e 415(15)
[G]
[LXIV] M+m/e 624 (30)
m/e 154 (36)
[I]
m/e 135 (22)[D]
m/e 132 (12)
[C]
m/e 181 (84)
[A]
m/e 311(45)[H]
m/e 195 (92)

CHART-XIII



[LXV]



[LXVI]

The fragmentation pattern of both (LXV) and LXVI) are shown in chart-XIV and the important peaks with relative percentage intensities are given in table V.

In LXVI, m/e 638(86) is very strong while m/e 624 constitute the base peak of LXV. Moreover, m/e 623(41) (M-15) is stronger than m/e 624(16) in the LXVI. Although fragment ions m/e 443 and m/e 431 are detectable in both the compounds, the peaks m/e 417 and m/e 207 are, as expected, more pronounced in LXV. The fragment ion m/e 195 is much stronger in LXVI and forms the base peak. The fragmentation pattern is shown in Chart-XIV

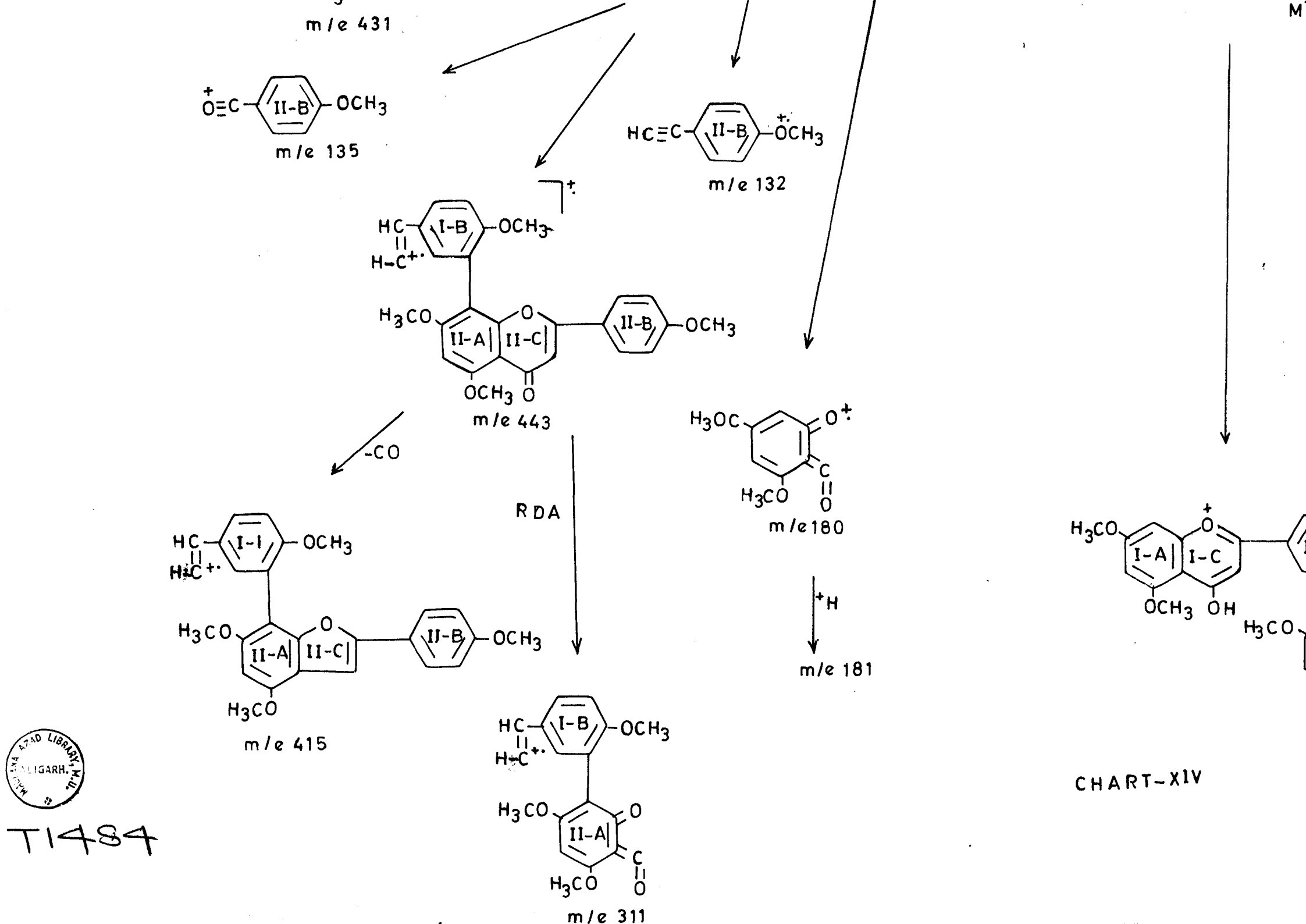


TABLE - VI

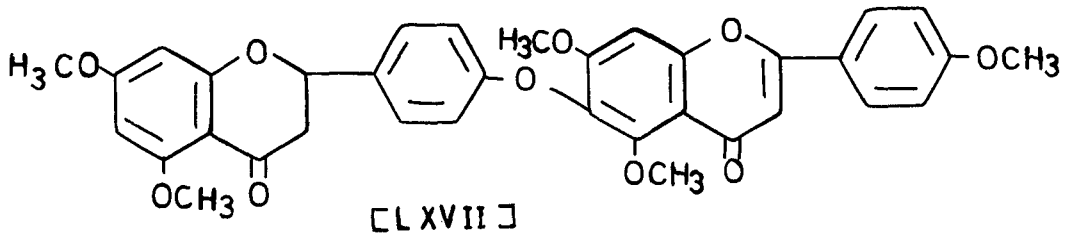
Peaks with relative % intensities:

LXV		LXVI
M^+	638 (33)	638 (86)
	624 (100)	624 (16)
	623 (28)	623 (41)
	610 (40)	610 (39)
	443 (86)	443 (38)
	431 (16)	431 (11)
	417 (15)	417 (5)
	415 (36)	415 (23)
	207 (15)	207 (6)
	195 (9)	195 (100)
	181 (34)	181 (32)
	135 (52)	135 (76)

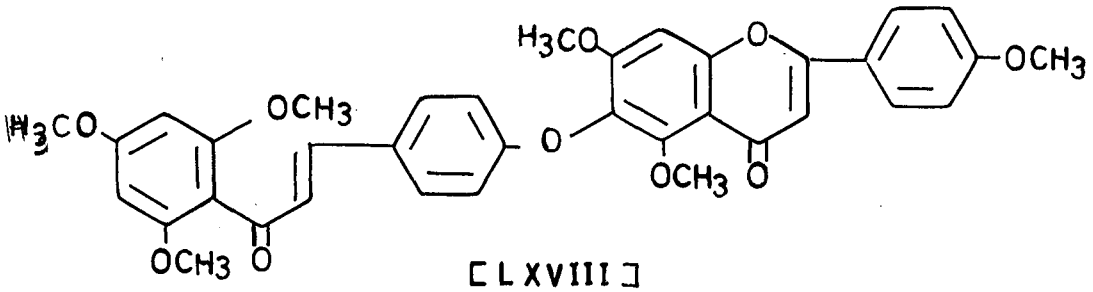
The main peaks in the mass spectrum of I-2,I-3-dihydrohinokiflavone pentamethyl ether (LXVII) are: 624(39); 610(82); 609(52); 576(73); 595(64); 582(60); 581(43); 429(57); 417(62); 403(70); 313(100); 311 (68); 299(60); 298(75); 297(64); 285(79); 283(72); 207(74); 195; 181 and 135.

The appearance of a weak peak at m/e 624, $C_{36}H_{32}O_{10}$ along with 610(82), $C_{35}H_{30}O_{10}$ suggested that I-2,I-3-dihydrohinokiflavone pentamethyl ether was slightly contaminated with the chalconeflavone hexamethyl ether (LXVIII) formed as a result of isomerization during methylation.

The fragmentation pattern is shown in Chart -XV.



[LXVII]



[LXVIII]

Thus mass spectral studies of biflavanoids reveal that their fragmentation patterns depend not only on the constituent monomeric flavanoid units but also on the nature and position of interflavanoid linkage. While the cracking patterns of simpler flavanoids are less complex, in application of these concepts to biflavanoids one has to take into consideration the influence of the additional structural and steric factors.

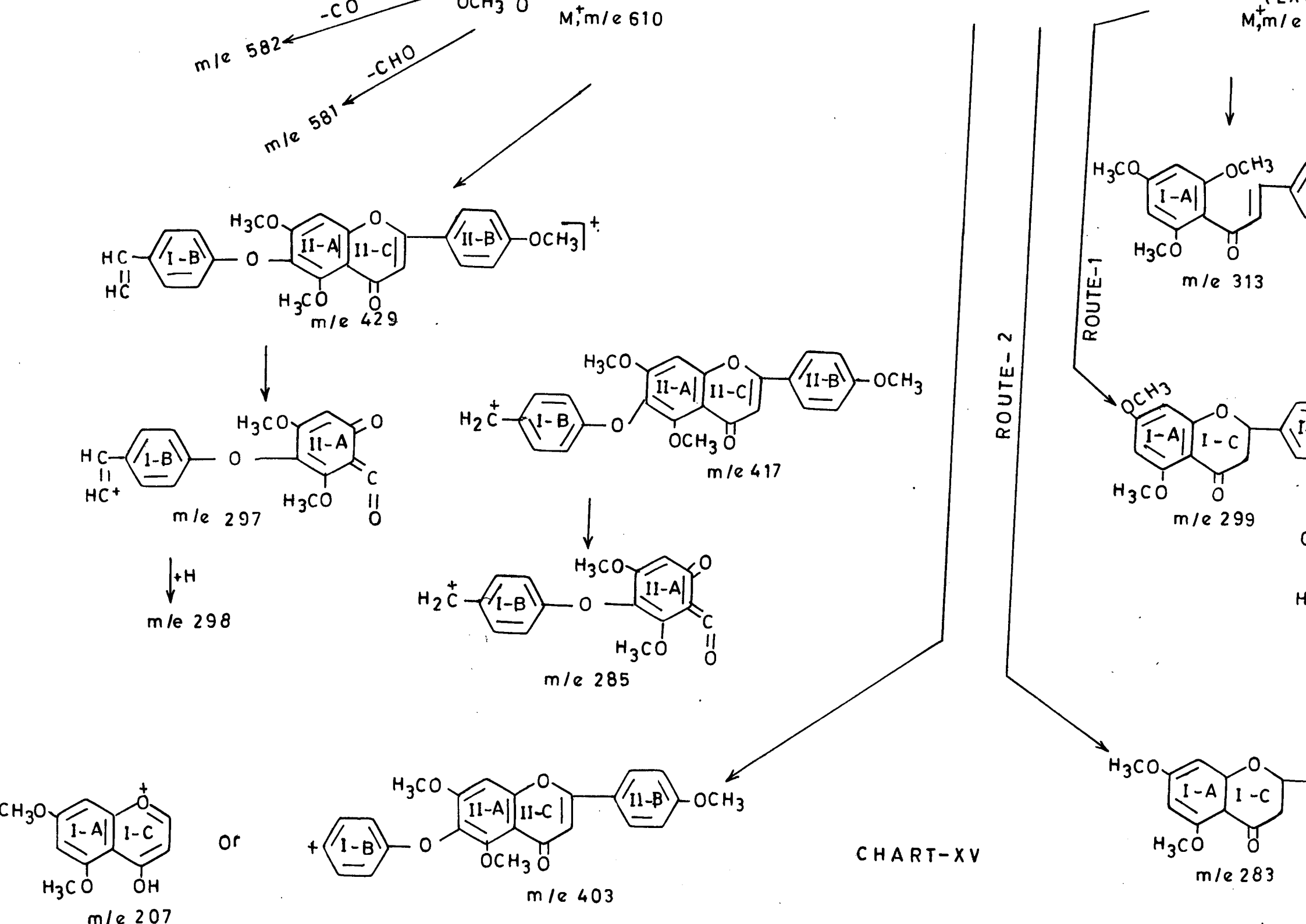

-CO
m/e 582
-CHO
m/e 581
M+ m/e 610
ROUTE-1
ROUTE-2
m/e 313
m/e 429
m/e 417
m/e 299
m/e 297
+H
m/e 298
m/e 285
m/e 283
m/e 207
Or
m/e 403

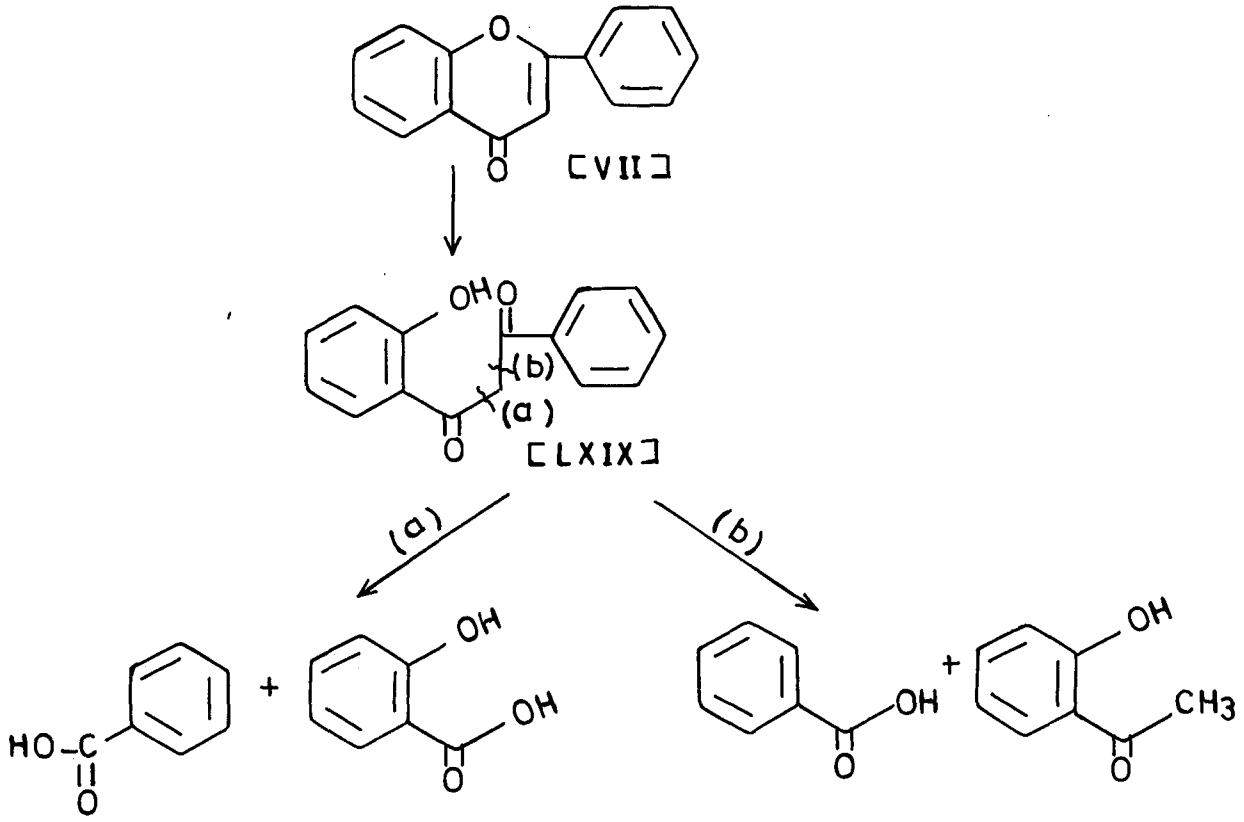
CHART-XV

(3) Degradation:

Degradation of flavanoids and biflavanoids can be brought about either by alkaline hydrolysis or oxidation with alkaline hydrogen peroxide.

Alkaline Hydrolysis:

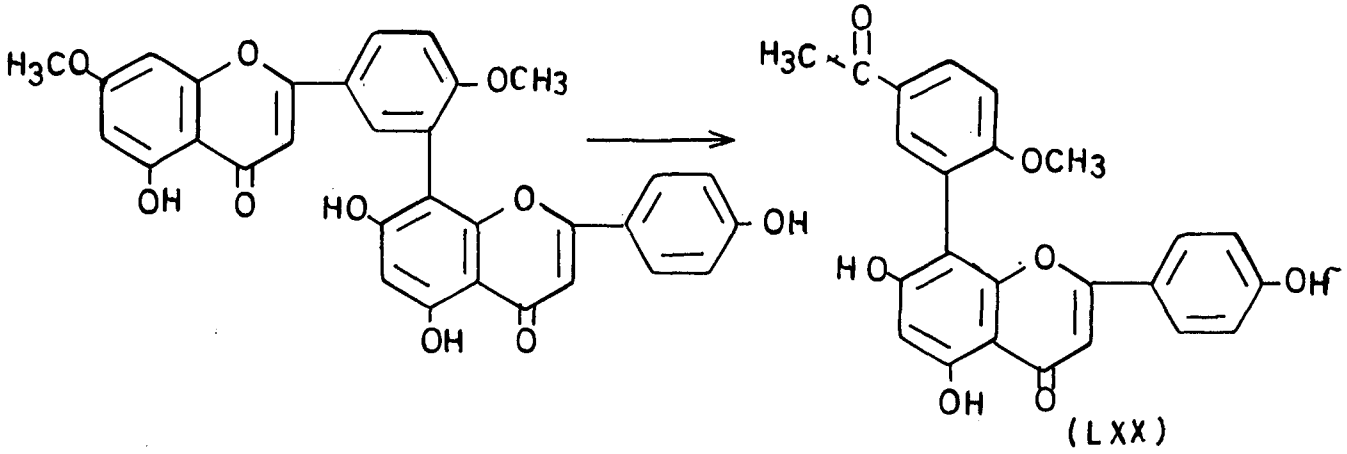
In general, a flavone (VII) gives four products which arise by opening of the pyrone ring followed by the fission of the intermediate, O-hydroxy-β-diketone (LXIX) by two different paths, (a) and (b).



The intermediate (LXIX) can be isolated if cold ethanolic solution of caustic soda is used.

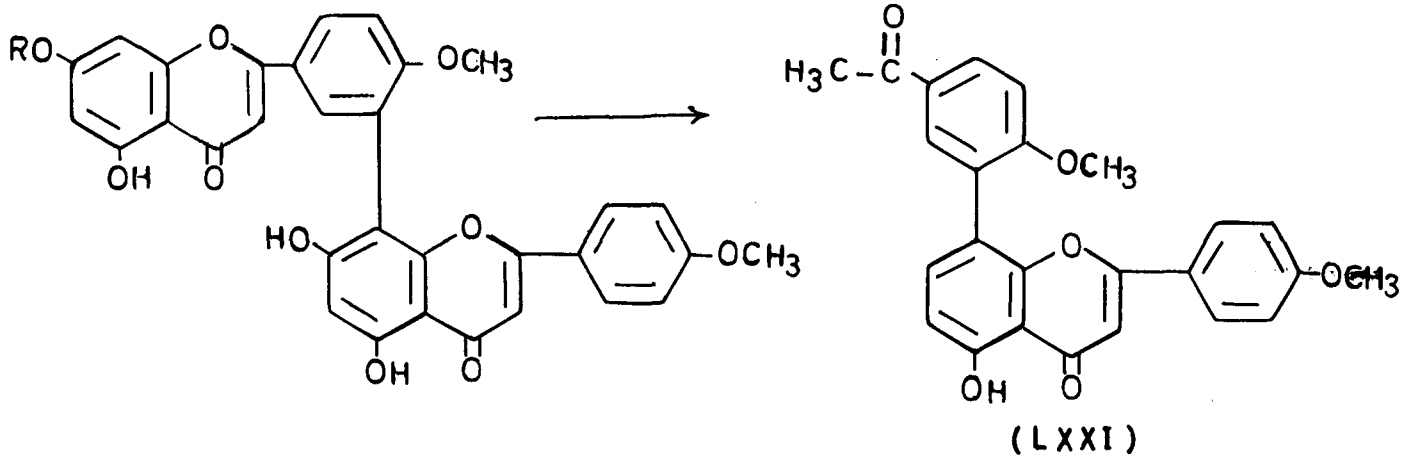
In the case of biflavanoids, 'Ketoflavones' are the characterstic degradation products of alkaline hydrolysis.

Hydrolysis of ginkgetin (XIV) gave p-hydroxyacetophenone, 2, 3-dihydroxy-4-methoxy acetophenone and a 'ketoflavone' $C_{24}H_{18}O_7$, whose structure was established as (LXX)[31].



(LXX)

Alkaline hydrolysis of both isoginkgetin (XIV.h) and sciadopitysin (XIV.m.) gave the same ketoflavone (LXXI), thus supporting the structures proposed for these biflavones.[31]



(LXXI)

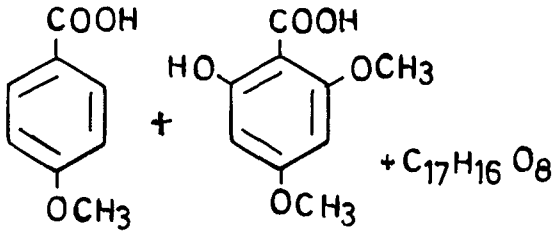
R=H Isoginkgetin (XIV.h.)
R=CH_3 Sciadopitysin (XIV.m.)

Oxidation with alkaline hydrogen peroxide:

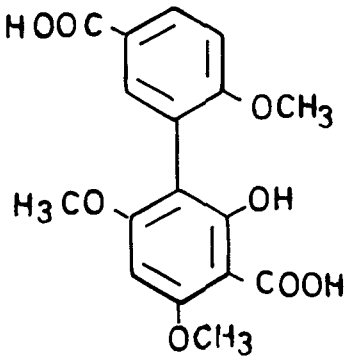
Alkaline hydrogen peroxide oxidation has been very useful in the determination of interflavanoid linkage. Jinkgetin (XIV.g.) tera-methyl ether on oxidation with alkaline H_2O_2 gave

anisic acid, 2-hydroxy-4, 6-dimethoxy benzoic acid and a compound, $C_{17}H_{16}O_8$.[30,99]

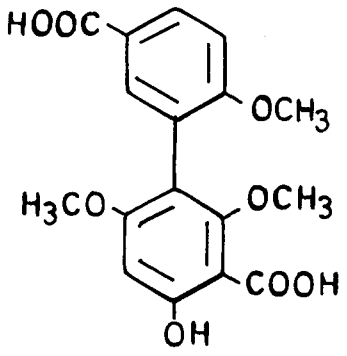
Ginkgetin (XIV.9.) tetra-methyl ether. ⟶



The compound $C_{17}H_{16}O_8$, was shown to be a dicarboxylic acid containing three methoxyl groups and one hydroxyl group. To fit in all the spectral data, two structures, (LXXII) and (LXXIII) were proposed for the diacarboxylic acid.



