This is to certify that the dissertation entitled "Chemistry of Natural Products" is the original work of the candidate and is suitable for partial fulfilment of the requirements for the degree of Master of Philosophy in Chemistry.

(M. KAMIL)
Co-supervisor

(M. ILYAS)
Supervisor
Dedicated to my Loving Parents
ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. M. Ilyas and Dr. M. Kamil who not only guided me but inspired me at all stages, without their keen interest, affectionate supervision and constant help throughout, it would have been impossible to complete this work.

It is an opportunity to express my sincere thanks to Prof. S.M.Osman, Chairman, Department of Chemistry, A.M.U., Aligarh for providing me the necessary facilities in the department for the execution of the work.

The continuous cooperation and fruitful discussions of Dr. M. Sarwar Alnm is gratefully acknowledged. Thanks are also due to Dr. M. A. Qasim for his help throughout the work.

Finally, I would like to thank the constant encouragement & the cheerful companionship of Miss. Amita Bajpai.

(NEERU JAIN)
CONTENTS

1. INTRODUCTION ............................................. 1-9
2. THEORETICAL .............................................
   a. Classification ........................................ 10-13
   b. Structure Determination ............................... 14-24
3. DISCUSSION .............................................. 25-40
4. EXPERIMENTAL ............................................ 41-46
5. BIBLIOGRAPHY .............................................. 47-51
6. LIST OF PUBLICATIONS ................................. 52
INTRODUCTION

The plant kingdom as a whole possesses a bewildering diversity of secondary chemical constituents and a corresponding obscurity in the biological reactions underlying their synthesis and function. Of all such secondary substances, by far the greatest diversity in structure is shown by those compounds that contain phenolic groups such as alkaloids, flavonoids, coumarins etc.

The field of flavonoids is one of the most fascinating areas of Natural Product Chemistry. Hundreds of new flavonoids are being reported every year from natural sources and many of them are being synthesized. The study of their stereochemistry, physiological activity and biosynthesis is augmenting the horizons of this field.

The term "flavonoid" covers a large group of naturally occurring compounds. The chemical structure of flavonoids are based on a C_{15} skeleton with a chromane ring bearing a second aromatic ring (ring B) in position 2, 3 or 4 (I). In few cases, the six membered heterocyclic ring (ring C) occurs in an isomeric open form or is replaced by a five membered ring.

(I)
The variation in oxidation state of the heterocyclic ring (ring C) and the position of ring B determines the properties and class of flavonoid compound. Examples of six major subgroups [chalcones (II), flavanones (III), flavones (IV), flavonol (V), anthocyanins (VI) and isoflavones (VII)] are given in chart 1. All of these bear ring B in position 2 except isoflavones, in which ring B occupies position 3. A group of chromane derivatives with ring B in position 4 (4-phenylcoumarins = neoflavonoids) (VIII), together with coumarin derivatives, oligomeric flavonoids, biflavonols (IX) and proanthocyanidins (X) are the other members of this family.

The major flavonoids occur almost universally in higher plants, including mosses and ferns. Despite several positive reports, the occurrence of flavonoids in bacteria, algae and fungi is doubtful.

The function of flavonoids in higher plants as attractants of animals involved in fertilization is obvious. Other important functions are attributed to flavonoids as protecting agent against UV light or infection by phytopathogenic organisms. The potent uses of flavonoids may be listed as heart stimulant, contraceptive drugs, coronary vasodilators, analgesic and vitamin P activity, effective inhibitors of blood cell aggregation, antitumor effects and several other more physiological activities.

The early steps in the biosynthesis of the various subgroups of flavonoids are closely related. Earlier experiments with radioactively labelled precursors established that the carbon skeleton
Chart I
of all flavonoids is derived from acetate and phenylalanine. Ring A
is formed from three acetate units (malonate), and phenylalanine
gives rise to ring B and C-2, C-3 and C-4 of the heterocyclic ring C.
A central intermediate in the formation of all flavonoids is the
chalcone or the isomeric flavanone. The biosynthetic relationship
of flavonoids as concluded mainly from isotope experiments is illus-
trated in chart-II. Most of the results from tracer studies have
been summarized in detailed review$^{10}$. 
\[ \text{OOC} \quad \text{NH}_2 \quad \text{OOC} \quad \text{OOC} \]

Phenylalanine \quad \text{Cinnamate} \quad 4\text{-Coumarate}

\[ \text{a} = \text{Phenylalanine ammonia-lyase} \]
\[ \text{b} = \text{Cinnamate 4-hydroxylase} \]
\[ \text{c} = 4\text{-Coumarate : CoA ligase} \]
\[ \text{d} = \text{Acetyl-CoA carboxylase} \]
\[ \text{e} = \text{Chalcone synthase} \]
\[ \text{f} = \text{Chalcone isomerase} \]

\[ 4\text{-Coumaroyl-CoA} \]

\[ \text{Acetate} \rightarrow \text{d} \rightarrow \text{Malonyl-CoA} \]

\[ \text{Naringenin chalcone} \]

**Flavonoid pathways**

flavones
flavonols
anthocyanins
isoflavonoids, etc.

\[ \text{Naringenin} \]

**Chart - II**
BIFLAVONOIDS

The biflavonoids which are dimer of monoflavonoids have lately been given more attention because of their physiological properties. During last two decades, a wealth of biflavonoids have been discovered, which differ from the classical biflavones (amentoflavone, cupressuflavone and agathisflavone) not only in the hydroxylation pattern of the aromatic rings, but also in the oxidation level of the centre heterocycle.

The formation of biflavonoids in the plants in all cases has been explained by Jackson et al. in terms of oxidation coupling of two chalcone units and subsequent modification of the central C3-units. For example, abstraction of an electron from C-4' anion of naringeninochalcone (XI) leads to the formation of a radical, represented by the three canonical structures (Chart-III). Abstraction of an electron from the C-7 anion of the same chalcone yields another radical (chart-III).

The precursors of all naturally occurring biflavonoids can be formed by pairing two of the above mentioned radicals.

Jackson et al. have also proposed a second mechanism as an alternative to radical pairing process, whereby the possibility of an electrophilic attack of one of the above mentioned radicals upon the phloroglucinol nucleus of a chalcone or flavanone has been discussed. However, this mechanism, which was initially suggested by Baker et al. to explain the oxidative dimerisation
Chart - III
of two apigenin molecules, would also account for the involvement of one 6 or 8 position in the interflavonyl link in naturally occurring biflavonoids. These two mechanisms, although speculative, provide a basis for grouping the various biflavonoids.
Theoretical
The biflavonoids known to date may be classified under two main headings:

(A) C-C linked biflavonoids
(B) C-O-C linked biflavonoids

(A) C-C linked biflavonoids

C-C linked biflavonoids are subdivided into the following series depending upon the nature of the constituent monomeric units and on the position of the linkage.

1. Agathisflavone Series: The series consists of six biflavonoids\(^{14}\). The parent member is agathisflavone derived from two apigenin units with [I-6, II-8] linkage.

2. Rhus flavanone\(^{20}\): This is derived from two naringenin units with [I-6, II-8] linkage.

3. Rhusflavone\(^{21}\): This flavanoflavone is derived from naringenin and apigenin units linked through [I-6, II-8].

4. Amentoflavone Series: The parent compound of this series is amentoflavone, derived from two apigenin units with [I-3', II-8] linkage and are represented by sixteen members\(^{13,22-39}\).

5. I-2, 3-Dihydroamentoflavone Series: The biflavonoid compound of this series are derived from a naringenin and an apigenin unit with flavanone [I-3', II-8] linkage, and are represented by four
members with I-2,3-dihydroamentoflavone as the parent compound.

6. **Tetrahydroamentoflavone Series**: I-2,3 II-2,3-tetrahydroamentoflavone has been isolated from the nuts of *Semecarpus anacardium*. Thirteen more closely related tetrahydrobiflavonoid compounds have also been isolated from other *Semecarpus* Species.

7. **Cupressuflavone Series**: Cupressuflavone Series comprises of dimers of apigenin and its partial methyl ether with [I-8, II-8] linkage. The parent compound is cupressuflavone.

8. **Musuaferone-A and Musuaferone-B**: Subramanyam et al have reported the isolation of I-2,3, II-2,3-tetrahydrocupressuflavone — Musuaferone-A and I-2,3-dihydrocupressuflavone — Musuaferone-B from the stamens of *Mesua ferrea*.