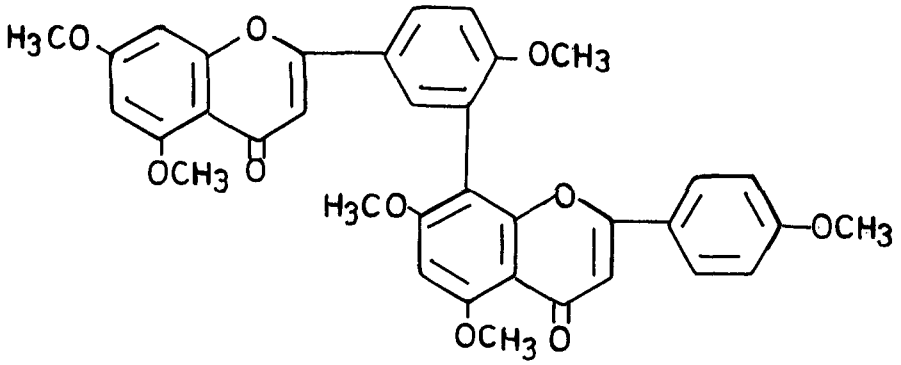
[LXXII]



[LXXIII]

These facts prove that a biphenyl residue must exist in the ginkgetin molecule and the interflavanoid linkage must involve position 3' of one flavanoid residue and position 6 or 8 of the other. The linkage (I-3', II-6) was considered unlikely since II-5-OH in a compound with this structure would be sterically hindered and there was no evidence that this hydroxyl group in ginkgetin was exceptionally difficult to methylate. The

structure (XIVq) with (I-3', II-8) linkage, was therefore, proposed for ginkgetin tetramethyl ether and structure (LXXII) for the dicarboxylic acid.[31]



[XIVq]

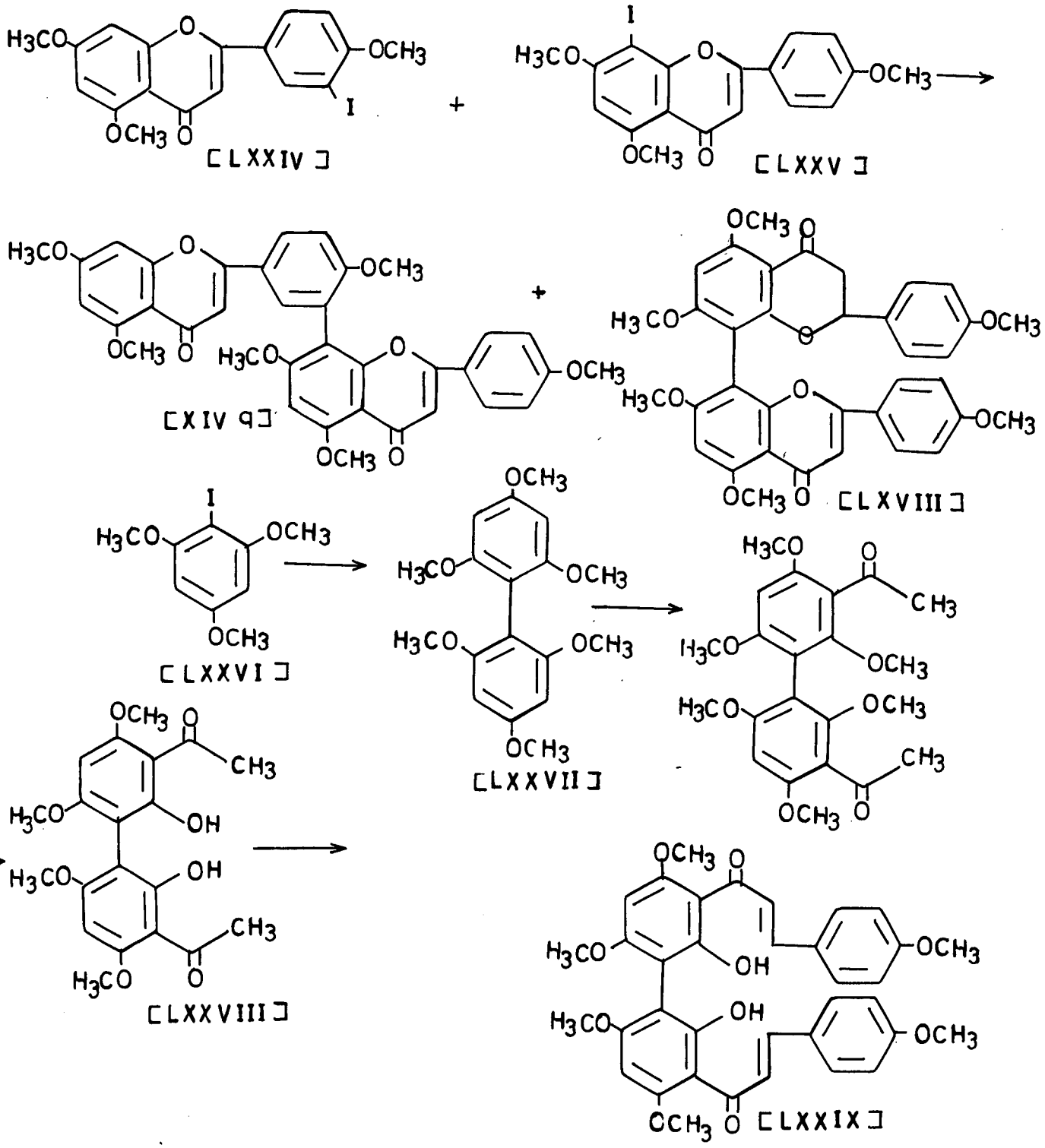
(4) Synthesis:

(a) biphenyl type biflavones

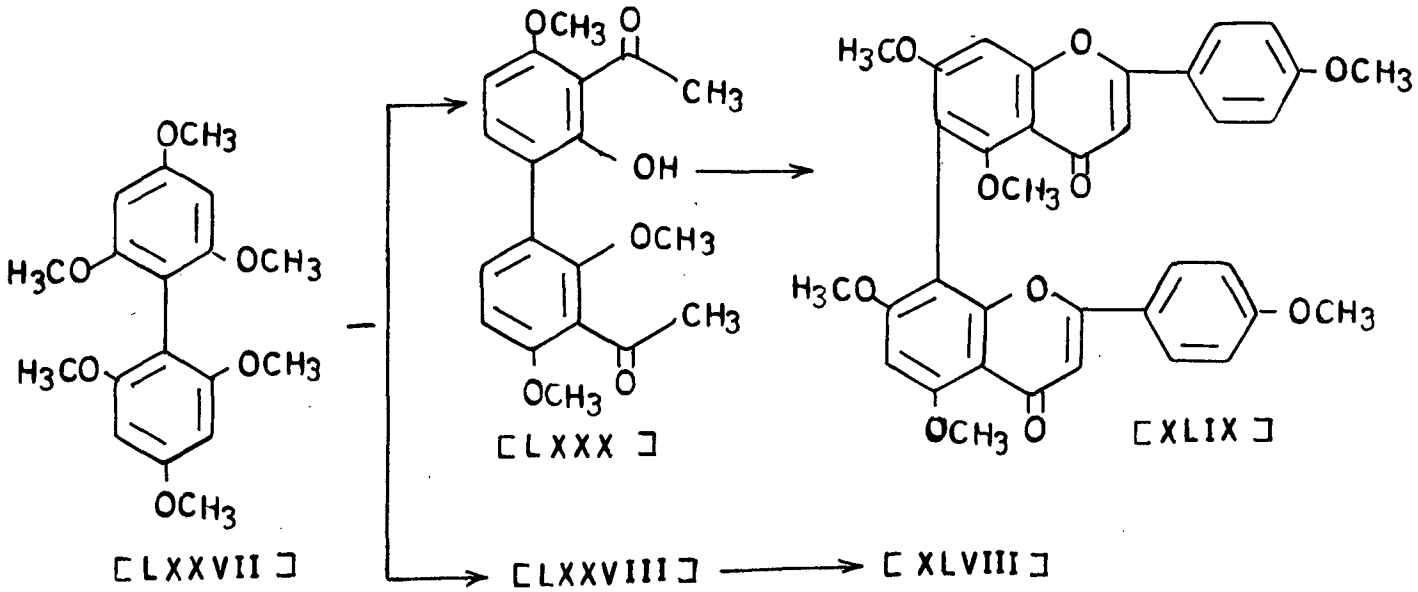
Synthesis of biphenyl type biflavones involves Ullmann reaction between two halogenated phenyl residues as one of the important steps. Nakazawa[100] accomplished the synthesis of amentoflavone hexamethyl ether by mixed Ullmann reaction between 3'-iodo-4', 5,7-Tri-O-methylflavone (LXXV). Cupressuflavone hexamethyl ehter was obtained as a biproduct and was found identical with the one obtained from natural sources. Later on Seshadri et al[19] have also synthesised cupressuflavone hexamethyl ether from 8-iodo-4',5,7-Tri-O-methylflavone (LXXV) under modified conditions of Ullmann reaction.

S. Ahmad and Razaq[101] and later on Kawano et al[94] have used an alternative route for the synthesis of cupressuflavone and agathisflavone hexamethyl ehhers. This involved Ullmann coupling between two molecules of 1-iodo-2,4,6-Tri-methoxybenzene (LXXVI)

to form a biphenyl system (LXXVII) as the first step. Subsequent Friedel Craft's acylation, partial demethylation and condensation with anisaldehyde gave a bichalcone (LXXIX). Oxidative cyclization of this bichalcone by SeO_2 gave cupressuflavone hexamethyl ether.[101]



From the Friedel Craft's acylation of (LXXVII) with acetylchloride and aluminum chloride in diethyl ether, the two compounds (LXXVIII) and (LXXIX) could be isolated. Subsequent acylation of these compounds with p-anisoyl-chloride, Baker-Venkataraman rearrangement and ring closure gave cupressuflavone hexamethyl ether&(XLIX)*, respectively.[102]

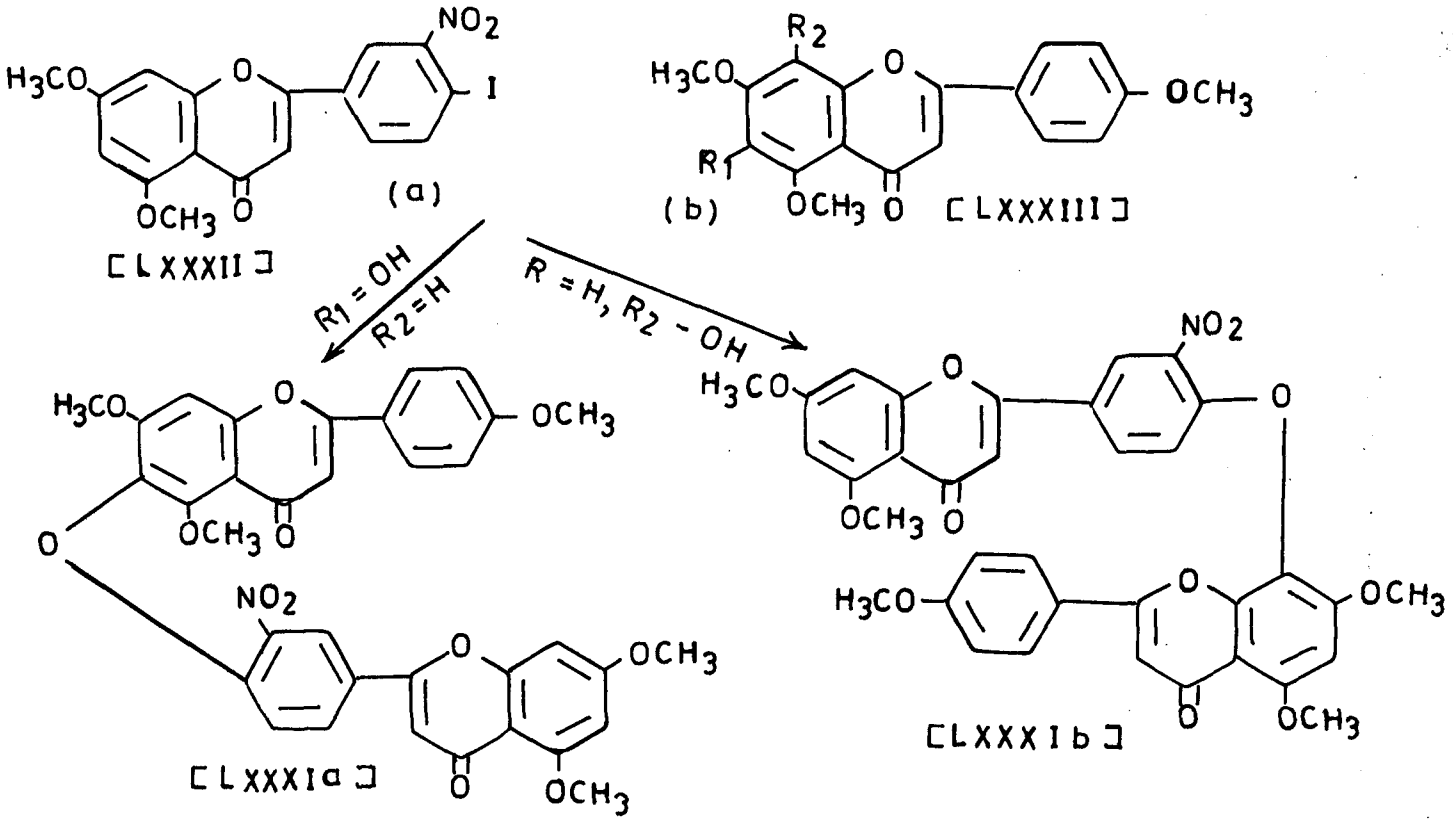


[XLIX] → agathisflavone hexamethyl ether

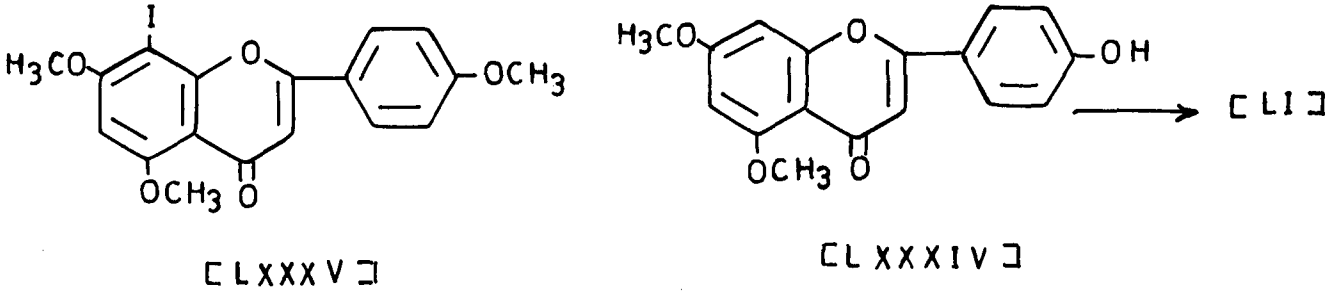
(b)Biphenyl ether type biflavones:

The synthesis of (I-4'-O-II-6) and (I-4'-O-II-8) linked hinokiflavone methyl ethers has been reported by Nakazawa.[52] The permethylated 3'-nitrobiflavone ether (LXXXI a or LXXXI b), the key intermediate, was obtained by condensation of 3'-nitro-4'-iodo-5, 7-Di-O-methylflavone (LXXXII) and 8-or 6-hydroxy-4,5,7-Tri-O-methylflavone (LXXXIII) in DMSO in the presence of K_2CO_3. The

nitro ethers were reduced by $Na_2S_2O_4$ in aqueous DMF, diazotized and decomposed with 50% H_3PO_2 to give pentamethyl hinokiflavone.



Seshadri et al[103] have reported that demethylation and Wessely-Moser rearrangement occurred during Ullmann condensation between 8-iodoapigenin trimethyl ether (LXXXV) using activated copper bronze and potassium carbonate in isoamyl alcohal, to give natural hinokiflavone pentamethyl ether (LI), after methylation of the reaction product.

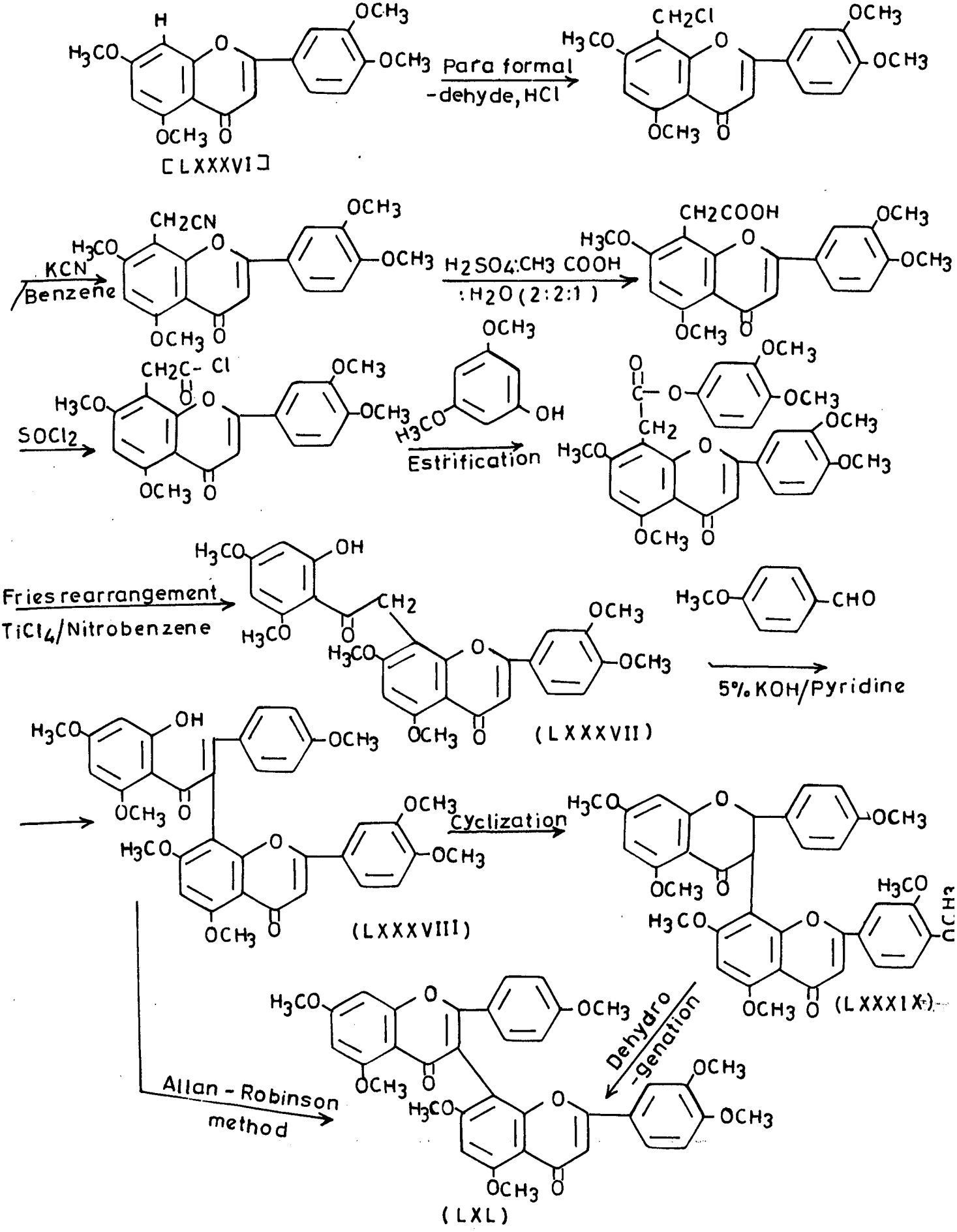


Konoshima et al[104] have recently reported the synthesis of II-3',I-4',II-4',I-5,II-5,I-7,II-7-hepta-O-methyl-flavanone (I-3,II-8)flavone (hepta-O-methylfukugetin)(LXXXIX) and its dehydrogenated derivative (LXL)(hepta-O-methylsaharanflavone). Luteolin tetra methyl ether(LXXXVI) was converted into 8-chloromethyl compound by chloromethylation with paraformaldhyde and HCl which was heated with KCN in benzene to give a cyanide which on hydrolysis with sulphuric acid-acetic acid water (2:2:1) gave an acid which was converted into acid chloride. Estrification of it with phloroglucinol dimethyl ether gave a compound which underwent the Fries arrangement in the presence of titanium tetrachloride in nitrobenzene at room temperature to give the required ketoflavone (LXXXVII). The ketoflavone was condensed with anisaldehyde to give a bichalcone (LXXXVIII) which on cyclization gave hepta-O-methylfukugetin (LXXXIX). Cyclization of LXXXVIII with anisic anhudride and sodium acetate (Allan-Robinson method) or dehydrogenation with iodine-potassium acetate gave hepta-O-methylsaharan flavone (LXL).

Biogenetic type synthesis:

Recently, attention of Organic chemists has focused on the synthesis of natural products by procedures which simulate certain steps of a proposed biosynthetic pathway rather than discovering new reagents and reactions. The application of phenol oxidation to synthetic chemistry has, therefore, been extensively studied.[105-107]

It has been experimentally established that in the phenol oxidation mechanism, the phenolate ion is oxidised by an electron

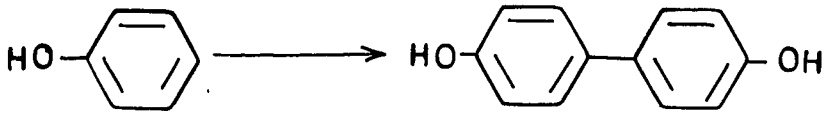

Para formal -dehyde, HCl
[LXXXVI]
KCN / Benzene
H2SO4:CH3 COOH : H2O (2:2:1)
SOCl2
Estrification
Fries rearrangement
TiCl4/Nitrobenzene
5% KOH/Pyridine
(LXXXVII)
Cyclization
(LXXXVIII)
(LXXXIX)
Allan - Robinson method
Dehydro -genation
(LXL)

oxidant like ferric chloride or potassium ferricyanide, to a phenoxy radical. The free electron in the phenoxy radical may be shown at various places by mesomeric effect. The free radicals are then coupled rapidly and irreversibly to dimeric and polymeric products under kinetic control. It is reasonable to assume that coupling occurs fastest at the positions of highest density of the free electron except where there is steric hinderence of approach.

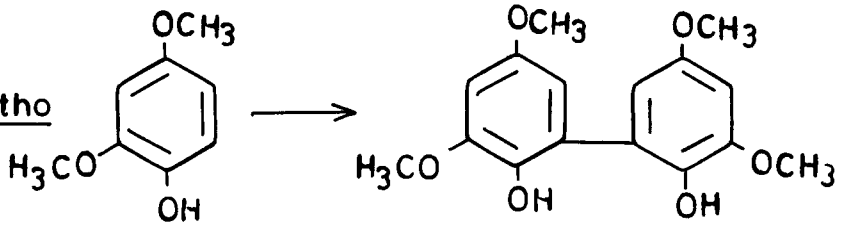
Dimerization of phenols may involve carbon-carbon, carbon-oxygen or oxygen-oxygen bonding as illustrated in the following simple examples:

(a) Carbon-Carbon Coupling

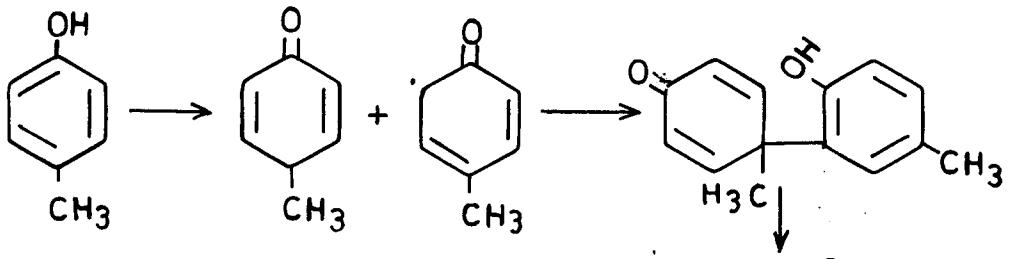
Para – Para



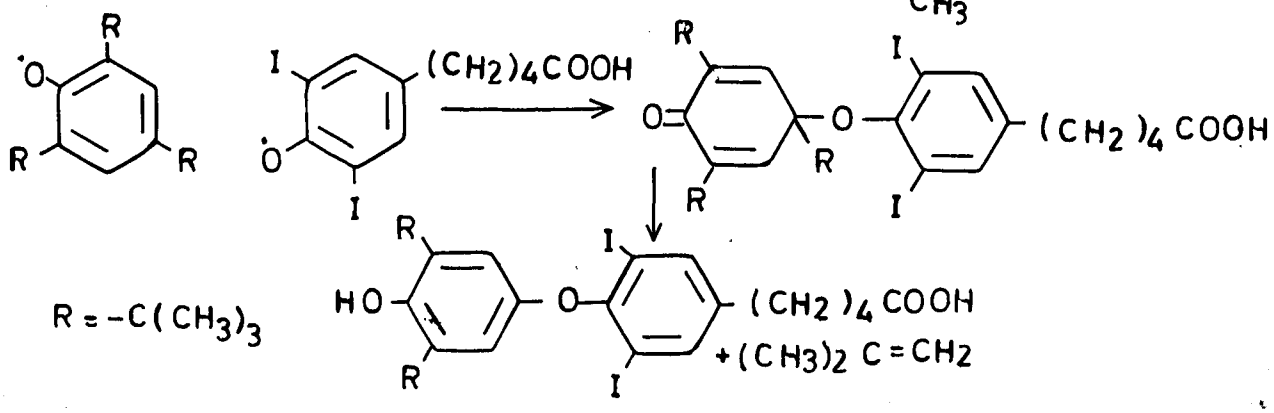
Ortho - Ortho



Ortho-para

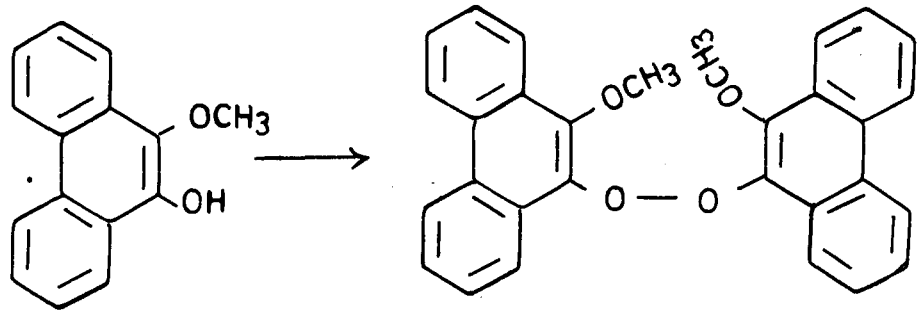


(b) Carbon-oxygen Coupling

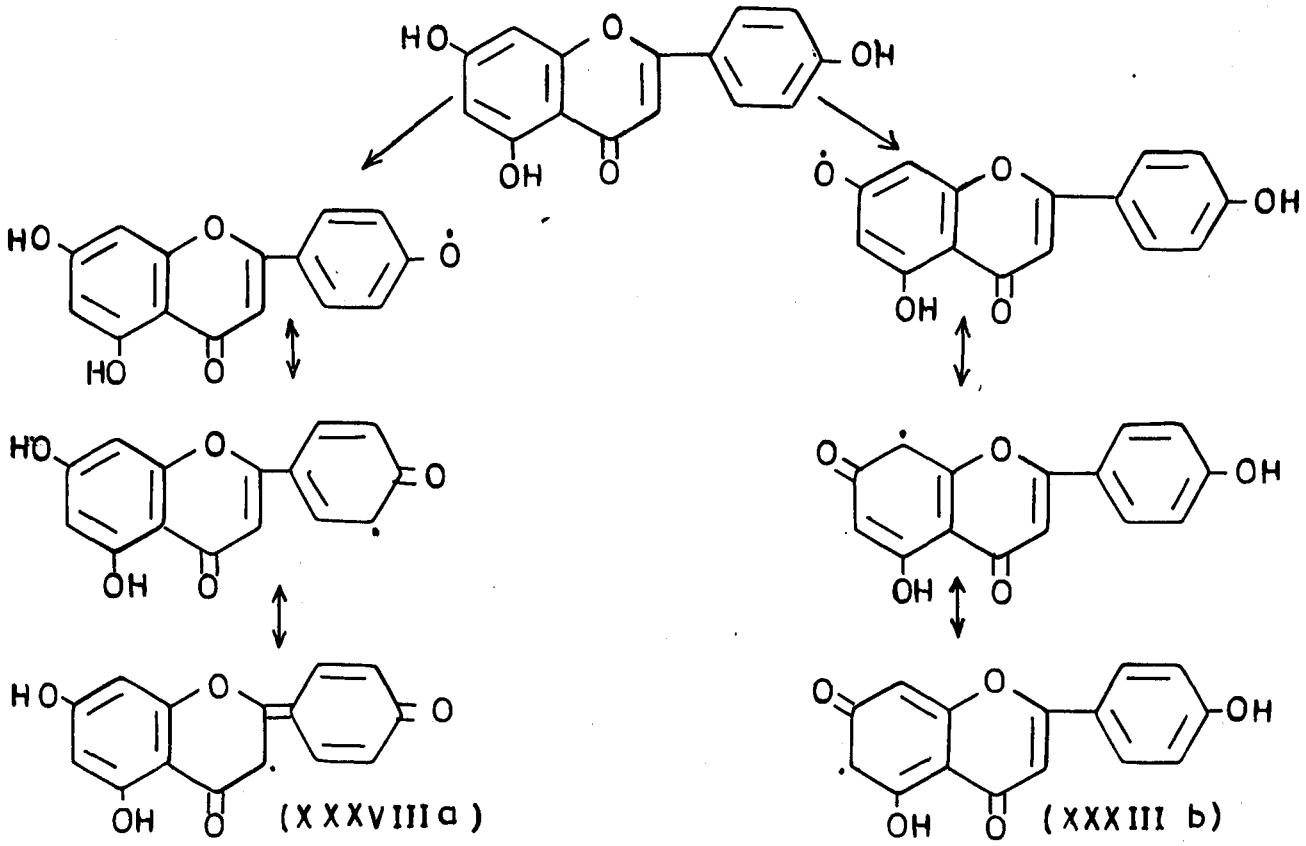


R = -C(CH3)3

(c) Oxygen-Oxygen Coupling

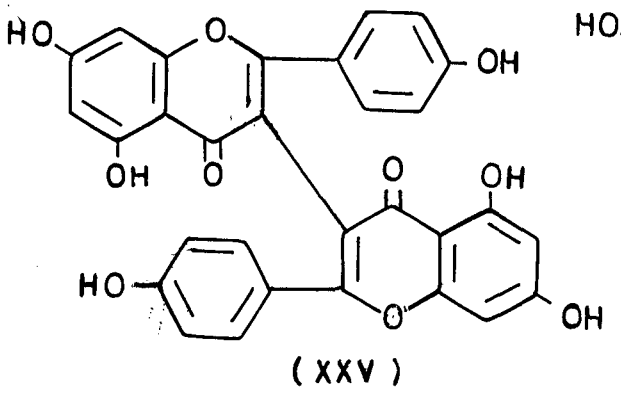


A large number of examples of biogenetic type synthesis involving C-C,C-O and O-O coupling have been reported in literature. These include bisnaphthols, bisterpenoids, bisanthraquinones, and biflavanoids. The parent biflavones, amentoflavone,cupressuflavone, agathisflavone and hinokiflavone together with their various O-methyl ethers exhibit either C-C or C-O linkage between the flavanoid units which might be expected to arise through oxidative coupling of an apigenin derived radical (XXXVIIIa or XXXVIIIb) by three of the many modes of dimerization theoretically possible, as shown below:-

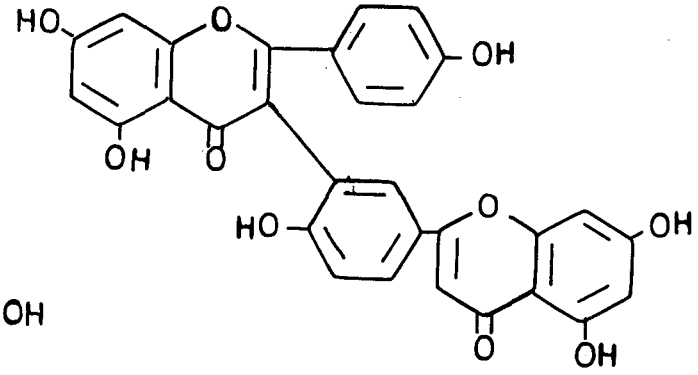


Molyneux et al[50] have reported that the oxidation of apigenin with alkaline potassium ferricyanide gave two novel dimers, namely, (I-3,II-3) (XXV) and (I-3,II-3') (XXVI) biapigeninyls, but no naturally occurring biapigeninyl was formed.

The synthetic compounds (XXV) and XXVI) appear to arise by oxidative coupling of the radical (XXXVIIIa), although none of the symmetrical (I-3',II-3')linked dimer, which might also be expected to be formed, could be isolated.



(XXV)



(XXVI)

These observations are consistent with the findings of kuhnle et al[108] who studied the EPR spectra of flavanoid anion radicals (derived from polyhydroxyflavones and having a 5-hydroxy function) and concluded that the electron density is mostly located in the 3-position of the heterocyclic ring and in the side phenyl positions. Molyneux et al have, therefore, suggested that biosynthesis of natural biflavonoids does not involve radical coupling of apigenin but probably takes place by the electrophillic attack of an apigeninyl radical on the 6-or the 8-position of another molecule of apigenin.

Pelter et al[109] tried oxidative coupling of 4'-hydroxy 7-methoxyflavone using alkaline potassium ferricyanide and got back the starting material. Seshadri et al[110] have recently carried out oxidative coupling of apigenin-7,4'-dimethyl ether with ferric chloride and isolated a dimer in 6% yield. The structure of its methyl ether has established as (I-8,II-8) biapigeninyl hexamethyl ether by NMR studies. However, a direct comparison of the dimer and its methyl ether with authentic cupressuflavone tetra and hexamethyl ethers showed that they are not identical. It appears to be definite that cupressuflavone tetra and hexamethyl ethers are isomers with the synthetic ones. Studies are under way to settle this problem.

On the basis of these findings, Seshadri et al[110] have suggested that when hydroxyl groups are protected e.g. by methylation (leaving only the 5-OH free), dimerization takes place through 6-or 8-positions of the A rings. It is reasonable to expect that in nature adequate mechanisms are available for protecting the hydroxyl groups and bringing about the coupling through the positions in A-rings.

D I S C U S S I O N

BIFLAVONES FROM THE LEAVES OF OCHNA SQUARROSA LINN (OCHNACEAE).

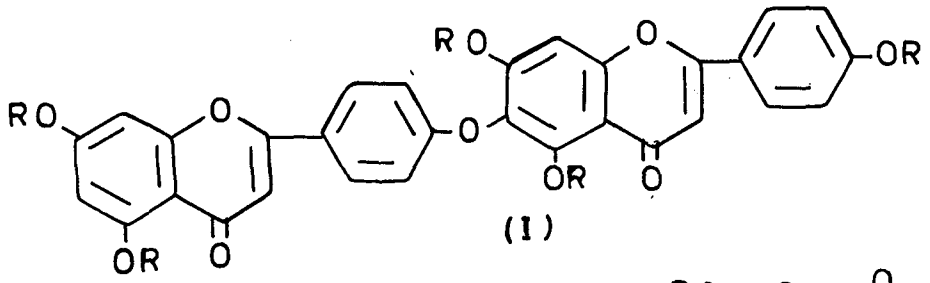
The biflavanoids are found distributed mainly in the leaves of Gymnospermae.[40,41,107] The heartwood, bark, latex and leaves of certain Angiosperms such as casuarinaceae,[123] Euphorbiaceae,[12,13] Guttiferae,[10,11] Caprifoliaceae,[14] Archegoniatae[15] and Anacardiaceae[17] have also been reported to contain biflavanoids. Selaginella[18] constitutes the only example of cryptogam containing such constituents. A dimeric proanthocyanidin was reported as the sole biflavone constituent from Ouratea species of Ochnaceae plants.[124] The present discussion deals with the isolation and characterization of biflavanoids from the leaves of Ochna squarrosa Linn belonging to the family Ochnaceae (Angiosperm) and Ochna suarrosa is found in Assam and Western Peninsula. The leaves are boiled and used in emollient cataplasm and bark as digestive tonic. The root is used as antidote to snake-bite, decoction is given in menstrual complaints and asthma.

It is worthy of mention that hinokiflavone (Ia) (I-4'-O-II-6) constitutes the sole example of naturally occurring biphenyl ether type of biflavones.[25,51,52,69] The biflavones from Ochna squarrosa linn (Ochnaceae) furnish the second example of new biphenyl ether type (I-3'-O-II-4') of biflavanoids.[16] The parent biflavone having the hitherto unknown interflavanoid ether linkage has been designated as "Ochnaflavone"(IIa). The occurrence of mono, and dimethyl ethers of Ochnaflavone has also been established in the original plant extract. The trimethyl ochnaflavone, I-5, II-5-Dihydroxy-I-4',I-7,II-7-tri-O-methyl(I-3'-O-II-4') biflavone(IIg)

has been obtained by methylation of the mixture of the above biflavones with diazomethane.

Leaves of Ochna squarrosa linn were procured from Horticulture Research Centre Saharanpur and Forest Research Institute Dehradun, U.P., India. The acetone extracts of the fresh leaves were purified by solvent fractionation and column chromatography (silica gel) to give a biflavone mixture which was separated by preparative layer chromatography into three fractions labelled as OSI, OSII and OSIII in the order of increasing R_f values using BPF(36:9:5).[62] Homogeneity of the components was checked by TLC using different solvent systems. Each component responded to the usual flavanoid colour reactions. The chromatographically homogeneous fractions OSI, OSII and OSIII on methylation gave the same methyl ether(m.p.167-169°), which was characterized as I-4', I-5, II-5, I-7, II-7-penta-O-methyl(I-3'-O-II-4') biflavone (IIb).

The components OSI, OSII and OSIII were characterized as I-4', I-5, II-5, I-7, II-7-pentahydroxy(I-3'-O-II-4')biflavone(IIa), I-5, II-5, I-7, II-7-tetrahydroxy-I-4'-O-methyl(I-3'-O-II-4')biflavone(IIc) and I-5, II-5, II-7-trihydroxy-I-4', I-7-Di-O-methyl(I-3'-O-II-4') biflavone (IId) respectively. The structure of each compound has been fully elucidated by U.V., I.R., N.M.R. including lanthanide induced shifts (LIS) and mass spectral studies. Further support to the structure is furnished by the synthesis of Ochnaflavone pentamethyl ether and its comparison with the methyl ether of the natural product. (IIb).

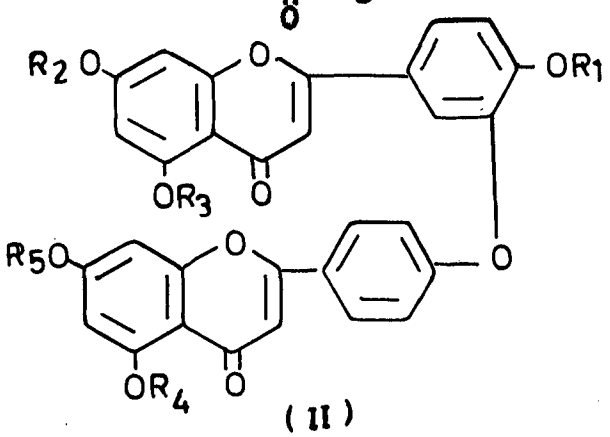


(I)

(a) R = H

(b) R = CH_3

(c) R = $-\overset{O}{\overset{\|}{C}}-CH_3$



(II)

(a) $R_1=R_2=R_3=R_4=R_5=H$

(b) $R_1=R_2=R_3=R_4=R_5=CH_3$

(c) $R_1=CH_3; R_2=R_3=R_4=R_5=H$

(d) $R_1=R_2=CH_3; R_3=R_4=R_5=H$

(e) $R_1=R_2=R_3=R_4=R_5=-\overset{O}{\overset{\|}{C}}-CH_3$

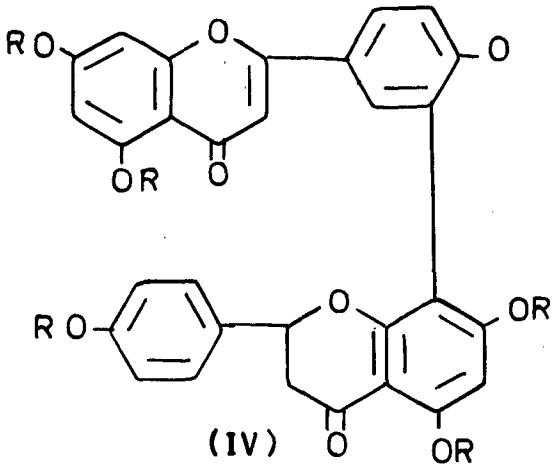
(f) $R_1=CH_3; R_2=R_3=R_4=R_5=-\overset{O}{\overset{\|}{C}}-CH_3$

(g) $R_1=R_2=CH_3; R_3=R_4=R_5=\overset{O}{\overset{\|}{C}}-CH_3$

(h) $R_1=R_2=R_5=CH_3; R_3=R_4=H$

(i) $R_1=R_2=R_5=CH_3; R_3=R_4=-\overset{O}{\overset{\|}{C}}-CH_3$

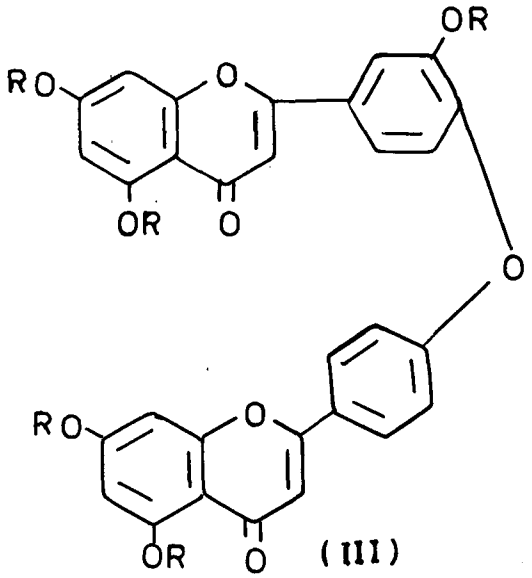
(j) $R_1=R_5=CH_3; R_2=R_3=R_4=H$



(IV)

(a) R = H

(b) R = CH_3



(III)

(a) R = H

(b) R = CH_3

I-4',I-5,II-5,I-7,II-7-Pentahydroxy(I-3'-O-II-4')biflavone (IIa) OSI:

Compound	m.p,	R_f
OSM (methyl ether)	167-169°	0.44
OSIA(acetate)	239-241°	-

The U.V.spectra of OSI λ max (ethanol); 271 and 339 nm (log ϵ 4.24 & 4.25),OSM (ethanol), 267 and 328 nm (log ϵ 4.60 & 4.65) and OSIA (ethanol)256 and 312 nm (log ϵ 4.28 & 4.43) are similar to those of apigenin (5,7,4'-trihydroxyflavone)derivatives but the intensities of absorption for corresponding bands in OSI,OSM and OSIA were approximately double those of apigenin derivatives.[99] This demonstrated the presence of two isolated flavanoid chromophores permolecule in OSI,OSM & OSIA thus indicating biflavanoid structure. IR spectrum of OSI showed strong absorption bands at 2930(OH) and 1655 $C\bar{m}^1$(CO) the latter being characterstic of 5-hydroxyl group chelated with carbonyl group. The shift of the corbonyl frequency to 1648 $C\bar{m}^1$on acetylation and 1640 $C\bar{m}^1$on methylation further supported the presence of chelated carbonyl function. The foregoing evidences suggested a 5,7,4'-oxygenation pattern.[19,99,28a]

On methylation with dimethyl sulphate and potassium carbonate, OSI yielded a methyl ether (OSM)$C_{35}H_{28}O_{10}$(IIb),m.p.167-169°, whose mass spectrum showed a parent peak (608) and fragment peaks, 592,580, 327,311,304(M^{++}),297,281 and so on, quite similar to those of penta -O-methyl hinokiflavone as shown in Chart-I.

NMR spectrum (Fig.1) of OSI methyl ether (OSM)showed methoxy signals between τ6.08 to τ6.12 which integrated for five methoxyls. It also showed the presence of six protons at I-3,II-3,I-6,II-6,I-8

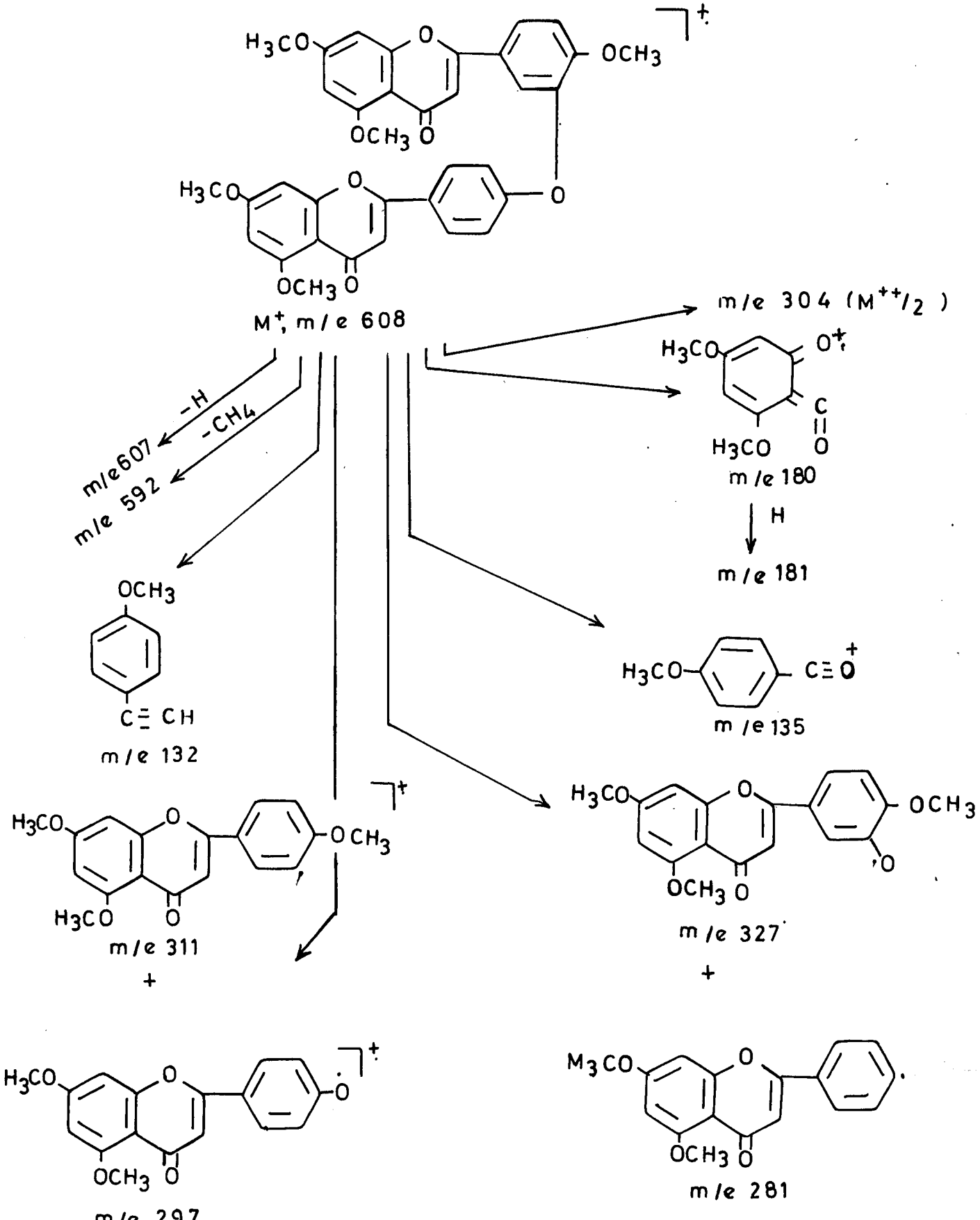

M+, m/e 608
m/e 304 (M++/2)
m/e 180
H
m/e 181
-H
-CH4
m/e607
m/e 592
m/e 132
m/e 135
m/e 311
+
m/e 327
+
m/e 297
m/e 281

CHART - I

and II-8 positions and seven protons at side phenyl groups as shown in table -I.