9. **Robustaflavone Series**: This class is represented only by robustaflavone and is derived from two apigenin units with [I-3', II-6] linkage. Before the isolation of robustaflavone from natural source, its hexamethyl ether had been prepared by Wesseley-Moser rearrangement of amentoflavone hexamethyl ether.

10. **Abiesin**: Chatterjee et al have recently isolated abiesin from *Abies webbiana*. It is derived from a flavone and a flavonol unit with [I-3', II-6] linkage.

11. **Succedaneaflavanone**: This is derived from two naringenin units with [I-6, II-6] linkage and isolated from *Rhus succedanea*. However, its hexamethyl ether has been synthesized by Parthasarthy et al.
12. **Taiwaniaflavone Series**: A new series of naturally occurring biflavone have been derived from *Taiwania cryptomerioides* Hayata. These are derived from two apigenin units with [I-3, II-3'] linkage. Although [I-3, II-3'] biapigenin has been reported\(^6^0\) earlier but this constitutes the first example of its occurrence from a natural source.

13. **G.B. Series\(^6^1-6^6\)**: This series comprises of reduced heterocyclic system and are derived from a naringenin linked with naringenin or aromadendrin or taxifolin or eriodictyol through [I-3, II-8] linkage.

14. **BGH Series\(^3^4,6^7-7^2\)**: These are derived from a naringenin and an apigenin or luteolin units with flavanone [I-3, II-8] flavone linkage and are represented by BGH-II and BGH-III as the parent compound respectively.

15. **WGH Series\(^6^7,7^1\)**: Two biflavones, WGH-II and WGH-III have been synthesized by dehydrogenation of BGH-II and BGH-III respectively.

16. **I-4', I-5, II-5, I-7, II-7-Pentahydroxyflavanone [I-3, II-8] Chromone\(^7^3\)**: This compound has been isolated from the leaves of *Garcinia dulcis* Kurz. It is a dimer of naringenin and 5,7-dihydroxy chromone linked through [I-3, II-8]. Its isolation has introduced a new series comprising of flavanone chromone structure.

(B) **C-O-C linked biflavonoids**

1. **Hinokiflavone Series\(^3^2,3^3,7^4-7^8\)**: These are derived from two apigenin units with [I-4'-0-II-6] linkage. Hinokiflavone is the parent compound with six others as its partial methyl ethers.
2. **I-2,3-Dihydrohinokiflavone**: The sole member has been isolated from *Metasequoia glyptostroboides* and *Cycas* species\(^{30,40}\).

3. **Ochnaflavone Series**: This class is represented by five members\(^{23,74,79}\). Kamil et al. have isolated II-7-O-methyl ochnaflavone\(^{80}\) from the leaves of *Ochna pumila*. 
STRUCTURE DETERMINATION OF BIFLAVONOIDS

There are some problems encountered during the process of determining the structure of biflavonoids that have made the job a complicated one. They are as given below.

(a) Presence of more than one biflavone in chromatographically homogeneous fractions, and their isolation in pure form.
(b) Insoluble in usual organic solvents.
(c) The difficulties in exact location of O-methyl group in partially methylated derivatives of biflavones.
(d) The intricate problem of establishing the interflavonoid linkage.

The methods which are usually applied in determining the structures are as follows:

1. Colour reactions
2. Spectroscopic methods
3. Degradation
4. Synthesis

Since mainly colour reactions and spectroscopic techniques (1H-NMR, Mass and UV) have been used in the identification and structure determination of the products isolated from the plant source during the course of present work, a short review of each techniques is given.
(1) **Colour reactions**\(^8^1\): Various colour reactions are reported in the literature for the detection of certain structural features among flavonoids. As the colour depends upon the pattern of hydroxylation and substitution, its diagnostic value is only a broad indication. The reagent generally used for colour reactions are magnesium hydrochloric acid\(^8^2\), sodium amalgam-hydrochloric acid\(^8^3\), Wilson boric acid\(^8^4\) and zinc hydrochloric acid\(^8^5\). Biflavonoids are found to give more or less the same colour reactions as monomers.