TABLE-I

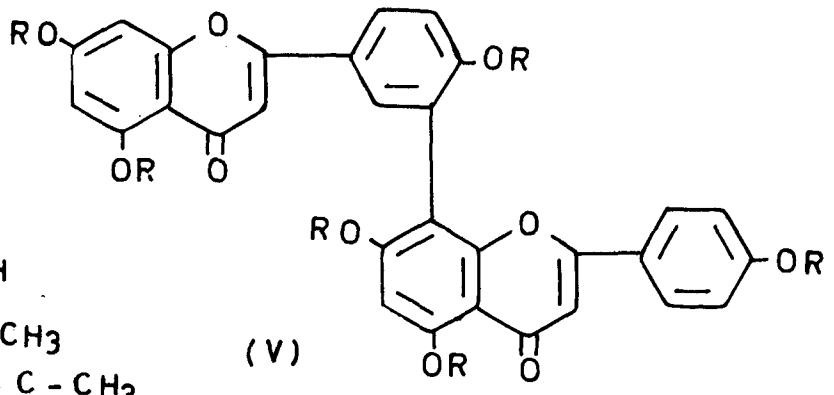
The NMR$_\lambda$(τ) data of methyl ether OSM (IIb), synthesized methyl ethers (IIb,IIIb) in $CDCl_3$ and S-Values by $Eu(fod)_3$.

Assigned Positions.	Signals	J(Hz)	OSM (IIb)	Synthesized (IIb)*	Synthesized (IIIb)*	S-values by $Eu(fod)_3$ (IIb)
I-4'	3H,s		6.12	6.12	6.20	-0.14
I-5	3H,s		6.08	6.08	6.17	10.26
II-5	3H,s		6.08	6.08	6.17	11.56
I-7	3H,s		6.12	6.12	6.17	0.80
II-7	3H,s		6.12	6.12	6.17	0.96
I-3	1H,s		3.45	3.45	3.47	0.32
II-3	1H,s		3.41	3.41	3.41	0.24
I-6	1H,d	2.5	3.66	3.66	3.73	4.86
II-6	1H,d	2.5	3.66	3.66	3.70	5.58
I-8	1H,d	2.5	3.49	3.49	3.53	1.14
II-8	1H,d	2.5	3.46	3.46	3.50	1.26
II-3',II-5'	2H,d	9.0	2.99	2.99	2.99	-0.12
II-2',II-6'	2H,d	9.0	2.19	2.19	2.21	-0.12
I-5'	1H,d	9.0	2.89	2.89	2.91	-0.10
I-2'	1H,d	2.5	2.40	2.40	2.59	0
I-6'	1H,q	2.5& 9.0	2.28	2.30	2.54	-0.10

s= singlet, d= doublet,q=quarter. TMS- as internal standard τ=10.0
Spectra run in $CDCl_3$ at 100 MHz.
* Synthetic.

The side phenyl proton signals were explained by NMDR technique. When 2H(II-3',II-5') doublet at τ 2.99 was irradiated the other 2H(II-2',II-6') doublet at τ2.19 was changed to a singlet. The irradiation of IH(H-I-5') coublet at τ2.89 changed the quartet at τ2.28(J=9.0 & 2.5 H_z) to a doublet (J=2.5 H_z). This clearly showed that these signals of side phenyl protons were very similar to those of amentoflavone derivatives[39] indicating I-3'-substitution pattern of one of the apigenin molecules.

The assignment of NMR signals (Fig.1) to various methoxyls and aromatic protons of OSM(IIb) gained further support by comparing its NMR spectrum with the spectra of I-3'-substituted unit of amentoflavone hexamethyl ether (Vb) and I-4'-substituted unit of hinokiflavone pentamethyl ether (Ib) as shown in table II. Similar comparison of NMR spectrum (Fig.2) of Ochnaflavone acetate OSIA(IIe) with the corresponding units of amentoflavone hexaacetate (VC) and hinokiflavone pentaacetate (Ic) is shown in table III.



(a) R = H

(b) R = $-CH_3$

(c) R = $-\overset{\overset{O}{\|}}{C}-CH_3$

(V)

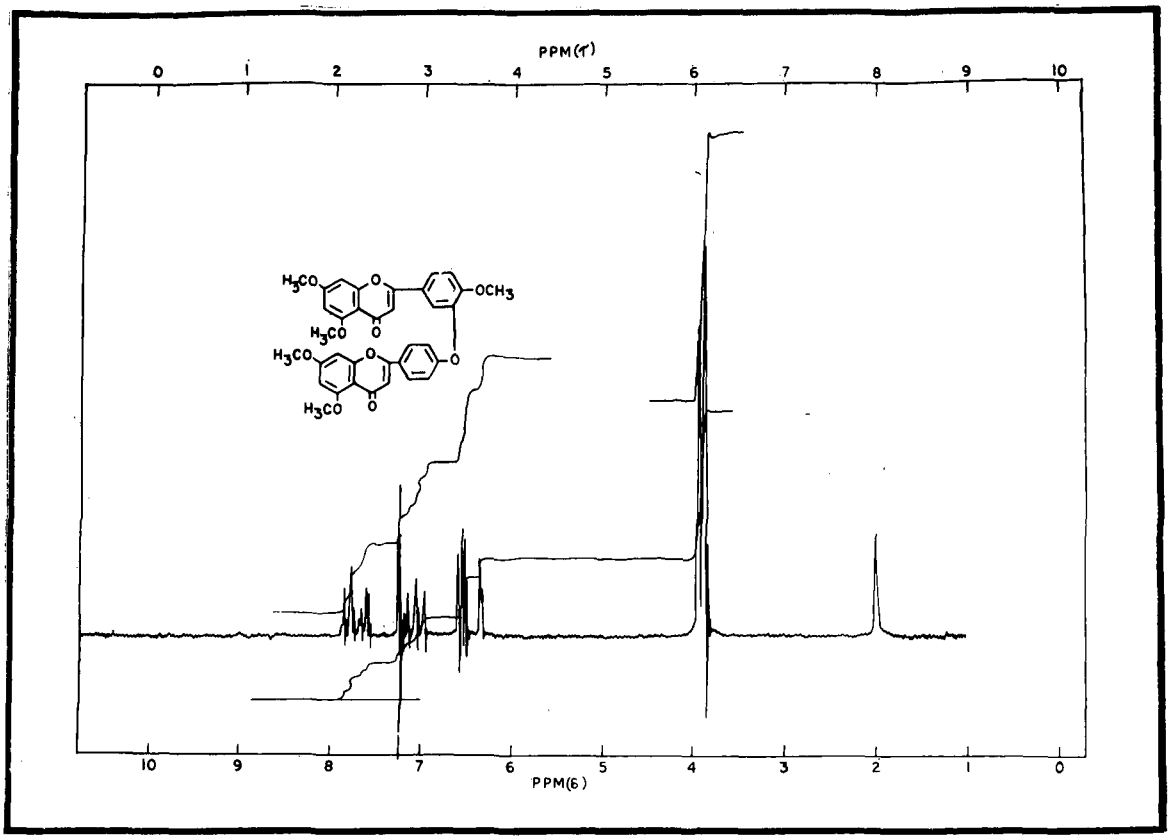

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
H3CO
OCH3
H3CO
O
H3CO
O
H3CO
O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG. 1

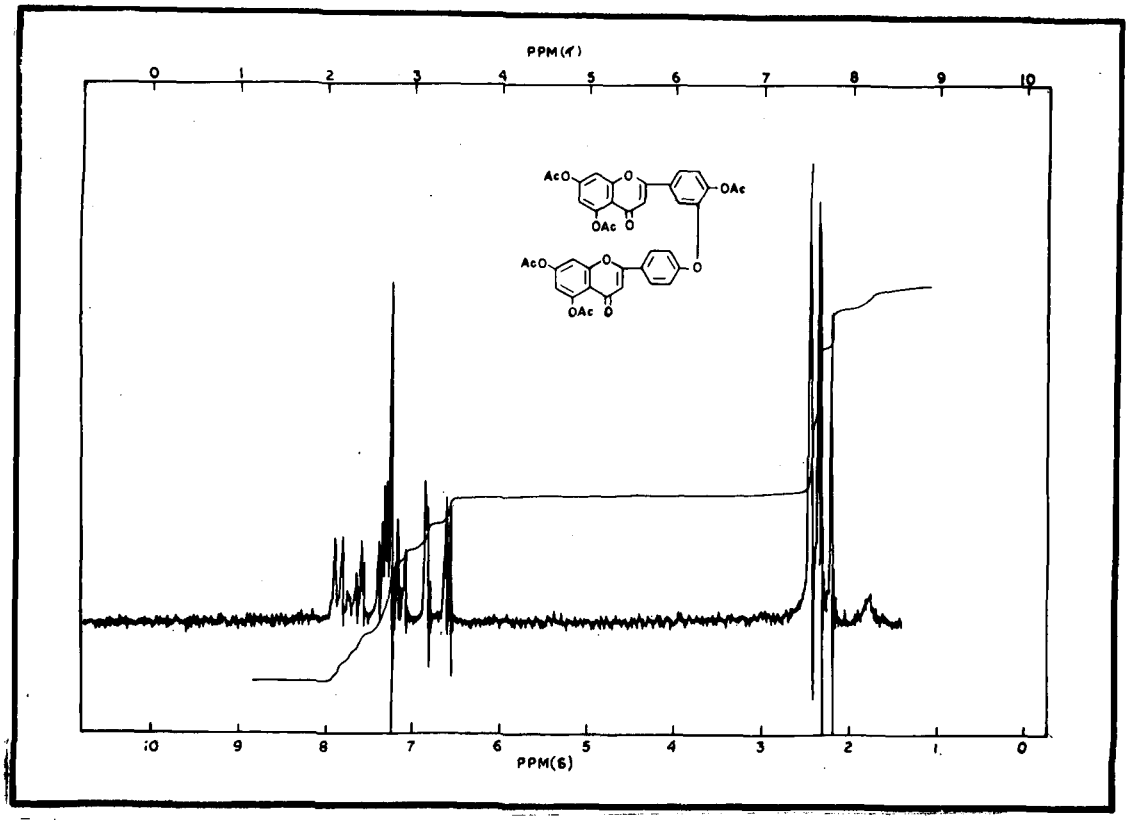

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
AcO
OAc
OAc
O
AcO
O
OAc
O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG. 2

TABLE-II

Comparison of NMR Spectra of Compound IIb with Vb and IIb with Ib, (Values on τ Scale).

Unit- I (Vb)	Assigned position	Unit-I (IIb) (OSM)	Unit-I ($I\text{-}4'$-linked) (Ib)	Unit-II (IIb) (OSM)
3.66,d	H-I-6	3.66,d	3.63,d	3.66,d(HII-6)
3.52,d	H-I-8	3.49,d	3.45,d	3.46,d(H-II-8)
2.16,d	H-I-$2'$	2.40,d	2.12,d	2.19,d(H-II-$2'$)
2.10,q	H-I-$6'$	2.28,q	2.12,d	2.19,d(H-II-$6'$)
2.88,d	H-I-$5'$	2.89,d	2.98,d	2.99,d(H-II-$5'$)
___	H-I-$3'$	___	2.98,d	2.99,d(H-II-$3'$)
3.47,s	H-I-3	3.45,s	3.38,s	3.41,s(H-II-3)
6.25,s	MeO-I-$4'$	6.12,s	___	___ (MeO-II-$4'$)
6.08,s	MeO-I-5	6.08,s	6.06,s	6.08,s(MeO-II-5)
6.12,s	MeO-I-7	6.12,s	6.10,s	6.12,s(Meo-II-7)

Spectra run in $CDCl_3$ at 100MHz.
s= singlet, d= doublet, q=quartet.

TABLE-III

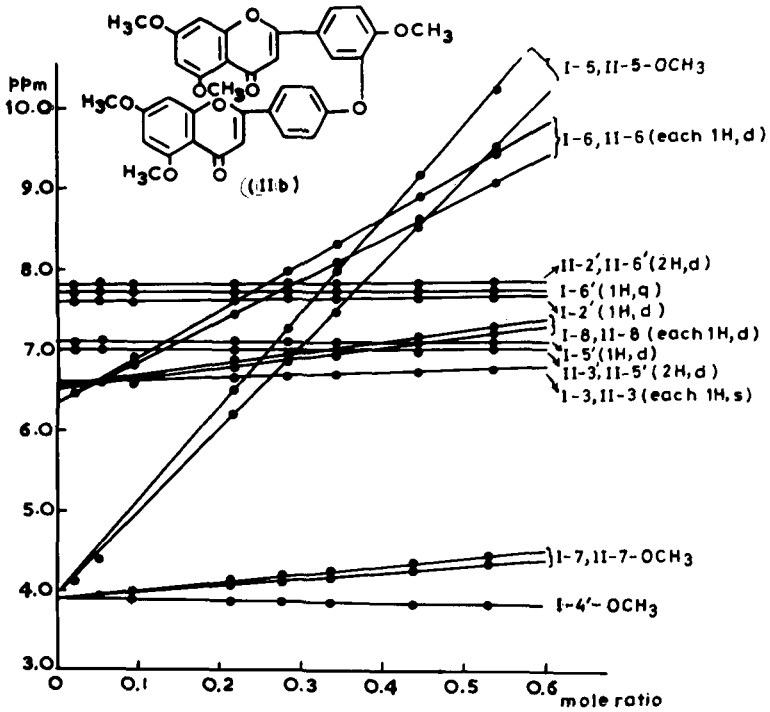
Comparison of NMR spectra of OSIA with Vc and OSIA with Ic (Values on τ scale).

I-3'-linked Vc(Unit-I)	Assigned position	Unit-I OSIA(IIe)	I-4' linked Ic(Unit-I)	Unit-II OSIA(IIe)
3.16,d	H-I-6	3.17,d	3.18,d	3.16,d(H-II-6)
2.74,d	H-I-8	2.68,d	2.60,d	2.66,d(H-II-8)
1.97,d	H-I-2'	2.38,d	2.11,d	2.14,d(H-II-2')
2.20,q	H-I-6'	2.28,q	2.11,d	2.14,d(H-II-6')
2.52,d	H-I-5'	2.65,d	2.80,d	2.87,d(H-II-5')
___	H-I-3'	___	2.80,d	2.87,d(H-II-3')
3.32,s	H-I-3	3.38,s	3.40,s	3.43,s(H-II-3)
7.59,s	OAc-I-5	7.58,s	7.58,s	7.56,s(OAc-II-5)
7.72,s	OAc-I-7	7.68,s	7.68,s	7.65,s(OAc-II-7)
7.99,s	OAc,I-4'	7.80,s	___	___ (OAc-II-4')

Spectra run in $CDCl_3$ at 100MHz.
s=singlet, d=doublet, q=quartet.

The relative upfield values of H-I-2' and H-I-6' both in OSM(IIb) and OSIA(IIe) as compared to those of amentoflavone derivatives (Vb&Vc) for the same protons may be attributed to the presence of an oxygen bridge in Ochnaflavone.

The mode of interflavanoid linkage (I-3'-O-II-4') in OSM(IIb) was established by lanthanide induced shift (LIS) studies using $Eu(fod)_3$. It was reported[73,125] that when a shift reagent $Eu(fod)_3$ [tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-octane -4,6-dionato) europium (III)] was added to the $CDCl_3$ solution, the NMR signals of Meo-I-5,II-5 of fully methylated biflavones showed much larger downfield shifts than those of Meo-I-7,II-7 and the protons H-I-6,H-II-6 showed a considerable downfield shift in comparison with H-I-8,H-II-8 and H-I-3,H-II-3 while the side phenyl protons or methoxyls were shifted to a very small extent. Chemical shift changes found in every proton signal of the methyl ether (OSM,IIb) on addition of $Eu(fod)_3$ were expressed by S-values[96] and sloped lines[125] shown in table-I and figure-3 respectively. S-values of the five methoxy groups of compound IIb(10.26,11.56,0.80,0.96 and -0.14) were all compatible with reported values of MeO-I-5,II-5, MeO-I-7,II-7 and a methoxy group in the side phenyl group respectively, meaning that the ether linkage between two apigenin molecules was in neither I-5, II-5-nor I-7,II-7-positions. Similarly, H-I-3, H-II-3, H-I-6, H-II-6, H-I-8 and H-II-8 in compound IIb all showed satisfactory S-values (table -I). The two pairs of marked by sloped lines in the figure assigned to Meo-I-5,II-5 and H-I-6,II-6 showed that coordination between $Eu(fod)_3$ and both the carbonyl groups (CO-I-4,II-4) occurred almost evenly without disturbance by other flavone unit which was linked at I-3-Or I-6-position.[125] The other lines, as shown in figure-3, were all compatible with reported slopes. Accordingly, the location of ether linkage between two



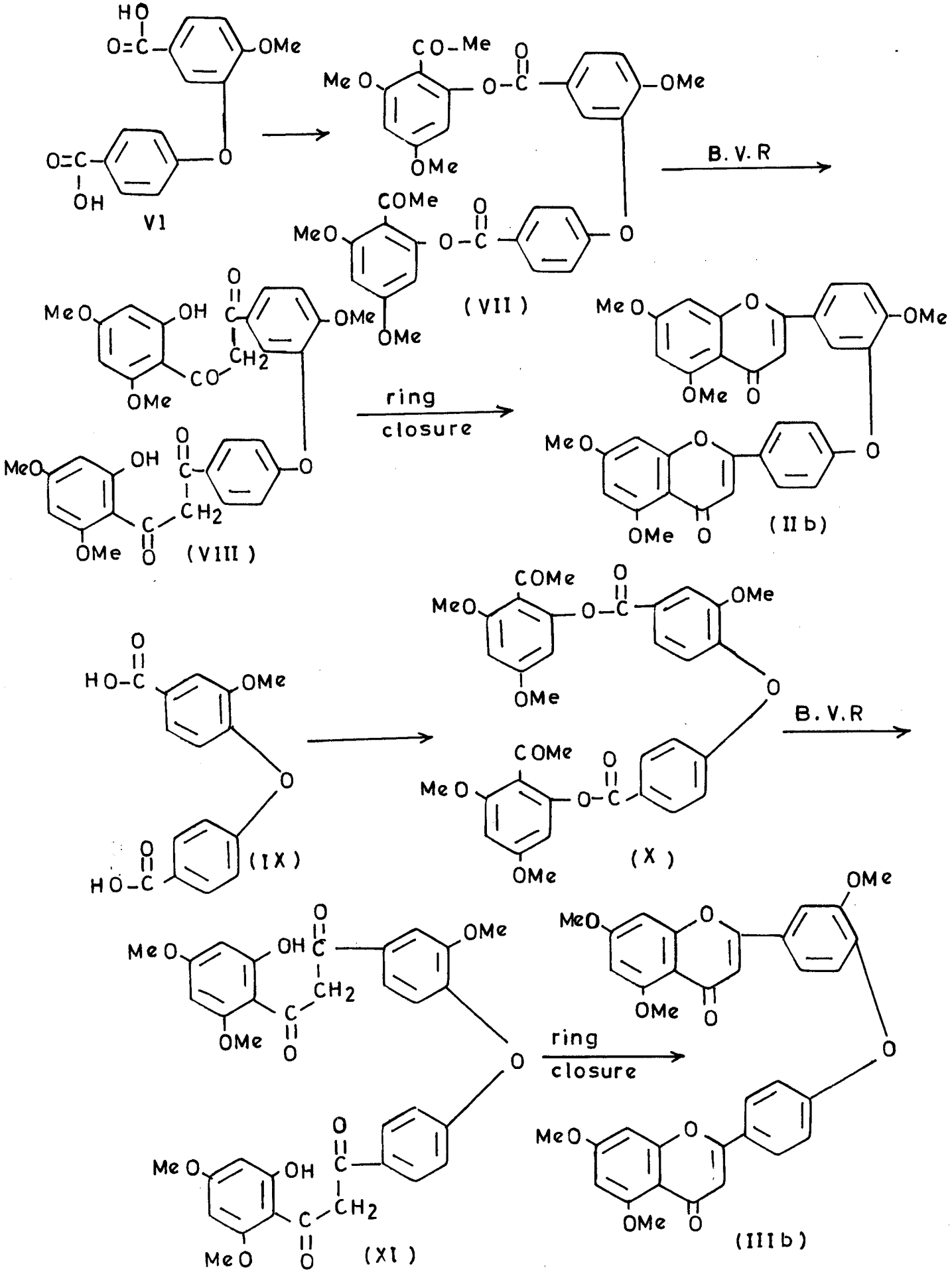
CHEMICAL SHIFT CHANGES OF OSM (II b) WITH INCREASING CONCENTRATION OF Eu (fod)$_3$

FIG. 3

flavone units would be confined to 3'- and 4'- positions of two side phenyl rings. Therefore, two possible structures, IIb and IIIb might be proposed for penta-O-methylochnaflavone, the former structure (IIb) being more preferable in the light of biosynthetic considerations because the structure (IIb) is obtainable by dehydrogenation between two molecules of apigenin like all other biflavones whereas the other structure (IIIb) is formed by, dehydration between apigenin and luteolin (5,7,3',4'-tetrahydroxy-flavone). This was confirmed by synthetic evidence.

Starting from corresponding biphenyl ether dicarboxylic acids (VI and IX)[126], the two compounds (IIb and IIIb) were synthesized by Baker-Venkataraman rearrangement (B.V.R.)[127] of diesters (VII & X) and subsequent ring closure of β-diketones (VIII & XI). The synthesized compound (IIb), m.p.169-171° was identified with methyl ether of natural ochnaflavone (OSM) by m.p.& m.m.p.169-171° and by comparisons of U.V.,I.R. and NMR spectra (table -I). The other isomer (IIIb), m.p.154-156° showed NMR signals of aromatic and methoxy protons (table -I) incompatible with those of the methyl ether of natural biflavone.

In the light of the aforesaid evidence OSM(IIb) was assigned the structure of I-4',I-5,II-5,I-7,II-7-penta-O-methyl (I-3'-O-II-4')biflavone. Ochnaflavone, the parent member of the new series, should subsequently have the structure IIa.


VI
(VII)
B.V.R
ring closure
(VIII)
(IIb)
(IX)
(X)
B.V.R
(XI)
ring closure
(IIIb)

I-5,II-5,I-7,II-7-tetrahydroxy-I-4'-O-methyl(I-3'-O-II-4') biflavone (OSII,IIc).

OSM(methyl ether)	167.169°	0.44
OSIIA(acetate)	248-250°	-

Methylation of OSII yielded the same methyl ether (OSM) as OSI. This was supported by m.p.,m.m.p.,R_f values, and NMR spectroscopy. The NMR spectrum of the corresponding acetate, OSIIA showed the presence of four acetoxy and one methoxy group (Fig-4). The signals due to all the protons and acetoxy groups were compatible with those of NMR signals in OSIA except the absence of an acetoxy proton signal at τ7.80 and the appearance of a new methoxy proton at τ6.12 in OSIIA. A doublet at τ2.65 in OSIA was also different with the doublet arising at τ2.87 in OSIIA as listed in table IV.

OSIA contained five acetoxy groups, meaning thereby that the signal assigned for I-4' acetoxy group was substituted by a methoxyl group at τ6.12 in OSIIA at the same position. This was supported by the upfield shift of H-I-5 from τ2.65 in OSIA to τ2.87 in OSIIA(table IV). A comparison of the NMR spectra of I-3'-substituted monoflavanoid unit of OSIIA and bilobetin pentaacetate[36] further supported the substitution pattern in OSIIA. These studies led to structure IIc for OSII.

TABLE- IV

Chemical shifts (τ values) of NMR spectra of Ochnaflavone acetate derivatives.

Assigned positions.	Signals	J(Hz)	OSIA	OSIIA	OSIIIA	OSIVA*
I-4'	3H,s		7.80	6.12	6.12	6.13
I-7	3H,s		7.68	7.69	6.12	6.12
II-7	3H,s		7.65	7.67	7.66	6.12
I-5	3H,s		7.58	7.57	7.57	7.58
II-5	3H,s		7.56	7.57	7.57	7.58
I-3	1H,s		3.38	3.46	3.51	3.53
II-3	1H,s		3.43	3.42	3.43	3.49
I-6	1H,d	2.5	3.17	3.18	3.42	3.43
II-6	1H,d	2.5	3.16	3.18	3.18	3.41
I-8	1H,d	2.5	2.68	2.71	3.18	3.19
II-8	1H,d	2.5	2.66	2.69	2.70	3.17
II-3',II-5'	2H,d	9.0	2.87	2.98	2.99	2.99
I-5'	1H,d	9.0	2.65	2.87	2.88	2.90
I-2'	1H,d	2.5	2.42	2.40	2.39	2.40
I-6'	1H,q	2.5 & 9.0	2.29	2.28	2.28	2.30
II-2',II-6'	2H,d	9.0	2.14	2.19	2.21	2.21

s= singlet, d= doublet, q= quartet.
Spectra run in $CDCl_3$ at 100MHz, TMS as an internal standard = τ10.0
* Synthetic.

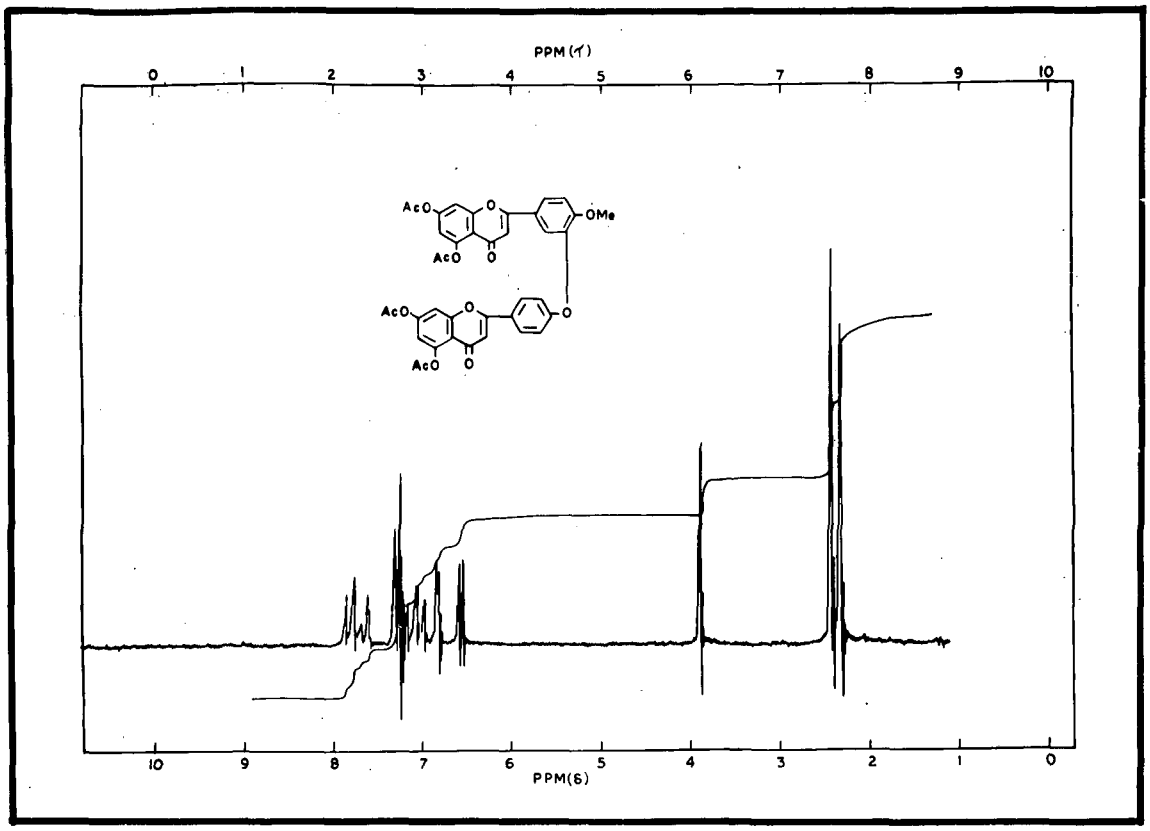

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
AcO
OMe
AcO
O
AcO
O
AcO
O
AcO
O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG.4

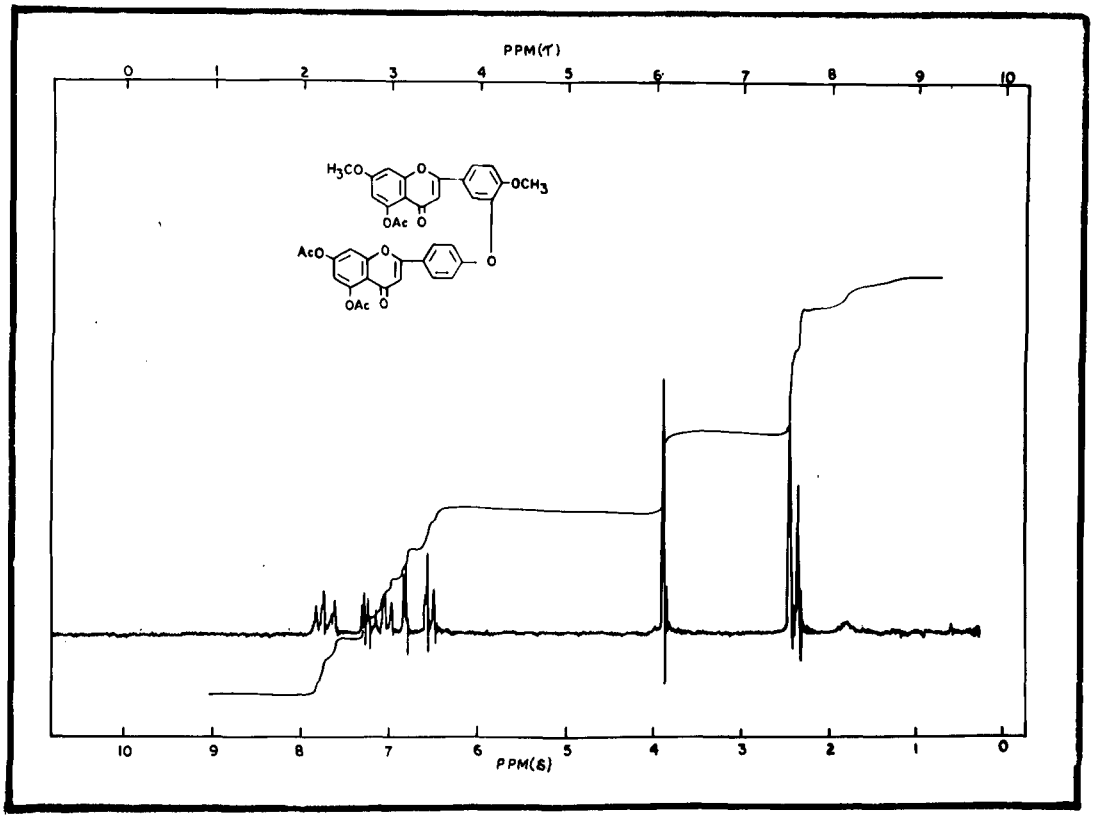

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
H3CO
OCH3
OAc
O
AcO
O
OAc
O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG.5

I-5,II-5,II-7-trihydroxy-I-4',I-7-Di-O-methyl (I-3'-O-II-4') biflavone (OSIII,IId):

Compound	m.p.	R_f
OSM(methyl ether)	167.169°	0.44
OSIIIA(acetate)	212.214°	-

Methylation of OSIII also gave the same methyl ether (OSM) as obtained in case of OSI and OSII.

The NMR spectra of OSIIIA showed the presence of three acetoxy and two methoxy groups (Fig.5). The positions of methoxy groups were assigned by a comparison with the NMR spectra of OSIA, OSIIA. The NMR signals (Table-IV) clearly showed that one of the methoxy groups was attached to position I-4' and the other might be at the positions I-7 or II-7, The chemical shift changes of H-I-6 or H-II-6(τ3.18 $\rightarrow$ τ3.42) and H-I-8 or H-II-8(τ2.71 $\rightarrow$ τ3.18) for OSII to OSIII suggested the presence of Meo-I-7 or Meo-II-7, indicating the structure IId or IIj for OSIII. In order to decide the structure of OSIII deuteriomethylation[131] of OSIII was carried out for mass spectrometric studies which gave the data shown in table V.

TABLE -V.

OSIIIDM	OSM
617(M^+), $C_{35}H_{19}D_9O_{10}$	608(M^+), $C_{35}H_{28}O_{10}$
330, $C_{18}H_{12}D_3O_6$	327, $C_{18}H_{15}O_6$
314, $C_{18}H_{12}D_3O_5$	311, $C_{18}H_{15}O_5$
303, $C_{17}H_7D_6O_5$	297, $C_{17}H_{13}O_5$
287, $C_{17}H_7D_6O_4$	281, $C_{17}H_{13}O_4$

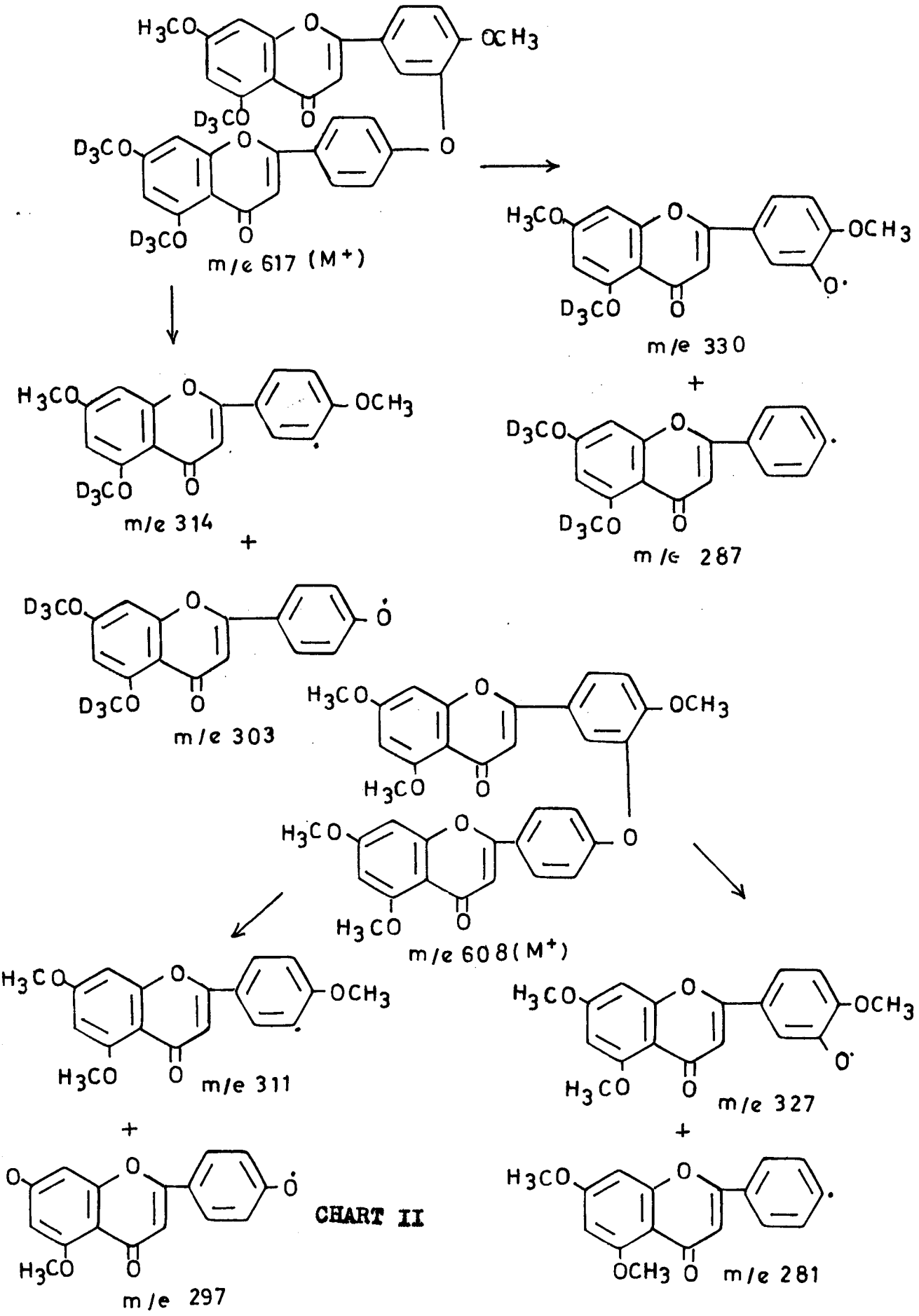

m/e 617 (M+)
m/e 330
m/e 314
m/e 287
m/e 303
m/e 608 (M+)
m/e 311
m/e 327
m/e 297
m/e 281

CHART II

The comparison of mass spectral data of deuteriummethylated product of OSIII(OSIIIDM) with that of penta-O-methylochnaflavone (OSM),(as shown in table V and chart II) indicated the position of deuteriomethyl groups at I-5,II-5 and II-7 positions in OSIII. The structure I-7,I-4'-Di-O-methylochnaflavone (IId) was therefore deduced for OSIII. This was further supported by a comparison of NMR spectra of the relevent units of OSIIIA(IIg) and ginkgetin triacetate [13c,28a].

I-5,II-5-Dihydroxy-I-4',I-7,II-7-tri-O-methyl(I-3'-O-II-4')biflavone (OSIV.IIh).

Compound	m.p.	R_f
OSIVM(methyl ether)	167.169°	0.44
OSIVA(acetate)	170-172°	—

A mixture of OSI,OSII & OSIII gave a major component of R_f value (BPF) 0.87 on methylation with diazomethane. This was separated from the resulted methylated product mixture by PLC using the solvent system BPF(36:9:5)[62] on silica gel (NCL poona) and was labelled as OSIV.

OSIV on methylation produced the same methyl ether (OSM) with dimethyl sulphate as in case of the above biflavone components. The NMR spectrum (Fig-6) of OSIVA showed the presence of two acetoxy, three methoxy groups. The positions of different protons, methoxy and acetoxy groups were assigned by comparing the NMR spectra of OSIA,OSIIA,OSIIIA and OSIVA(tableIV). The structure I-4',I-7,II-7-Tri-O-methylochnaflavone(IIh) was subsequently deduced for OSIV.

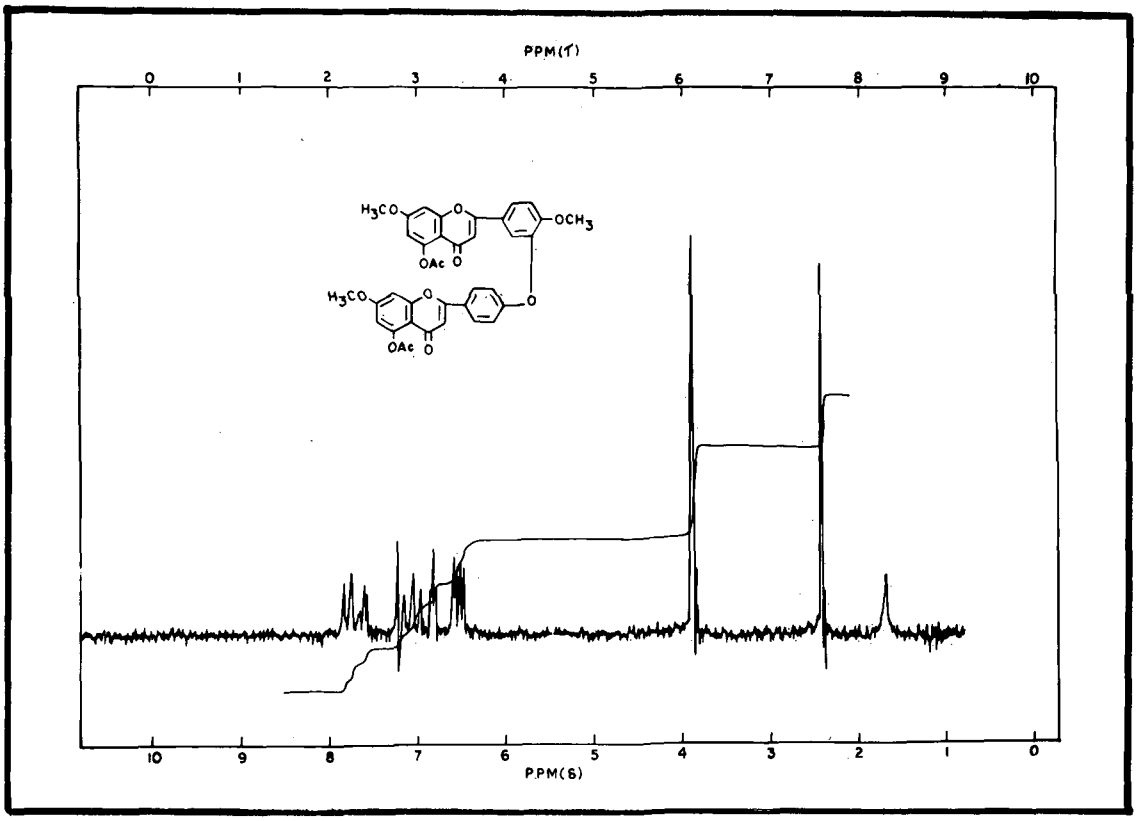

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
H3CO
OCH3
OAc O
H3CO
OAc O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG. 6

Although all types[128](amentoflavone, hinokiflavone, cupressuflavone, agathisflavone, and robustaflavone[41]) of naturally occurring biflavones so far isolated were found in gymnosperm, Ochnaflavone was found in angiosperm and it is noteworthy that the Ochnaflavone is a biflavanoid ether which has a flavone-flavone linkage between side phenyls with no relation with chromone ring, whereas the linkage of all other known biflavones is related with chromone ring of flavone nucleus.

BIFLAVONES FROM CEPHALOTAXUS HARRINGTONIA (FORBES).K.KOCH (CEPHALOTAXACEAE):

The genus Cephalotaxus (Cephalotaxaceae) consists of only seven species and nearly eleven varities. They are mostly evergreen trees or shurbs and are found in China, Japan, Korea, the Khasia hills and Assam. The wood, although used locally for various purposes is of no particular economic value. A fatty oil is obtained from the seeds of Cephalotaxus harringtonia and probably from those of other species.

The taxonomic position of Cephalotaxus is still debatable. Despite superficial resemblance to the Taxaceae, with which it is united by many authors, Cephalotaxus is regarded by others as the sole genus of a distinct family, the Cephalotaxaceae to be placed in the coniferales rather than the Taxales.

The debatable taxonomic position of Cephalotaxus stimulated the chemists for the chemical investigation of the plants of Cephalotaxaceae and Taxaceae.

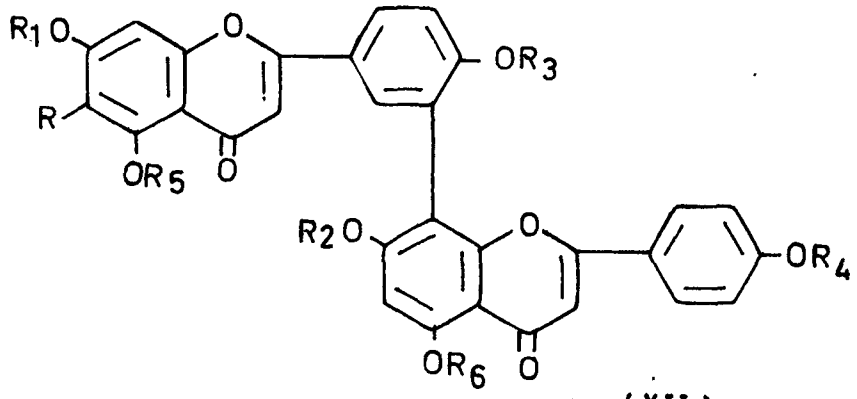
Cephalotaxus drupacea Sieb and Zuce and Cephalotaxus nana Nakai were reported to contain Kayaflavone[99](XIId) as the sole biflavone constituent along with apigenin-5-rhamnoglucosyl in the former.[129] Recently the isolation and characterization of sequoiaflavone (XIIb), ginkgetin(XIIe), Sciadopitysin(XIIf) and amentoflavone (XIIa)(in traces only) from the leaf extracts of C.drupacea[13c] was reported. Apigenin glycoside was isolated as one of the major components but it was not further investigated.

The present discussion is devoted to the investigations on biflavanoids from the leaf extracts of Cephalotaxus harringtonia (Forbes)K.Koch procured from the Forest Research Institute, Dehradun, U.P. India.

The phenolic extractives of the coarsely powdered leaves by solvent fractionation, column chromatography (silica gel,NCL Poona) followed by preparative layer chromatography(Silica gel, BDH) using the solvent system Benzene-Pyridine-Formic acid (BPF,36:9:5)[62] yielded four components, the usual colour reactions and UV spectra indicated them to be flavanoids. They were labelled as CHI,CHII,CHIII & CHIV.

The four components were comparable (TLC) to those isolated earlier[13c]. One of the chromatographically homogeneous fractions CHII on methylation using methyl sulphate-potassium carbonate-acetone mixture followed by TLC examination revealed the presence of a mixture of hexa-O-methylamentoflavone and a new biflavone methyl ether. The fraction CHII was, therefore, subjected to repeated P.L.C. on silica gel using the same solvent system. This resulted in the separation of two components, which were labelled CHIIa & CHIIb.