(2) **Spectroscopic methods:**

(a) **Ultra-violet spectroscopy:** The ultra-violet spectra of different flavonoids are very characteristic\(^8^6\) and alongwith colour reactions\(^8^1\), have been used extensively to distinguish the various groups of this class of compounds. The absorption maxima of flavones have been correlated to the presence of a benzoyl (XII) and cinnamoyl (XIII) grouping\(^8^7\). The former giving rise to the low wavelength band at 240-270 nm and the latter to the high wavelength band at 320-380 nm.

\[
\begin{align*}
\text{XII} & \quad \text{XIII} \\
\end{align*}
\]

On the basis of this generalisation, important deducation have been made about the location of substituents in the two rings.
Substitution in the B ring specially at 4' stabilizes the cinnamoyl chromophore resulting in a bathochromic shift of band I whereas substitution in the A ring has a similar effect on the position of band II. Compounds having a free 5-hydroxyl absorb at higher wavelength and methylation of this hydroxyl group brings about a hypsochromic shift of 10-12 nm of both maxima. The presence of a hydroxyl group at this position is routinely established by measuring the spectra in presence of AlCl$_3$\textsuperscript{88}. Hydroxyl groups at 7,4' are more acidic than others and a bathochromic shift of band I or band II on addition of fused sodium acetate is a good indication of the presence of hydroxyl groups at these positions but the result of these measurements have to be interpreted with caution. Presence of hydroxyl group at 4' is also confirmed by a large bathochromic shift in band I without a decrease in intensity on the addition of sodium methoxide\textsuperscript{88}. Ortho hydroxyl groups in ring A and ring B are identified by a bathochromic shift in band I in the presence of AlCl$_3$ and sodium acetate/boric acid respectively.

In flavanones and isoflavones, absence of cinnamoyl chromophore has the effect of supressing the high wave length band which is either totally absent or present only as inflection.

The ultra-violet spectra of biflavonyls are very similar to that of the monoflavonoid unit with the only difference that the molecular extinction coefficient ($\epsilon$) of the biflavone is approximately double as compared to the corresponding monoflavonoid. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of biflavonoid.
NMR Studies on biflavonoids:

The application of NMR spectroscopy has proved to be most powerful and indispensable tool in the structure determination of flavonoids. A lot of useful informations can be obtained in the structure elucidation of biflavonoids by making a comparison of their NMR spectra with those of their corresponding monomers. Double irradiation technique helps to assign each and every proton in the molecule.

Comparison of the NMR spectra of methyl and acetyl derivatives of a biflavonoid with those of biflavonoids of the same series as well as with those of biflavonoids of the other series in which at least one monoflavonoid unit is similarly constituted, is very helpful in assigning each and individual protons of the methoxy groups. The problem of interflavonoid linkage has been successfully solved by following two techniques.

(a) The solvent induced shift studies of methoxy resonance.
(b) The lanthanide induced shift studies

Benzene induced shifts of aromatic methoxy groups are a useful aid in the elucidation of the structures of various classes of natural products.

Wilson et al, while measuring the PMR spectra first in CDCl$_3$ and then in C$_6$H$_6$ have observed that size of the benzen-induced shift ($\Delta$) of certain methoxy signals was to some extent indicative of the position of the methoxy group on the flavone
nucleus. Methoxy groups at C-5, C-7, C-2', C-4' exhibits large positive values ($\Delta = \delta_{\text{CDCl}_3} - \delta_{\text{C}_6\text{H}_6} \approx 0.5 - 0.8$ ppm) in the absence of OMe or OH substituents ortho to these groups. In contrast, OMe group at C-3 or those flanked by two ortho OMe functions (or one ortho -OH and one ortho -OMe function) show small positive or negative values. An OMe at C-5 suffer a drastic algebraic decrease in solvent shift upon the introduction of an OMe group at C-6.

The benzene induced solvent shift [$\Delta (\text{CHCl}_3 / \text{C}_6\text{H}_6)$] of certain methoxy groups in flavones are appreciably enhanced by the addition of small quantity (3% v/v) of trifluoroacetic acid (TFA) to the solution of flavone in benzene.

The possibility of using other solvents to obtain additional information has been considered and accordingly Lanthanide shift reagents$^{93,94}$ (LSR) have been extensively used for the structural and conformational studies of organic natural products$^{94-97}$.