CHIIa and CHIIb were characterized as I-7-O-methylamento-flavone (sequoiaflavone,XIIb) and I-6-C-methyl-I-7-O-methylamento-flavone (Cephalotaxoflavone XIIC), respectively by spectral studies of their acetates and methyl ethers.



(XII)

(a) $R_1=R_2=R_3=R_4=R_5=R_6=R=H$

(b) $R_1=CH_3$; $R_2=R_3=R_4=R_5=R_6=R=H$

(c) $R_1=R=CH_3$; $R_2=R_3=R_4=R_5=R_6=H$

(d) $R_2=R_3=R_4=CH_3$; $R_1=R_5=R_6=R=H$

(e) $R_1=R_3=CH_3$; $R_2=R_4=R_5=R_6=R=H$

(f) $R_1=R_3=R_4=CH_3$; $R_2=R_5=R_6=R=H$

(g) $R_1=R_2=R_3=R_4R=_5R_6=CH_3$; $R=H$

(h) $R_1=CH_3$; $R_2=R_3=R_4=R_5=R_6=-\overset{O}{\overset{\|}{C}}-CH_3$; $R=H$

(i) $R=R_1=R_2=R_3=R_4=R_5=R_6=CH_3$

(j) $R=R_1=CH_3$; $R_2=R_3=R_4=R_5=R_6=-\overset{O}{\overset{\|}{C}}-CH_3$

(k) $R_2=CH_3$; $R_1=R_3=R_4=R_5=R_6=R=H$

(l) $R_2=CH_3$; $R_1=R_3=R_4=R_5=R_6=-\underset{O}{\overset{\|}{C}}-\underset{3}{CH}$; $R=H$

I-4',II-4',I-5,II-5,II-7-Pentahydroxy -I-7-O-methyl(I-3',II-8) biflavone (CHIIa):

Compound	m.p.	R_f value	MolWt.(M^+)
CHIIa(parent)	300-302°	0.37	552
CHIIaM(methyl ether)	223-225°	0.40	622
CHIIaA(acetate)	260-262°	-	762

TLC examination of CHIIa and its complete methyl ether and NMR spectra of its acetate and methyl derivatives, indicated CHIIa to be a monomethyl ether of amentoflavone. The results of NMR studies of CHIIa acetate, amentoflavone hexaacetate and hexamethyl ether are given in table VI.

On the basis of the NMR values of H-I-6 and H-I-8 in the three compounds, CHIIa was assigned the structure of I-4',II-4',I-5,II-5,II-7-pentahydroxy-I-7-O-methyl(I-3',II-8) biflavone (sequoiaflavone, XIIb).

TABLE VI

Chemical shifts of Protons (τScale) of CHIIa acetate amentofflavone hexaacetate and amentoflavone hexamethyl ether.

Assigned Positions	CHIIaA (acetate)	Amentoflavone hexaacetate.	Amentoflavone hexamethyl ether
H-I-2'	1.96(d,1H) J=3Hz	1.94(d,1H) J=3Hz	2.16(d,1H) J=3Hz
H-I-6'	2.08(q,1H) J_1=9Hz J_2=3Hz	1.99(q,1H) J_1=9Hz J_2=3Hz	2.10(q,1H) J_1=9Hz J_2=3Hz
H-II-2',II-6'	2.50(d,2H) J=9Hz	2.50(d,2H) J=8Hz	2.63(d,2H) J=9Hz
H-I-5'	2.52(d,1H) J=9Hz	2.52(d,1H) J=9Hz	2.88(d,1H) J=9Hz
H-II-6	2.92(s,1H)	2.97(s,1H)	3.38(s,1H)
H-II-3',II-5'	3.00(d,2H) J=9Hz	2.94(d,2H) J=9Hz	3.24(d,2H) J=9Hz
H-I-8	3.20(d,1H) J=3Hz	2.74(d,1H) J=3Hz	3.52(d,1H) J=3Hz
H-I-3,II-3	3.34,3.36(s,2H)	3.30,3.32(s,2H)	3.42,3.48(s,2H)
H-I-6	3.41(d,1H) J=3Hz	3.13(d,1H) J=3Hz	3.66(d,1H) J=3Hz
I-4',II-4'	7.96,7.74(s,6H)	7.99,7.77(s,6H)	(6.24),(6.26)(s,6H)
I-5,II-5	7.52,7.56(s,6H)	7.55,7.59(s,6H)	(6.07),(5.94)(s,6H)
I-7,II-7	(6.16),7.92(s,3H) each	7.72,7.95(s,6H)	(6.17),(6.12)(s,6H)

s=singlet,d=doublet,q=quartet.

Spectra run in $CDCl_3$ at 100MHz,TMS as internal standard=τ10.00

Figures in parenthesis show chemical shifts of methoxy protons.

I-6-C-methyl-I-4',II-4',I-5,II-5,II-7-pentahydroxy-I-7-O-methyl (I-3',II-8) biflavone (CHIIb):

Compound	m.p.	R_f value	Mol.Wt.(M^+)
CHIIb (parent)	above 340°	0.39	566
CHIIbM(methyl ether)	234-236°	0.45	636
CHIIbA(acetate)	250-252°	___	774

The mass spectrum of CHIIbM showed the molecular ion peak at M^+636.2007 and base peak at m/e 621. The NMR spectrum CHIIbM shoed the presence of six methoxy and one C-methyl groups (Fig.-7). The aromatic proton signals were found almost similar to those of hexo-O-methylamentoflavone (XIIg) except a singlet at τ3.33 instead of two meta coupled doublets (XIIg) due to H-I-6 and H-I-8 at τ3.66 and τ3.52 respectively, indicating I-6-or I-8-C-methylamentoflavone hexamethyl ether for CHIIbM (table VII).

Recently it was reported[73] that the NMR signals of H-I-6 and H-II-6 of methylated biflavones on adding Eu(fod)3, showed considerable downfield shifts (large-S-values) in comparison with those of H-I-3,II-3,H-I-8 and H-II-8. In order to establish the S-values of C-methyl protons at I-6-or I-8-positions of flavone nucleus, lanthanide-induced shift (LIS) studies were carried out with I-6-and I-8-C-methyl derivatives of tri-O-methylapigenin, showing a large difference in S-values between Me-I-6(5.04 ppm) and Me-I-8(0.80 ppm). This observation provided a method of distinguishing between I-6-and I-8-C-methyl groups in flavone nucleus. The techniques using $Eu(fod)_3$ were extended to CHIIbM (XIIi) giving the results shown in the table VIII. Although the

TABLE VII

Chemical shifts of protons (τ Scale)CHIIbA(XIIj), I-7-O-methylamentoflavone pentaacetate (XIIh) and II-7-O-methylamentoflavone pentaacetate (XIIl).

Assignment	CHIIbA(XIIj)	(XIIh)	(XIIl)
H-I-$2'$	1.94(d,1H) J=3Hz	1.96(d,1H) J=3Hz	1.99(d,1H) J=3Hz
H-I-$6'$	2.06(q,1H) J_1=9Hz J_2=3Hz	2.08(q,1H) J_1=9Hz J_2=3Hz	1.99(q,1H) J_1=9Hz J_2=3Hz
H-II-$2'$,II-$6'$	2.50(d,2H) J=8Hz	2.50(d,2H) J=8Hz	2.48(d,2H) J=8Hz
H-I-$5'$	2.56(d,1H) J=9Hz	2.52(d,1H) J=9Hz	2.53(d,1H) J=9Hz
H-II-$3'$,II-$5'$	2.97(d,2H) J=9Hz	2.98(d,2H) J=9Hz	2.95(d,2H) J=9Hz
H-II-6	3.00(s,1H)	3.01(s,1H)	3.25(s,1H)
H-I-8	3.24(s,1H)	3.22(d,1H) J=3Hz	2.75(d,1H) J=3Hz
H-I-3,II-3	3.35(s,2H)	3.34,3.36(s,2H)	3.33,3.41(s,2H)
I-6	(7.91)(s,3H)	3.41(d,1H) J=3Hz	3.16(d,1H) J=3Hz
I-$4'$,II-$4'$	7.95,7.73 (s,3H each)	7.96,7.74 (s,3H each)	7.62,7.68 (s,3H each)
I-5,II-5	7.51,7.51 (s,6H)	7.52,7.56 (s,3H each)	7.44,7.50 (s,3H each)
I-7,II-7	(6.13),7.91 (s,3H each)	(6.16),7.92 (s,3H each)	7.95(6.09) (s,3H each)

s= singlet, d= doublet, q= quartet

Spectra run in $CDCl_3$ at 100MHz,TMS as internal standard = τ10.00

Figures in parenthesis show chemical shifts of methoxy protons.

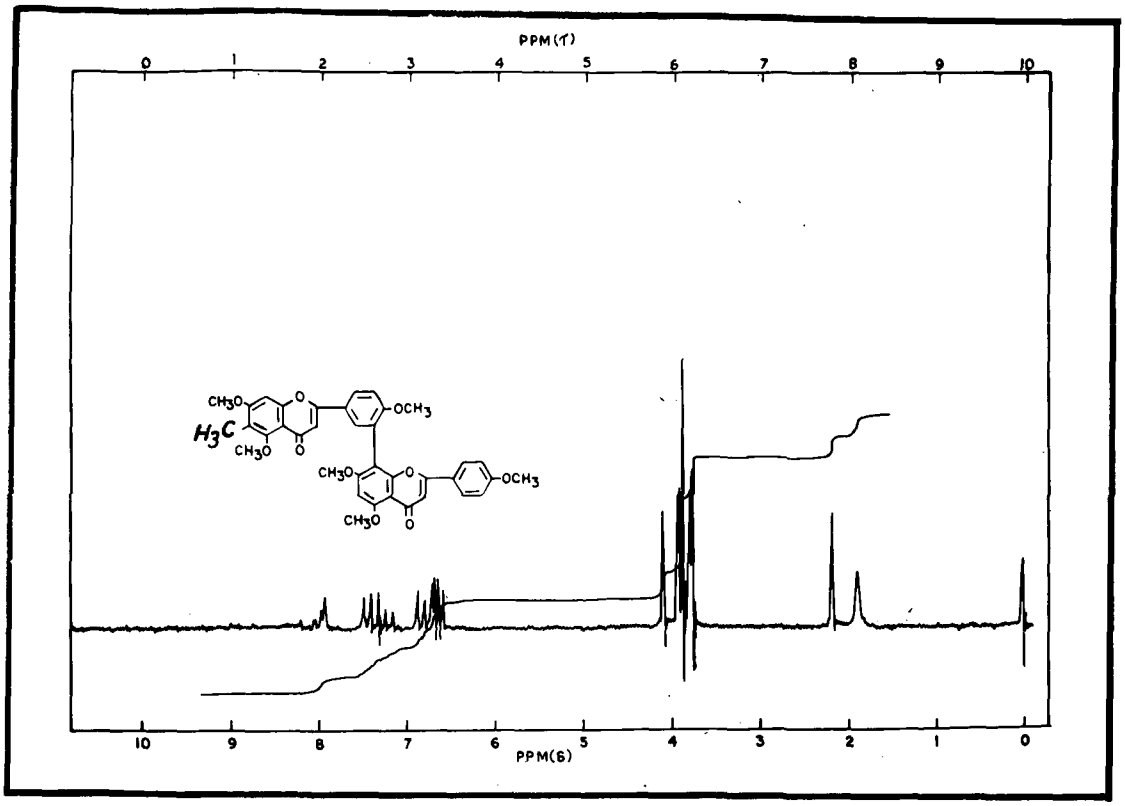

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
CH3O
H3C
CH3O
OCH3
CH3O
CH3O
OCH3
O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG. 7

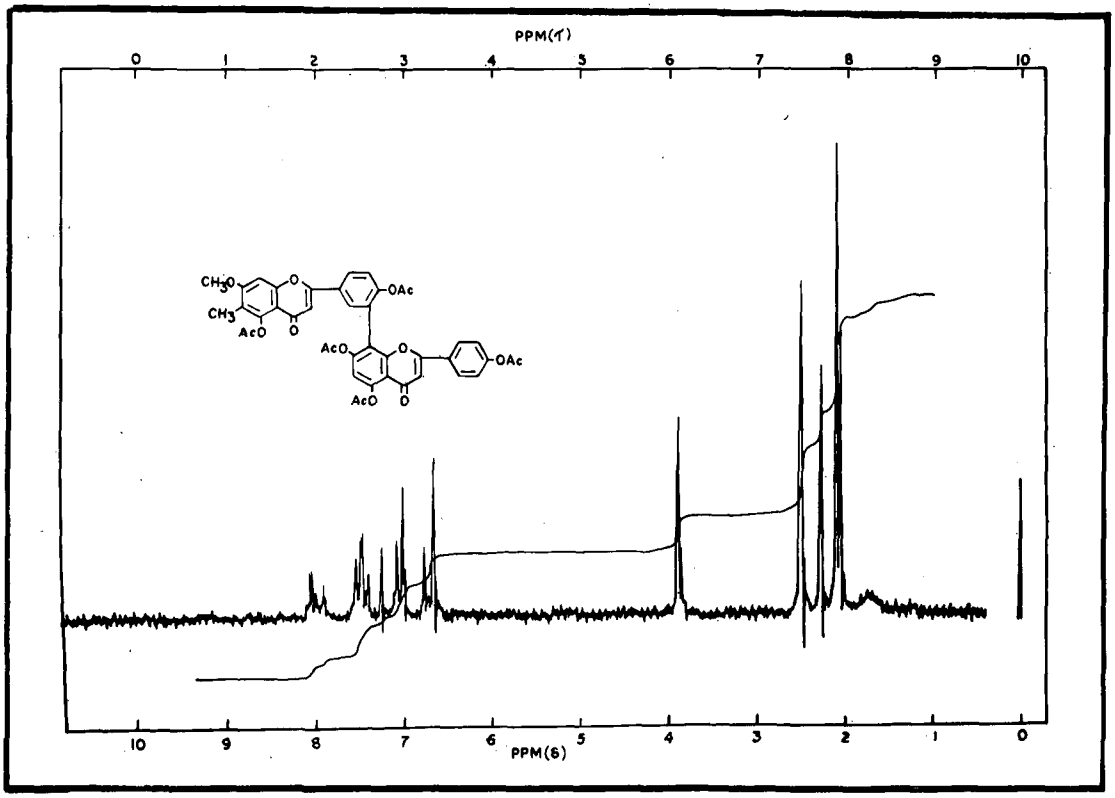

PPM(τ)
0 1 2 3 4 5 6 7 8 9 10
CH3O
CH3
AcO
AcO
OAc
AcO
OAc
O
10 9 8 7 6 5 4 3 2 1 0
PPM(δ)

FIG. 8

TABLE VIII

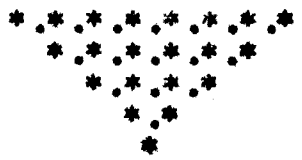
NMR data of I-6-C-Methylamentoflavone Hexamethyl ether (XIIi) and Amentoflavone Hexamethyl ether (XIIg).

Assigned position.	(ppm) (XIIi)	S-values by $Eu(fod)_3$ (XIIi)	(ppm) (XIIg)	S-values by $Eu(fod)_3$ (XIIg)
MeO-I-5	6.13(3H,s)	5.76	6.07	6.12
-II-5	5.89	9.36	5.95	8.78
-I-7	6.13	0.22	6.17	0.36
-II-7	6.08	1.20	6.12	1.06
-I-4'	6.20	0.20	6.24	0.12
-II-4'	6.22	-0.20	6.26	-0.08
Me or H-I-6	7.82 (3H,s)	2.20	3.66	2.76
H-I-3	3.40 (1H,s)	-0.20	3.42	0.02
-II-3	3.33	-0.20	3.48	-0.16
-II-6	3.26	4.98	3.38	4.24
- I-8	3.29	0.56	3.52	0.50

S-value (2.20 ppm) of methyl signal of CHIIbM(XIIi) was much smaller than that (5.04 ppm) of 6-C-methylapigenin trimethyl ether it was reasonable to propose I-6-C-methyl structure (XIIc) for CHIIb because about a half amount of used reagent was effective to each flavone nucleus of CHIIbM when the same molar ratio of the reagent was used.

The NMR spectrum of acetate CHIIb(Fig-8) showed the presence of one methoxy, one C-methyl and five acetoxy groups. In comparison of τ values of aromatic protons (table VII), especially H-II-6 (τ3.00 and H-I-8 (τ3.24) CHIIbA with those of two related

compounds,[130] I-7-O-methylamentoflavone pentaacetate (XIIh) (τ3.01s & τ3.22d) and II-7-O-methylamentoflavone pentaacetate (XIIl) (τ3.25 & τ2.75d), compound (XIIh)found to be more similar to compound CHIIbA than (XIIl) suggesting that I-6-C-methyl-I-7-O-methylamentoflavone was preferable structure for CHIIb. It was also supported by NMR studies of a deuteriomethylated[131] product of CHIIb, revealing a sharp signal at τ6.13 assigned to Meo-1-7 by the S-values (table-VIII). Therefore, CHIIb was assigned the structure of I-6-C-methyl-I-4', II-4', I-5, II-7-pentahydroxy-I-7-O-methyl (I-3', II-8) biflavone (XIIc) and constitutes the first example of naturally occuring C-methyl biflavones.



C O N C L U S I O N S

C O N C L U S I O N S.

BIFLAVONES FROM THE LEAVES OF OCHNA SQUARROSA LINN (OCHNACEAE).

From the phenolic extractives of the leaves of Ochna squarrosa, three new biflavones have been isolated and characterized as their complete methyl ethers and acetate derivatives. The corresponding parant biflavones having the hitherto unknown interflavanoid linkage (I-3'-O-II-4') between the two apigenin units, has been named as "Ochnaflavone". The other two new biflavones are identified as a mono-and a dimethyl ether of Ochnaflavone. Further, the formation and characterization of a trimethyl ether of Ochnaflavone after the diazomethylation of previously isolated biflavones has also been carried out. This is shown as under:

i. Ochnaflavone
ii. Ochnaflavone monomethyl ether
iii. Ochnaflavone dimethyl ether
iv. Ochnaflavone trimethyl ether (Synthetic)

Ochnaflavone and its partial methyl ethers, thus constitute a second example of naturally occurring biflavones ether type biflavanoids, the first one being hinokiflavone.

BIFLAVONES FROM THE LEAVES OF CEPHALOTAXUS HARRINGTONIA (FORBES) K.KOCH(CEPHALOTAXACEAE).

The phenolic extractives of the fresh leaves of C.harringtonia have been examined. The biflavones isolated and characterized are detailed below:

*i. Amentoflavone
ii. Amentoflavone monomethyl ether (sequoiaflavone).

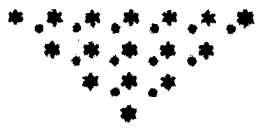
iii. I-6-C-methyl-I-7-O-methylamentoflavone (Cephalotaxoflavone), a new biflavone.

*iv. Amentoflavone dimethyl ether(Ginkgetin)

* v. Amentoflavone trimethyl ether,(Sciadopitysin).

I-6-C-methyl-I-7-O-methylamentoflavone constitutes the first report of the isolation and characterization of the naturally occurring C-methyl biflavones.

* *Those marked with astrisk are only detected (TLC).



EXPERIMENTAL

E X P E R I M E N T A L

All m.ps. were measured on a Kofler hot microscopical stage and are uncorrected. NMR spectra were recorded on Jeol 4H-100 instrument. Chemical shifts are expressed in values relative to TMS as internal standard. Mass spectra were obtained on JEOL-OISG double focus high resolution mass spectrometer with a direct inlet system and operating at an ionization energy of 75 eV. TLC and preparative TLC were carried out on silica gel (E.Merck), or silica gel (N.C.L.Poona) or silica gel (B.D.H.) using benzene-Pyridine-formic acid (BPF,36:9:5)[62] and toluene-ethyl formate-formic acid (TEF,5:4:1)[62]. All the reagents used were of 'ANALAR' or 'BDH' grade except formic acid (E.Merck).

Extraction of biflavones from the leaves of Ochna squarrosa Linn (Ochnaceae):-

Fresh leaves (10 Kg) procured from Horticulture Research Centre, Saharanpur, U.P.India and Forest Research Institute, Dehradun, U.P.,India, were completely exhausted with hot acetone and the acetone extracts were concentrated first at atmospheric pressure and then under diminished pressure.

A gummy dark green mass was obtained. This was refluxed with petroleum ether($40-60^{\circ}$), benzene and chloroform successively till the solvent in each case was almost colourless. The residue left behind was then treated with boiling water. The insoluble mass was dissolved in alcohol and dried under reduced pressure. A solid brownish residue (30 gms) thus obtained responded to usual flavanoid colour tests.

Purification of biflavone mixture-Column Chromatography:-

A well stirred suspension of silica gel (400 gms.) in dry petroleum ether (40-60°) was poured into a column. When the obsorbent was well settled, the excess petroleum ether was allowed to pass through the column. The crude biflavone mixture (30 gms.) was dissolved in dry acetone (150 ml.) and was adsorbed on silica gel (100 gms.) in a china dish. The excess solvent was allowed to evaporate untill a dry residue obtained. This adsorbed silica gel was added to the column. The column was eluted successively with petroleum ether (40-60°), benzene, chloroform, benzene ethylacetate (1:1, and 1:2), ethyl acetate and acetone. The last four fractions gave usual flavanoid colour tests; they were combined and the solvent distilled off to give yellowish brown residue (6.0 gms.).

Separation of biflavone mixture-Preparative thin layer chromatography:-

Using thin layer spreader (Desaga-Heidelberg), glass plates (20x20 Cm. and 40x20 Cm.) were coated with a well stirred suspension of silica gel (50 g) in water (95.0 ml.) to give a layer approximately 0.5 mm in thickness. After drying for 3 hours at room temprature, the plates were activated at 110-120° for 1 hour and preserved in a desiccator until required.

The complexity of the crude biflavone mixture obtained after purification by column chromatography was examined by TLC using the following systems.

a- Benzene-Pyridine-formic acid (36:9:5).[62]

b- Toluene-ethyl formate-formic acid(5:4:1)[62]

e- Toluene-Pyridine-acetic acid (10:1:1)

d- Benzene-ethylacetate-acetic acid (8:5:2)

e- Benzene-Pyridine-ethyl formate-dioxan (5:1:2:2)

In solvent system(a) , the biflavone mixture showed three compact brown spots in UV light, and the differences in R_f values were so marked as to make it the developing system of choice for quantitative separation.

Solution of the biflavone mixture (5%) in pyridine was applied to plates (40x20 cm) with the help of mechanical applicator (Desaga-Heidelberg) 2Cm from the lower edge of the plates. The plates mounted on a stainless steel frame were placed in a Desaga glass chamber (45x22x22 Cm.) containing 500 ml of the developing solvent (BPF,36:9:5). When the solvent front travelled 18 Cm. from starting line the development was interrupted and the plates were dried at room temprature. The zn positions of the bands were marked in UV light. The marked pigment zones were scraped with the help of a spatula and eluted in separate columns with dry acetone. The eluates were evaporated to give oily liquids which on addition of water yielded yellow precipitates in each case. The precipitates were filtered, washed with water and dried. Homogeneity of the pigments was again checked by TLC using five solvent systems already listed. The components were labelled as OSI(R_f0.56,1.0 gm), OSII(R_f0.72,2.0 gm) and OSIII(R_f0.81,0.4 gm).