The most commonly used lanthanide reagents are trischelates of lanthanide ions with -diketones, 2,2,6,6-tetramethylheptane-5,5-dione (dipivaloylmethane) and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dione. Typical shift reagents are tris-(dipivaloylmethanato) europium and tris- 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dianato europium, the name of which are normally abbreviated to Eu(dpm)$_3$ and Eu(fod)$_3$. 
Mass spectrometry:

Mass spectrometry plays a very important role in evaluating the structure of monoflavonoid as well as biflavonoids by fragmentation pattern relationship. The retro Diel's-Alder (RDA) reaction is the principal mode of fragmentation in flavones and biflavones. Most flavonoids yield intense peaks for the molecular ion (M⁺) and indeed this is often the base peak. In addition, flavonoids usually afford major peak for (M-H)⁺ and, when methylated (M-CH₃⁺). The common fragmentation processes of flavone is shown in chart-IV using apigenin (XVII)⁹⁸ as a typical example.

Biflavonoids

A more specific study of the mass spectral fragmentation of the permethyl ether derivatives of amentoflavone, Cupressuflavone and hinokiflavone have been made by Seshadri⁹⁹ and co-workers. The mass spectra of hexamethyl ether of amentoflavone (XVIII), cupressuflavone (XIX) are similar, molecular ion being the base peak in each case. Together with RDA reaction these compounds also undergo (a) fission of the C-C or C-O-C linkage between aromatic residues (b) elimination of CO and CHO from the biphenyl ethers (c) rearrangement involving condensation between the phenyl rings.

C-C linked biflavonoids:
Amentoflavone hexamethyl ether (XVIII):

The mode of fragmentation of amentoflavone hexamethyl ether is shown in chart-V.
Amentoflavone hexamethyl ether

m/e 621(3)  m/e 607(33)  \(-\mathrm{CH}_3\)  \(\rightarrow\)  m/e 592(8)

\(-\mathrm{H}^*\)

\(\mathrm{H}_3\mathrm{CO}\)

\(\mathrm{H}_5\mathrm{CO}\)

\(\mathrm{H}_7\mathrm{CO}\)

m/e 135(16)

M\(^+\), m/e 622(100)

\(-\mathrm{OCH}_3\)  \(-\mathrm{CH}_3\)

m/e 180(3)

m/e 576(10)

Chart - V
Main peaks (m/e; intensity): 622(100), 621(31), 607(33), 592(1), 576(10), 312(2), 245(5), 181(2), 135(16), and 132(3).

Cupressusflavone hexamethyl ether:

The mode of fragmentation of cupressusflavone hexamethyl ether (XIX) is given in chart-VI.

Main peaks: 622(100), 621(38), 607(8), 592(18), 576(4), 312(7), 311(14), 245(11), 135(26), and 132(14).
Cupressuflavone hexamethyl ether

\[ \text{M}^+, \text{m/e 622(100)} \]

\[ \text{m/e 245(11)} \]

(490\textsuperscript{++} appears at m/e 245)
C-O-C linked biflavonoids:

Hinokiflavone pentamethyl ether:

The mode of fragmentation of hinokiflavone pentamethyl ether (XX) which contain a biphenyl ether system, is considerably different from those of amentoflavone, cupressuflavone and agathisflavone hexamethyl ethers, (Chart-VII).

Main peaks: 608(39), 607(12), 593(36), 560(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(11), 180(3), 135(19), and 132(18).
Discussion
BIFLAVONES FROM JUNIPERUS BEDFORDIANA (CUPRESSACEAE)

The genus Juniperus Linn (Cupressaceae) consists of about seventy species of evergreen trees or shrubs, distributed chiefly in the northern hemisphere from the arctic zone to the mountains of the tropics. Five species occur in India and a few more exotics have also been introduced.

An essential oil is obtained by distillation from wood and leaves, often used for perfumary. Junipers has been employed in medicine for the treatment of various diseases. Earlier reports show that the extractive of the plant were used as face lotions and masks for beautification. Its pharmaceutical preparations are used for treatment of hypertension and also as hypnotic, besides fungicidal and bacterial properties. Most Junipers are found to possess interesting physiological properties.

The present discussion deals with the isolation and characterization of complex mixture of biflavonoids from the leaf extracts of Juniperus bedfordiana. The biflavonoid constituents identified in other Juniperus species are given in chart-VIII.