Ochnaflavone...OSI(1g) was recrystallized from Pyridine-methanol to give yellow needles (0.7g),m.p.233-235°.

λ max$^{(EtOH)}$ 271 and 339 nm(logϵ 4.24 and 4.25).

ν max$^{(KBr)}$ 3100-3400,2940,1655,1612,1500 and 835 Cm^{-1}.

A mixture of OSI (50 mg), dimethyl sulphate (0.5 ml), anhydrous — Potassium carbonate (2 g) and dry acetone (150 ml.) was refluxed on a water bath for about 8 hours. A small portion of reaction mixture was taken out in a test tube and tested for alc.$Fecl_3$ reaction. Refluxing continued until it gave negative alc.$Fecl_3$ test. It was then filtered and the residue washed several times with hot acetone. The filtrate and washings were combined and evaporated to dryness. The yellow residue washed 2-3 times with petroleum ether and then taken up in chloroform (50 ml) and washed several times (i.e.till washings became neutral) with water. The chloroform solution dried over anhyd.Sodium sulphate, concentrated and purified on a silica gel column using chloroform as the eluent.

TLC examination of the methylated product revealed in UV light a compact flurescent spot different from the methyl ethers of the all biflavones.

I-4',I-5,II-5,I-7,II-7-Penta-O-methyl(I-3'-O-II-4') biflavone (OSIM).

A mixture of OSI (100 mg.), dimethyl sulphate (1.0 ml.), anhydrous potassium carbonate (5 g) and dry acetone (250 ml) was refluxed together for about 8 hours.

After usual work up and purification by silica gel column, the methylated product was crystallised from ethanol into colourless needles (60 mg), m.p.167-169°, mol.wt.608(mass) λmax (ethanol): 267 nm (log ϵ , 4.60) and 328 (4.65). $\nu_{max}^{(KBr)}$ 3010,2950,1738,1688, 1612 and 1500 Cm^{-1}.

$\tau(CDCl_3)$; 2.40(d,J=2.5 Hz,1H,H-I-$\dot{2}$); 2.89(d,J=9.0 Hz,1H,H-I-$\dot{5}$); 2.28(q,J_1=2.5 Hz & J_2=9.0Hz,H-I-$\dot{6}$);2.19(d,J=9.0Hz,2H,H-II-$\dot{2}$,H-II-$\dot{6}$); 2.99(d,J=9.0Hz,2H,H-II-$\dot{3}$,II-$\dot{5}$) 3.49(d,J=2.5Hz,1H,H-I-8); 3.46(d,J=2.5Hz,1H,H-II-8); 3.66(d,J=2.5Hz,1H,H-I-6); 3.66(d,J=2.5Hz,1H,H-II-6); 3.45(s,1H,H-I-3);3.41(s,1H,H-II-3); 6.12(s,3H,OCH_3-I-$\dot{4}$);6.12(s,3H,OCH_3-I-7);6.12(s,3H,OCH_3-II-7); 6.08(s,3H,OCH_3-I-5); 6.08(s,3H,OCH_3-II-5).

<u>I-$\dot{4}$,I-5,II-5,I-7,II-7-Pentaacetyl(I-$\dot{3}$-O-II-$\dot{4}$) biflavone (OSIA)</u>:

A mixture of OSI (100 mg), pyridine (1.0 ml) and Ac_2O (2.0 ml) was refluxed on a water bath for two hours. The mixture was cooled and poured onto crushed ice. The colourless solid was filtered off, washed with water and dried. It crystallized from ethylacetate-chloroform as colourless prisms (70 mg) m.p. 239-241°. λ max(ethanol) 256 and 312 nm (log ϵ 4.28 and 4.34). ν max (^{KBr})2930,1770,1648,1615, 1501 and 840 Cm^{-1}. $\tau(CDCl_3)$: 2.42(d,J=2.5Hz,1H,H-I-$\dot{2}$); 2.65(d,J=9.0Hz, 1H,H-I-$\dot{5}$);2.29(q,J_1=2.5Hz & J_2= 9.0Hz,1H,H-I-$\dot{6}$); 2.14(d,J=9.0Hz,2H, H-II-$\dot{2}$,II-$\dot{6}$);2.87(d,J=9.0Hz,2H,H-II-$\dot{3}$,II-$\dot{5}$); 3.38(s,1H,H-I-3); 3.43(s,1H,H-II-3); 3.17(d,J=2.5Hz,1H,H-I-6);3.16(d,J=2.5Hz,1H,H-II-6); 2.68(d,1H,J=2.5Hz,H-I-8);2.66(d,1H,J=2.5Hz,H-II-8);7.80(s,3H,OAc-I-$\dot{4}$); 7.68,7.65,7.58,7.56(s,3H each,OAc-I-7,II-7,I-5,II-5 respectively).

<u>I-4-O-Methylochnaflavone (OSII)</u> — Yellow needles (1.5 g)(from pyridine), m.p.297-299°. λ max$^{(EtOH)}$271.5 and 332 nm(logϵ4.24 and 4.42), ν max $^{(KBr)}$3360,3060,2930,1655,1615,1500,1357 and 1025 Cm^{-1}.

<u>I-$\dot{4}$,I-5,II-5,I-7,II-7-Penta-O-methyl(I-$\dot{3}$-O-II-$\dot{4}$) biflavone (OSIIM)</u>:

A mixture of OSII(300 mg), dimethyl sulphate (1.0 ml), dry acetone (400 ml) and anhydrous potassium carbonate (10.0 gms) was

refluxed on a water bath for 8 hours. After the methylation the mixture was filtered off and the residue was washed with dry acetone till the colour comes. The solvent was distilled off, the residue was recrystallized into colourless needles (220 mg) from ethanol. m.p. 167-169°, mol.wt. 608 (mass) $\lambda max^{(EtOH)}$: 267 nm logϵ 4.60) and 328 (4.65) $\nu max^{(KBr)}$ 2930, 1640, 1605, 1505, and 830 Cm^{-1}. $\tau(CDCl_3)$: 2.40(d, J=2.5Hz, 1H, H-I-$\dot{2}$); 2.89(d, J=9.0Hz, 1H, H-I-$\dot{5}$); 2.28(q, J_1=2.5Hz & J_2=9.0Hz, 1H, H-I-$\dot{6}$); 2.19(d, J=9.0Hz, 2H, H-II-$\dot{2}$, II-$\dot{6}$); 2.99(d, J=9.0Hz, 2H, H-II-$\dot{3}$, II-$\dot{5}$); 3.49(d, J=2.5Hz, 1H, H-I-8); 3.46(d, J=2.5Hz, 1H, H-II-8); 3.66(d, J=2.5Hz, 1H, H-I-6); 3.66(d, J=2.5Hz, 1H, H-II-6); 3.45(s, 1H, H-I-3); 3.41(s, 1H, H-II-3); 6.12, 6.12, 6.12, 6.08, 6.08(s, 3H each, OCH_3-I-$\dot{4}$, I-7, II-7, I-5, II-5 respectively).

<u>I-5, II-5, I-7, II-7-tetraacetyl-I-$\dot{4}$-O-methyl(I-$\dot{3}$-O-II-$\dot{4}$) biflavone (OSIIA)</u>:

A mixture of OSII(100mg), pyridine (1.0ml) and Ac_2O(2.0 ml). was refluxed on a water bath for two hours. The mixture was cooled and poured onto crushed ice. The colourless solid was filtered off, washed with water and dried. It crystallized from ethlacetate-chloroform as colourless prisms (67 mg) m.p. 248-250° $\lambda max^{(EtOH)}$ 22.5, 255 and 320 nm(logϵ 4.30, 4.22 and 4.34); $\nu max^{(KBr)}$ 2950, 1770, 1645, 1610 and 1504 Cm^{-1}. $\tau(CDCl_3)$: 2.40(d, J=2.5Hz, 1H, H-I-$\dot{2}$); 2.87(d, J=9.0Hz, 1H, H-I-$\dot{5}$); 2.28(q, J_1=2.5Hz & J_2= 9.0Hz, 1H, H-I-$\dot{6}$); 2.19(d, J=9Hz, 2H, H-II-$\dot{2}$, II-$\dot{6}$); 2.98(d, J=9.0Hz, 2H, H-II-$\dot{3}$, II-$\dot{5}$); 3.46(s, 1H, H-I-3); 3.42(s, 1H-II-3); 3.18(d, J=2,5Hz, 1H, H-I-6); 3.18(d, J=2.5Hz, 1H, H-I-8); 2.69(d, J=2.5Hz, 1H, H-II-8); 6.12(s, 3H, OCH_3-I-$\dot{4}$); 7.69, 7.67, 7.57, 7.57 (s, 3H each, OAc-I-7, II-7, I-5, II-5 respectively).

I-7,I-4'-Di-O-methylochnaflavone (OSIII)...Brownish yellow prisms (0.2g) (from Pyridine-methanol), m.p.288-290° λ max (EtOH). 271.5 and 332 nm (log ϵ 4.28 & 4.27), ν max (KBr) 3240,2940,1658, 1623,1603 and 1350 Cm^{-1}.

I-4',I-5,II-5,I-7,II-7-Penta-O-methyl (I-3'-O-II-4') biflavone(OSIIIM).

A mixture of OSIII (50 mg), dimethyl sulphate (1.0 ml), dry acetone (400 ml) and anhydrous potassium carbonate (10.0 g) was refluxed on a water bath for 8 hours. After the methylation the mixture was filtered and residue was washed with dry acetone till the colour comes. The solvent was distilled off, the residue was recrystallized into colourless needles (23 mg) from ethanol m.p. 167-169°, mol.wt. 608 (mass) λ max (EtOH) 267 nm (log ϵ, 4.60) and 328 (4.65) ν max (KBr) 2930,1640,1605,1505 and 830 Cm^{-1}. τ ($CDCl_3$): 2.40(d,J=2.5Hz,1H,H-I-2'); 2.89(d,J=9.0Hz,1H,H-I-5'); 2.28(q,J_1=2.5Hz & J_2=9.0Hz,H-I-6'); 2.19(d,J=9.0Hz,2H,H-II-2',II-6'); 2.99(d,J=9.0Hz,2H,H-II-3',II-5');3.49(d,J=2.5Hz, 1H,H-I-8); 3.46(d,J=2.5Hz,1H,H-II-8); 3.66(d,J=2.5Hz,1H,H-I-6); 3.66(d,J=2.5Hz,1H,H-II-6); 3.45(s,1H,H-I-3); 3.14(s,1H,H-II-3); 6.12,6.12,6.12,6.08,6.08(s,3H each,OCH_3-I-4',I-7,II-7,I-5,II-5 respectively).

I-5,II-5,II-7-Triacetyl-I-4',I-7-O-methyl (I-3'-O-II-4') biflavone (OSIIIA):

A mixture of OSIII(100 mg), Pyridine (1.0 ml) and Ac_2O(2.0 ml) was refluxed on a water bath for two hours. The mixture was cooled and poured onto crushed ice. The colourless solid was filtered off, washed with water and dried. It crystallized from ethylacetate as colourless needles (46 mg) m.p. 212-214°. λ max (EtOH) 260 and

329 nm(log 4.30 and 4.31); $\nu_{max}^{(KBr)}$ 2930,1730,1658,1603 and 828 cm^{-1}. $\tau(CDCl_3)$: 2.39(d,J≈2.5Hz,1H,H-I-2'); 2.88(d,J=9.0Hz, 1H,H-I-5'); 2.28(q,J_1=2.5Hz & J_2=9.0Hz,1H,H-I-6'); 2.21(d,J=9.0Hz,2H,H-II-2',II-6'); 2.99(d,J=9.0Hz,2H,H-II-3',II-5'); 3.51(s,1H,H-I-3); 3.43(s,1H,H-II-3); 3.42(d,J=2.5Hz,1H,H-I-6); 3.18(d,J=2.5Hz,1H,H-II-6); 6.12(s,3H,OCH_3-I-4'); 6.12(s,3H,OCH_3-I-7) 7.66,7.57,7.57(s,3H each OAc-I-5,II-5,II-7 resepectively).

Deuteriomethylation of OSIII.. The ether solution (10.0 ml) of diazo methane prepared from nitrosomethlurea (5g) was mixed with dioxane (15 ml) and D_2O (1.0 ml) and kept for 3 hours under ice-cooling. OSIII (10 mg) was dissolved in dioxane (10.0ml), mixed with two drops of D_2O (99%, Merck), and added to the above dioxane-ether solution. After two days the solvent was distilled off and the residue was purified by PLC (silica gel G nach Stahl, Merck and chloroform-methanol 97:3). The band corresponding to penta-O-methylochnaflavone was collected to give colourless needles (4 mg) (from ethanol), m.p.160-165°, mass spectral data obtained as: 617.2229(M^+)($C_{35}H_{19}D_9O_{10}$); 330($C_{18}H_{12}D_3O_6$); 314($C_{18}H_{12}D_3O_5$); 303($C_{17}H_7D_6O_5$); 287($C_{17}H_7D_6O_4$).

I-5,II-5-Dihydroxy-I-4',I-7,II-7-O-methyl (I-3'-O-II-4') biflavone (OSIV):

A biflavone mixture (400 mg) of OSI,OSII and OSIII obtained from benzene-ethyl acetate eluate was mixed with methanol (5.0 ml) and excess of diazomethane etheral solution (50.0 ml) and kept in an ice box for 12 hour. The solvent was evaporated to give a reaction mixture, which showed the presence of one major (R_f 0.87) and three minor components (R_f 0.81,0.72 and 0.56) on TLC

(NCL,BPF). The major component (OSIV) was obtained by PLC as brown plates (150 mg) (from methanol), m.p.250-252°. On methylation with dimethyl sulphate by the same way with the case of OSM,OSIV gave the same penta-O-methylochnaflvaonve.

I-5,II-5-Diacetyl-I-4',I-7,II-7-O-methyl (I-3'-O-II-4') biflavone (OSIVA):

A mixture of OSIV (100 mg), Pyridine (1.0 ml) and Ac_2O (2.0 ml) was refluxed on a water bath for two hours. The mixture was cooled and poured onto crushed ice. After usual work up the solid was recrystallized from ethylacetate—chloroform as colour-less plates (60 mg)m.p. 170-172° $\tau(CDCl_3)$: 2.41(d,J=2.5Hz, 1H, H-I-2'); 2.90(d,J=9.0Hz,1H,H-I-5'); 2.30(q,J_1=2.5Hz & $J_2$9.0Hz, 1H,H-I-6'); 2.21(d,2H,J=9.0Hz,H-II-2',II-6'); 2.99(d,J=9.0Hz,2H,H-II-3', II-5');3.53(s,1H,H-I-3);3.49(s,1H,H-II-3);3.43(d,1H,J=2.5Hz,H-I-8); 3.17(d,J=2.5Hz,1H,H-II-8); 6.13,6.12(s,3H each, OCH_3-I-4',I-7,II-7 respectively); 7.58(s,6H,OAc-I-5 & II-5).

Di-(2-acetyl-3,5-dimethoxyphenyl)2-methoxy -1,1'-biphenyl ether-4',5-dicarboxylate (VII):

2-Methoxy -1,1'-biphenyl ether-4',5-dicarboxylic acid(VI, 2.93) and thionyl chloride (5.0 ml) were mixed and refluxed for about 1.5 hour. Excess of thionyl chloride was distilled off under reduced pressure to give brownish viscous residue, which was mixed with 2-hydroxy-4,6-dimethoxyacetophenone (4g) and pyridine (20.0 ml) and kept for 15 minutes in an oil bath at 100°. The cooled reaction mixture was poured into ice water and extracted with chloroform (100 ml) two times. The chloroform layer

was combined, washed with 10% hydrochloric acid and water, dried and evaporated under reduced pressure. The reaction mixture was chromatographed on silica gel(30 g). The fraction eluted with chloroform-methanol (98:2) gave non-crystalline ester (VII,3.2g). $\tau(CDCl_3)$: 7.55,6.22, and 6.17(6H,s each),6.14(3h,s), 3.68 and 3.61(2H, d each, J=2.5Hz), 3.05(2H,d,J=9Hz), 2.92(1H,d, J=9Hz),2.14(1H,d,J=2.5Hz), 1.94(1H,q,J_1=2.5Hz and J_2=9Hz) and 1.92(2H,d,J=9Hz).

4',5-Bis-(2''-hydroxy-4'',6''-dimethoxybenzoylacetyl)-2-methoxy-1,1'-biphenyl ether (VIII):

The above biphenyl ether (VII) (1g) dissolved in pyridine (5ml) was well mixed with powdered potassium hydroxide (1g) and kept for 10 minutes at 120°. The cooled mixture was poured into ice-water and extracted with chloroform. The chloroform layer was washed with 10% hydrochloric acid and water and evaporated under reduced pressure to give yellow crystals (VIII) (650 mg) (from chloroform-methanol), m.p.123-129°.

Penta-O-methylochnaflavone (IIb):

The biphenyl ether (VIII) (500 mg) dissolved in acetic acid (5 ml) was added to a mixture (0.5 ml) of Conc.sulphuric acid and acetic acid (1:4 w/w) and kept for 20 minutes on a steam bath. The mixture was poured into ice-water to yield a precipitate which was collected, w ashed with water, and purified by PLC te give colourless needles (280 mg) (from ethanol), m.p.169-171°. No depression on admixture with penta-O-methyl-Ochnaflavone (OSM). $\tau(CDCl_3)$: 2.40(d,J=2.5Hz,1H,H-I-2'); 2.89(d,J=9.0Hz,1H,H-I-5');

2.30 (q,J_1=2.5Hz & J_2=9.0Hz,H-I-6'); 2.19 (d,J=9.0Hz,2H,H-II-2',II-6'); 2.99(d,J=9.0Hz,2H,H-II-3',II-5'); 3.49(d,J=2.5Hz,1H,H-I-8); 3.46(d,J=2.5Hz,1H,H-II-8); 3.66(d,J=2.5Hz,1H,H-I-6); 3.66(d,J=2.5Hz,1H,H-II-6); 3.45(s,1H,H-I-3); 3.14(s,1H,H-II-3); 6.12,(s,9H,OCH_3-I-4',I-7,II-7); 6.08(s,6H,OCH_3-I-5 & II- 5).

Di-(2-acetyl-3,5-dimethoxyphenyl)2-methoxy-1,1'-biphenyl ether-4,4'-dicarboxylate (X):-

2-Methoxy-1,1'-biphenyl ether -4,4'-dicarboxylic acid (IX) (1.2g) and thionyl chloride (3.0 ml) were refluxed for 3 hours. After excess of thionyl chloride was removed off under diminished pressure the resulting acid chloride was mixed with pyridine (10.0 ml) and 2-hydroxy-4,6-dimethoxyacetophenone (1.7g) and kept for 15 minutes in an oil bath at 100°. The cooled mixture was treated as described in the case of compound (VII) to give non-crystalline ester (X)(2.3g) $\tau(CDCl_3)$:
7.57 &7.54(3H,s each); 6.09-6.03(15H); 3.55-3.49(4H);2.89(2H, d,J=9.0Hz); 2.98(1H,d,J=9.0Hz);2.12(1H,d,J=2.5Hz); 2.10(1H,q,J_1=2.5Hz & J_2=9.0Hz); and 1.80(2H,d,J=9Hz).

4,4'-Bis-(2"-hydroxy-4",6"-dimethoxybenzoylacetyl)-2-methoxy-1,1'-biphenyl ether (XI):-

To the biphenyl ether (X) (1,5 g) dissolved in pyridine (10 ml) was added powdered potassium hydroxide (1.5 g) and kept for 10 min. In an oil bath at 120°. The cooled mixture was poured into ice-water (30 ml), acidified with hydrochloric acid, and extracted with chloroform. The chloroform layer was washed with water, dried and evaporated under diminished pressure to give a resinous substance, which was chromatographed on silica gel. A fraction

eluted with chloroform-methanol (95.5) gave yellow crystals (XI) 1.2g), m.p. 130-135°(from chloroform-methanol).

<u>I-3',I-5,II-5,I-7,II-7-Penta-O-methyl(I-4'-O-II-4') biflavone (IIIb)</u>: The above ether (500 mg) dissolved in acetic acid (5 ml) was added to a mixture (1 ml) of conç.sulphuric acid and acetic acid (1:4 w/w) and kept for 20 min. at 100°. After cooling the reaction mixture was poured into ice-water to afford a precipitate, which was collected, washed with water, and purified by column chromatography on silica gel. A fraction eluted with chloroform-methanol (98:2) mixture gave colourless needles, m.p. 154-156° (310 mg) (from chloroform-methanol). $\tau(CDCl_3)$: 2.59(d,J=2.5Hz,1H, H-I-2'); 2.91(d,J=9.0Hz,1H,H-I-5'); 2.54(q,J_1=2.5Hz &J_2=9.0Hz,1H,H-I-6') 2.21(d,J=9.0Hz,2H,H-II-2',II-6'); 2.99(d,J=9.0Hz,2H,H-II-3',II-5'); 3.47(s,1H,H-I-3); 3.41(s,1H,H-II-3); 3.53(d,J=2.5Hz,1H,H-I-8); 3.50(d,J=2.5Hz,1H,H-II-8); 3.70(d,J=2.5Hz,1H-H-I-6); 3.70(d,J=2.5Hz,1H,H-II-6); 6.20(s,3H,OCH_3-I-3'); 6.17(s,12H,OCH_3-I-5,II-5,I-7,II-7).

BIFLAVONES FROM CEPHALOTAXUS SPECIES:

Extraction of biflavanoids from the leaves of Cephalotaxus harringtonia K.Koch.

Fresh leaves (10 kg.), procured from Forest Research Institute, Dehradun U.P., India , were exhausted with hot acetone and the solvent distilled off. The dark green concentrate was dried under reduced pressure and then successively treated with petrolium ether (40-60°), benzene, chloroform and hot water to remove non-flavanoidic and resinous matter. The residue was then refluxed with ethylacetate for 8 hours, filtered and the filtrate concentrated. The dark coloured residue was purified on a silica gel coloumn eluting successively with petrolium ether (40-50°), benzene, chloroform and benzene-ethyl acetate (1:1 and 1:2). The last two fractions gave usual flavanoidic colour tests; they were combined and the solvent distilled off to give yellowish brown residue (5.0 gms).

TLC examination of the crude biflavone mixture in BPF (36:9:5)[62] revealed four brown spots in UV light. It was, therefore, subjected to preparative layer chromatography and the four bands were separated and labelled as CHI,CHII,CHIII and CHIV.

CHI:

It was separated from the whole residue obtained but was not enough to prepare all the derivatives so was methylated using dimethyl sulphate and anhydrous potassium carbonate as described earlier. TLC examination of the methylated product

showed only one spot in U.V.light, corresponding to hexamethyl ether of amentoflavone.

CHII:

This fraction on methylation using methyl sulphate-potassium Carbonate-acetone mixture followed by TLC examination revealed the presence of a mixture of hexa-O-methylamentoflavone and a new biflavone methyl ether. The fraction CHII was, therefore, subjected to repeated PLC on silica gel using the same solvent system. This resulted in the separation of two components, which were labelled as CHIIa & CHIIb.

I-4',II-4',I-5,II-5,I-7,II-7-Hexa-O-methyl(I-3',II-8)biflavone (CHIIaM):

A mixture of CHIIa (100 mg), dimethyl sulphate (1.0 ml), anhydrous, potassium carbonate (5 g) and dry acetone (400 ml) was refluxed on a water bath for about 8 hours. After usual work up purification by silica gel coloumn, the product was crystallized from chloroform-methanol as colourless needles(60 mg). m.p.170-171°. Mol.wt.622(mass). $\tau(CDCl_3)$: 2.13-2.30 (m,2H,H-I-2', I-6'); 2.96(d,J=9.0Hz,1H,H-I-5'); 2.72(d,J=9.0Hz,2H,H-II-2',II-6'); 3.32(d,J=9.0Hz,2H,H-II-3',II-5'); 3.74(d,J=3.0Hz,1H,H-I-6); 3.59(d,J=3Hz,1H,H-I-8);3.47,3.55(s,1H each, H-I-3,II-3); 3.41(s,1H,H-II-6); 5.95,6.11,6.18,6.24,6.28,6.29(s,3H each,OCH_3-II-5,I-5,I-7,II-7,I-4',II-4' respectively).

I-4',II-4',I-5,II-5,II-7-pentaacetyl-I-7-O-methyl(I-3',II-8) biflavone (CHIIa acetate):

A mixture of CHII a (100 mg) , pyridine (1.0 ml) and Ac_2O

(2.0 ml) was refluxed on a water bath for about 2 hours, cooled and poured onto crushed ice. The spparated solid was filtered, washed with water and dried. It was recrystallized from chloroform-methanol as colourless needles (70 mg).m.p. 260-62°.

$\tau(CDCl_3)$; 1.96(q,J_1=9Hz,J_2=3Hz,1H,H-I-6'); 2.08(d,J=3Hz,1H,H-I-2'); 2.52(d,J=9Hz,2H,H-II-2',II-6'); 2.68(d,J=9Hz,1H,H-I-5'); 2.92(s,1H,H-II-6); 3.0(d,J=9Hz,2H,H-II-3',II-5'); 3.20(d,J=3Hz,1H, H-I-8); 3.36(s,2H,H-I-3,II-3); 3.40(d,J=3Hz,1H,H-I-6); 6.16(s,3H,OCH_3-I-7); 7.52(s,3H,OAc-II-5); 7.56(s,3H,OAc-I-5); 7.74 (s,3H,OAc-II-4'); 7.92(s,3H,OAc-II-7); 7.96(s,3H,OAc-I-4')

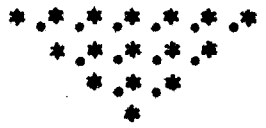
<u>I-6-C-methyl-I-4',II-4',I-5,II-5,I-7,II-7-Hexa-O-methyl(I-3',II-8) biflavone (CHIIbM)</u>:

A mixture of CHIIb (100 mg), dimethyl sulphate (1.0 ml), anhydrous potassium carbonate (5 g) and dry acetone (400 ml) was refluxed on a water bath for about 8 hours. After usual work up and purification by silica gel coloumn, the product was crystallized from chloroform-methanol as colourless needles (60 mg)m.p.234-236°, mol.wt. 636 (mass); $\tau(CDCl_3)$; 1.97(q,J_1= 9Hz & J_2=3Hz, 1H,H-I-6'); 2.06(d,J=3Hz,1H,H-I-2'); 2.53(d,J=9Hz,2H,H-II-2',II-6'); 2.78(d,J=9Hz,1H,H-I-5'); 3.16(d,J=9Hz,2H,H-II-3',II-5'); 3.26(s,1H,H-I-8); 3.29(s,1H,H-II-6); 3.34(s,1H,H-II-3); 3.40(s,1H,H-I-3); 5.89(s,3H,OCH_3-II-5); 6.07(s,3H,OCH_3-I-5); 6.11(s,6H,OCH_3-II-4',I-7); 6.80(s,3H,OCH_3-II-7); 6.22(s,3H,OCH_3-I-4'); 7.82(s,3H,CH_3-I-6).

<u>I-6-C-methyl-I-4',II-4',I-5,II-5,II-7-pentaacetyl-I-7-O-methyl (I-3',II-8) biflavone (CHIIbA)</u>

A mixture of CHIIb (100 mg), pyridine (1.0 ml) and Ac_2O(2.0 ml

was refluxed on a water bath for about 2 hours, cooled and poured onto crushed ice. The separated solid was filtered, washed with water and dried. It was recrystallized from chloroform-methanol as colourless needles (60 mg) m.p.250-252°: τ($CDCl_3$);
1.94(d,J=3Hz,1H,H-I-2')2.06(q,J_1=9Hz & J_2= 3Hz,1H,H-I-6');
2.50(d,J=9Hz,2H,H-II-2',II-6'); 2.56(d,J=9Hz,1H,H-I-5');
2.97(d,J=9Hz,2H,H-II-3',II-5'); 3.00(s,1H,H-II-6);
3.24(s,1H,H-I-8); 3.35(s,2H,H-I-3,II-3);
6.13(s,3H,OCH_3-I-7); 7.51(s,6H,OAc-I-5,OAc-II-5);
7.73(s,3H,OAc-II-4'); 7.91(s,6H,CH_3-I-6,OAc-II-7);
7.95(s,3H,OAc-I-4').



B I B L I O G R A P H Y

1. K.Venkataramn, in Progress in the Chemistry of Organic Natural Products XVII, Ed.L.Zechmeister, Springer-Verlag, (1959), p.46.

2. E.F.Kurth and F.L. Chan, J.Am. Oil, Chem.Soc., 28,433(1951).

3. A.C.Mehtal and T.R.Seshadri, J.Sci.Industr.Res., 188, 25 (1959).

4. A.Banerji,Ph.D.Thesis, Delhi University (1964).

5. J.Abbot and R. Waite, Chem.Abstr., 58,1853 (1963)

6. "The Chemistry of Flavanoid Compounds" T.A.Geissman. (Ed.) Pergamon Press (1962) (a) p.468,513; (b) p.546,583; (c) p.73; (d) p.107.

7. S.M.Kupchan,C.W.Sigel, R.J. Hemingway, J.R.Knoz and M.S.Udayamurthy, Tetrahedron, 25,1603 (1669).

8. J.B.Harborne "Comparative Biochemistry of the Flavonoids Academic Press, New York (1967).

9. T.Swain (Ed) "Chemical plant Taxonomy", Academic Press London (1963).

10. A. Pelter, R.Warren, K.K.Chexal, B.K.Handa and W.Rahman, Tetrahedron, 27, 1625 (1971) and references therein.

11. B.Jackson, H.D.Locksley and F.Scheinmann, J.Chem.Soc.(C) 3791 (1971) and references therein.

12. A.K.Varshney,M.Aqil, W.Rahman , M.Okigawa and N.Kawano, Phytochemistry 12, 1501 (1973)

13. (a) R.Madhav, Teterahedron Letters 2017 (1969).
(b) H.S.Garg and C.R.Mitra, Phytochemistry 10,2787 (1971).
(c) Nizam U.Khan, M.Ilyas, W.Rahman, M.Okigawa and N.Kawano, Phytochemistry , 10, 2541 (1971).

14. Von.L.Hörhammer, H.Wagner and H.Reinhardt, Bot.Mag., Tokyo, 79,510 (1966).

15. B.Voirin and P.Lebreton, Compt. Rend. 262(D), 707(1966).

16. M.Okigawa, N.Kawano, M. Aqil and W. Rahman, Tetrahedron Letters, 2003 (1973).

17. M.S.P.Rao, L.R.Row and R.T. Brown, Phytochemistry, 12, 671 (1973).

18. M.Okigawa , C.W.Hwa, N.Kawano and W. Rahman, Phytochemistry, 10, 3286 (1971)

19. V.V.S. Murti, P.V. Raman and T.R. Seshadri, Tetrahedron, 23, 397 (1967); Tetrahedron Letters, 2995 (1964).

20. T.Mashima, M.Okigawa, N.Kawano, N.U.Khan, M.Illyas and W.Rahman, Tetrahedron Letters, 2937 (1970).

21. N.U.Khan, W.H.Ansari, W. Rahman, M.Okigawa and N.Kawano, Chem. Pharm. Bull. Tokyo, 19, 1500(1971)

22. (a) M.Illyas, J.N.Usmani, S.P.Bhatnagar, M.Ilyas, W.Rahman and A.Pelter, Tetrahedron Letters, 5515 (1968).

(b) M.Ilyas, Ph.D. Thesis (1969), Aligarh Muslim University, Aligarh U.P., India.

23. S.Natarajan, V.V.S.Murti and T.R. Seshadri, Indian, J.Chem, 8, 113 (1970).

24. Von.L.Hörhammer, H.Wagner and H. Reinhardt, Natur Wissens Chaften, 52, 161 (1965).

25. Fa-Ching Chen, Yuh-Meeilin and Chi-Ming Liang., Phytochemistry, 13, 176 (1974).

26. (a) M.Aqil, W. Rahman , M.Okigawa and N.Kawano, Tetrahedron, (in Comm.).

(b) N.U.Khan, Ph.D. Thesis (1971) Aligarh Muslim University, Aligarh , U.P. India.

(c) N.Hameed , Ph.D. Thesis (1974), Aligarh Muslim University, Aligarh, U.P. India.

(d) W. Rahman unpublished results.

27. H.Miura and N.Kawano, J. Pharm. Soc., Japan, 88, 1489 (1968)

28. (a) W. Baker, A.C.M. Finch, W.D. Ollis and K.W. Robinson, J. Chem.Soc., 1477 (1963).

(b) Looker and Hanneman , J.Org. Chem, 27, 381 (1962).

29. H.Miura, T. Kihara and N. Kawano, Tetrahedron Letters, 2339 (1968); Chem.Pharm. Bull., Tokyo, 17, 150 (1969).

30. W. Baker, A.C.M. Finch, W.D. Ollis and K, W. Robinson, Proc. Chem. Soc., 91 (1959).

31. W. Baker, W.D. Ollis and K.W. Robinson, Proc. Chem. Soc., 269 (1959).

32. R. Hodges, Aust. J. Chem., 18, 1491 (1965)

33. S.F. Dossaji, E.A.Bell and J.W. Wallace, Phytochemistry, 12, 371 (1973).

34. N. Chandramouli, S. Natarajan , V.V.S. Murti and T.R. Seshadri, Indian J.Chem., 9, 895 (1971)

35. N. Kawano and N.Yamada, J. Am. Chem. Soc., 82, 1505 (1960).

36. B.K.Handa, K.K.Chexal, W. Rahman, M.Okigawa and N.Kawano., Phytochemistry, 10, 436 (1971).

37. A.K.Varshney, T.Mah, N.U.Khan, W. Rahman, M. Okigawa and N. Kawano, Indian. J. Chem., 11, 1209 (1973).

38. N.U.Khan, M.Illyas, W. Rahman, T.Mashima, M.Okigawa and N.Kawano, Tetrahedron, 28, 5689 (1972).

39. A. Pelter, R. Warren, J.N.Usmani, R.H. Rizvi, M.Illyas and W. Rahman, Experientia, 25, 351 (1969).

40. N.U.Khan, M.Illyas, W. Rahman, M.Okigawa and N.Kawano, Tetrahedron Letters, 2941 (1976).

41. A.K.Varshney, W. Rahman, M.Okigawa and N.Kawano, Experientia, 29, 784 (1973).

42. (a) H.Geiger and W.D.G. Pfleiderer, Phytochemistry, 10, 1936 (1971).

(b) S.Bechman, H. Geiger and W.D.G. Pfleiderer, Phytochemistry, 10, 2465 (1971).

43. C.G.Ksranjgaonkar, P.V. Radhakrishan and K.Venkataraman, Tetrahedron Letters, 3195 (1967).

44. M.Konoshima, Y.Ikeshiso, A. Nishinaga, T.Matsure, T.Kubota and N. Sakamaho, Tetrahedron Letters , 121 (1969).

45. M.Konoshima, and Y.Ikeshiso, Tetrahedron Letters, 1717,(1970).

46. H.D.Locksley and I.G.Murray, J.Chem.Soc.(C) 1332(1971).

47. G.A.Herbin, B.Jackson, H.D. Locksley, F.Scheinmann and W.A.Wolstenholme, Phytochemistry, 9, 221 (1970).

48. B.S.Joshi, V.N.Kamat and N.Vishwanathan, Phytochemistry, 9,881 (1970).

49. (a) B.Jackson, H.D.Locksley, F.Scheinmann and W.A. Wolstenholme, Tetrahedron Letters , 787 (1967), 3049 (1967).

(b) B.Jackson,H.D.Locksley and F.Scheinmann, Cehm. Commun., 1125 (1968), 1360 (1968).

(c) A.Pelter, Tetrahedron Letters, 1767 (1967).

50. R.J.Molyneyx, A.C. Waiss Jr. and W.P. Haddan, Tetrahedron 26, 1409 (1970).

51. (a) Y.Fukui and N.Kawano., J.Am. Chem.Soc., 81, 6331 (1959).

(b) N.Kawano and Y.Fukui, Yakugaku Zasshi, 80, 749 (1960);

(c) Y.Fukui, ibid, 80, 752,756 (1960).

52. K. Nakazawa, Chem.Pharm. Bull., Tokyo, 16, 2503 (1968).

53. (a) H.Miura and N.Kawano, Chem.Pharm.Bull., Tokyo,15, 232 (1967).

(b) H.Miura, Yakugaku Zasshi, 87, 871 (1967).

54. H.Miura, N.Kawano and A.C. Waiss Jr., Chem.Pharm.Bull., Tokyo; 14, 1404 (1966).

55. M.Konoshima, Y.Ikeshiro and S.Miyahara, Tetrahedron Letters, 4203 (1970).

56. J.Shinoda, J.Pharm. Soc. Japan., 48, 214 (1928).

57. V.Asahiva and M.Inubuse, Ber., 61 B, 1646 (1928).

58. (a) C.W.Wilson, J.Am. Chem.Soc., 61, 2303 (1939).

(b) J.C.Pew, J.Am.Chem.Soc., 70, 3031 (1948).

59. J.B.Harborne in 'Chromatographic Reviews' Vol.2 M.Lederer (Ed.), Elsevier-Amsterdam (1960), p.105.

60. A.C. Waiss Jr. , R.E.Ludlin and D.J. Stern, Tetrahedron Letters, 513 (1964).

61. T.Katagi, A. Horii, Y.Omura, H.Miyakawa, T.Kyu, Y.Ikeda and K.Isoi, J. Chromatog., 79, 45 (1973).

62. K.K.Chexal, B.K.Handa and W.Rahman, J.Chromatog., 48,484 (1970).

63. T.J.Mabry, K.R.Markham and M.B.Thomos, "The systematic identification of Flavanoids". Springer-Verlag, New York, Heidelberg (1970).

64. F.M. Dean, "Naturally occurring Oxygen Ring Compounds" London, Bulterworths (1963). (a) p.287,(b) p.325.

65. R.M. Horowitz , J.Am. Chem. Soc,, 79, 6561 (1951).

66. L.Jurd and R.M.Horwitz., J.Org. Chem., 22, 1618 (1957).

67. L.Jurd, Arch. Biochem., Biophys., 63, 376 (1956).

68. A.C.Waiss Jr. , H.E.Ludlin and D.J.Stern., Tetrahedron Letters, 513 (1964).

69. A.Pelter, R.Warren, J.N.Usmani, M.Ilyas and W.Rahman, Tetrahedron Letters, 4259 (1969).

70. N.Kawano, M.Okigawa, M.Aqil and W.Rahman , J.C.S.,(in Comms).

71. A.Pelter, R.Warren, M.Ilyas, J.N.Usmani, S.P. Bhatnagar and R.H.Rizvi, M.Ilyas and W.Rahman, Experientia, 25, 350 (1969)

72. E.Rodrignez, N.J. Carman and T.J. Mabry., Phytochemistyy 11, 409 (1972) and references therein.

73. M.Okigawa, N.Kawano, W. Rahman and M.M.Dhar, Tetrahedron Letters, 4125 (1972).

74. T.J.Batterham and R.J. Highet, Aust. J. Chem., 17, 428 (1964).

75. T.J. Mabry, J.Kagan and R.Rosler, monograph,'Nuclear Magnetic resonance Analysis of Flavonoids' University of Texas Publication No.6418 (1964).

76. J. Massicot and J.P. Marthe, Bull. Soc.ChimFr., 1962(1962).

77. J.W.Clark-Lewis, L.M.Jackman and T.M.Spotswood, Aust. J.Chem, (a) 17,772 (1964) , (b) 21, 2059 (1968).

78. R.G.Wilson, J.H.Bowie and D.H.Williams, Tetrahedron 24, 1407 (1968) and references therein.

79. S.Natarajan, S.C.Datta , V.V.S.Murti and T.R. Seshadri, Indian J.Chem. , 9, 813 (1971).

80. R.G.Wilson and D.H.Williams, J.Chem. Soc. (C) 2377 (1968).

81. A.Pelter and P.I. Amenechi, J.Chem. Soc. (C)., 887 (1969).

82. C.S.Barnes and T.L.Occlowitz, Aust.J.Chem.16,219 (1963).

83. R.I.Reet and J.M.Wilson, J.Chem., Soc., 5949 (1963).

84. S.Natarajan, V.V.S.Murti and T.R.Seshadri, Indian J.Chem., 751 (1969).

85. A.Pelter, P.Stainton and M.Barbu., J.Heterocyclic Chem., 2, 262 (1965).

86. D.G.I.Kingston, Tetrahedron 27, 2691 (1971) and references therein.

87. A.Pelter and P.Stainton, J.Chem.Soc., (C) 1933(1967).

88. R.G. Wilson and D.H. Williams, J. Chem. Soc. (C), 2477 (1968).

89. S. Natarajan, V.V.S.Murti and T.R. Seshadri, Indian J. Chem., 7, 751 (1969).

90. G.de Modica, P.F. Rossi, A.M. Rivero and E.Berello, Chem. Abstr., 57, 16537 (1962).

91. (a) M.R.Peterson Jr., and G.H. Wahl, J. Chem. Edu, 49, 790 (1972).

(b) A.F.Cockerill, G.L.O.Davis and R.G.Harden, Chemical Reviews, 553 (1973).

92. R.V.Ammon and R.D.Fischer, Augew.Chem., 11, 675 (1972).

93. W.D.Horrocks.Jr, and J.P.Sipe, J.Am.Chem.Soc, 93, 6800(1971)

94. J.K.M.Sanders and D.H.Williams, J.Am.Chem.Soc., 93,641 (1971).

95. C.C.Hinckley, J.Am.Chem.Soc., 5160 (1969).

96. A.F.Cockerill and D.M.Rackham, Tetrahedron Letters, 5149 (1970).

97. G.H.Wahl Jr. and M.R.Peterson Jr., Chem.Commun., 1167 (1970).

98. M.Okigawa and N.Kawano, Chem.and Ind., 850 (1973).

99. W.Baker and W.D.Ollis in 'Recent Developments in the Chemistry of Natural Phenolic Compounds'. Ed. W.D.Ollis Pergamon Press, Oxford.P.152 (1961).

100. N.Nakazawa, Chem.Pharm. Bull., Tokyo, 10, 1032 (1962).

101. S.Ahmad and S.Razaq, Tetrahedron Letters 4633 (1971).

102. S.Moriyama , M.Okigawa and N.Kawano, Tetrahedron Letters, 2105 (1972).

103. S.Natarajan, V.V.S. Murti and T.R. Seshadri, Indian J.Chem., 8, 116 (1970).

104. Y.Ikeshiro and M.Konoshima, Tetrahedron Letters, 4389 (1972).

105. A.I.Scott., Quast. Rev (London), 19,1 (1965).

106. W.I.Taylor and A.R. Battershy, 'Oxidative coupling of phenols', Marcel Dekker, Inc., New York (1967).

107. C.H.Hassel and A.I. Scott in 'Recent Developments in the Chemistry of Natural Phenolic Compounds', Ed. W.D.Ollis , Pergamon Press, Oxford.p.119 (1961).

108. A.J.Kuhnle, J.J.Widdle and A.C. Waiss.Jr., J.Chem. Soc, (B),613 (1969).

109. A.Pelter, J. Bradstand and R.F.Warren, Phytochemistry 10,835 (1971)

110. S.Natarajan, V.V.S. Murti and T.R. Seshadri, Indian J. Chem ,9,383 (1971)

111. C.C.Hinekley, M.R.Klotz and F.Patil, J.Am. Chem.Soc., 93, 2417 (1971)

112. B.L.Shapiro, J.R.Hlubucek, G.R.Sullivan and L.F.Johnson, J.Am.Chem. Soc., 93,3281 (1971)

113. J.K.M.Sanders and D.H.Williams, Chem. Commun., 422 (1970).

114. J.Briggs, G.H.Prost, F.A.Hart, G.P.Moss and M.L.Stamforth, Chem.Commun., 749 (1970).

115. R.I.Reed and J.M.Wilson, J.Chem.Soc., (C), 5949 (1963).

116. A.Pelter,P.Stainton and M.Barber, J.Heterocyclic Chem, 2 262 (1965).

117. J.H.Bowie and D.W.Cameron, Aust.J.Chem.19, 1627 (1966).

118. J.H.Bowie and P.Y.White, J.Chem.Soc., (B), 89(1969).

119. B.S.Joshi and V.N.Kamat, Indian J.Chem., 7,639 (1969).

120. F.Bohlmann and C.Sdero, Tetrahedron Letters, 3239 (1967).

121. C.H.Brieskorn and M.Michel, Tetrahedron Letters, 3447 (1968).

122. J.C.Nielsen, Tetrahedron Letters, 803 (1970).

123. Flavanoids from the leaves of casuerina equisetifolia Linn. (Casuarinaceae),J.Ind.Chem.Soc.,47,179 (1970).

124. P.D.Monache,I.L.dAlbuquerque, F.Ferrari, and B.M.Bettolo, Tetrahedron Letters, 1967,4211.

125. M.Okigawa, N.U.Khan, N.Kawano and W.Rahman , J.C.S.,Perkin I, 1975 (5/082).

126. E.Spath and J.Pikl, Berichte, 62, 2251, (1929).

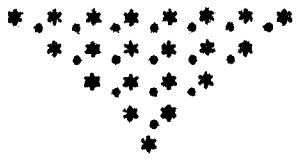
127. W. Baker, J.Chem.Soc., 1381, (1933) : H.S.Mahal and K.Venkataraman , ibid., 1767, (1934).

128. H.D.Locksley, 'Fortschritte der chemic org. Naturstoff', Springer Verlag, Wien, 1973, p.207.

129. Victor Pléuvier; C.R.Acad.Sci.Paris, 263(20); 1529 (1966).

130. H.D.Locksley in "Progress in the chemistry of Organic natural products 30 "W.Horz, H.Grisebach and G.W.Kirby, Ed., Springer Verlag Wien, 1973, p. 299.

131. K.J.Vander Merwe, P.S.Steyn and S.H.Eggers, Tetrahedron Letters, 1964, 3923.



LIST OF SPECTRA

DISCUSSION

LIST OF PUBLICATIONS

1. The structure of Ochnaflavone, A new type of biflavone and the synthesis of its Pentamethyl ether, Tetrahedron Letters 22, 2003-20006 (1973)
2. Biflavones from Putranjiva roxburghii, Phytochemistry, 12, 1501 (1973).
3. Ochnaflavone, a new series of biflavones from Ochnasquarrosa Linn, Comm., J.C.S., perkin I, (1975).
4. Cephalotaxoflavone, The first C-Methylbiflavone from Cephalotaxus and synthesis of its hexamethyl ether and use of LSR in orienting 6- and 8-C-Methylflavones, Comm., Tetrahedron (1975).