Survey of the literature showed that no work seems to have been done on Juniperus bedfordiana, therefore the present chemical investigation had been undertaken.

The phenolic extractives of the coarsely powdered leaves of Juniperus bedfordiana (procured from Forest Research Institute, Dehradun) after purification by solvent fractionation and column
## Chart - VIII

**Distribution of Biflavones in Juniperus species**

<table>
<thead>
<tr>
<th>Band</th>
<th>Biflavones</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Am</td>
<td>+a</td>
<td>+a</td>
<td>+a</td>
<td>+a</td>
<td>+a</td>
<td>+a</td>
<td>+a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(a) Amentoflavone</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>+b</td>
<td>+b</td>
<td>+b</td>
<td>+b</td>
<td>+b</td>
<td>+b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) Cupressuflavone</td>
</tr>
<tr>
<td></td>
<td>Ag</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c) Agathisflavone</td>
</tr>
<tr>
<td></td>
<td>Ro</td>
<td></td>
<td></td>
<td></td>
<td>+d</td>
<td>+d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) Robustaflavone</td>
</tr>
<tr>
<td>II</td>
<td>Hi</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M-Am</td>
<td></td>
<td>+f</td>
<td></td>
<td>+l</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(f) Podocarpusflavone-A</td>
</tr>
<tr>
<td></td>
<td>M-Cu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(g) Cryptomerin-A</td>
</tr>
<tr>
<td>III</td>
<td>M-Hi</td>
<td>+g</td>
<td></td>
<td></td>
<td>+h</td>
<td>+h</td>
<td>+h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(h) Isocryptomerin</td>
</tr>
<tr>
<td></td>
<td>Di-Am</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(i) 7,7'-Di-O-methyl-cupressuflavone</td>
</tr>
<tr>
<td></td>
<td>Di-Cu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+i</td>
<td></td>
<td></td>
<td>(j) Sciadopitysin</td>
</tr>
<tr>
<td>IV</td>
<td>Tri-Am</td>
<td>+</td>
<td></td>
<td>+j</td>
<td>+j</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Di-Hi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(l) Bilobetin</td>
</tr>
</tbody>
</table>

---

**Legend:**

- **Am** = Amentoflavone
- **Cu** = Cupressuflavone
- **Ag** = Agathisflavone
- **Ro** = Robustaflavone
- **Hi** = Hinokiflavone
- **M** = Mono
- **A** = *Juniperus chinensis* L.g.
- **B** = *J. chinensis* Cr. Sorgentin
- **C** = *J. chinensis* Cr. Kaizuku
- **D** = *J. conferta*
- **E** = *J. horizontalis*
- **F** = *J. procumbens*
- **G** = *J. recurva*
- **H** = *J. macropoda*
- **I** = *J. phoenecia*
- **J** = *J. virginiana*
- **K** = *J. utilis*
- **L** = *J. communis*
- **M** = *J. seudosabina*

- **+** = detected
- **+ with superscript** = fully characterized
chromatography (silica gel) revealed the presence of three major bands labelled as JBI, JBII and JBIII in order of increasing $R_f$ values. All the three bands were separated by preparative TLC (BPF, 36:9:5) and eluted by acetone mixed with pyridine (2%).

**JBI:**

JBI, although homogeneous in chromatographic behaviour in a number of solvent systems, was found on complete methylation followed by TLC examination, to be a mixture of two methyl ethers, labelled as JBIMI and JBIMII. They were separated by preparative TLC and characterized as hexa-O-methyl amentoflavone and hexa-O-methyl cupressuflavone respectively by comparison with authentic samples (m.p., m.m.p., $R_f$ Values, characteristic fluorescence in UV light and $^1H$-NMR).

**JBIMI:**

JBIMI (m.p. 210-12°) was characterized as amentoflavone hexamethyl ether by comparison with authentic sample. The $^1H$-NMR studies of JBIMI and amentoflavone hexamethyl ether are recorded in Table-1.

The $^1H$-NMR spectrum of JBIMI (Fig. 1) showed ABX and $A_2B_2$ pattern associated with ring I-B and II-B substituted at positions I-3', 4', and II-4' respectively. Thus rings I-B and II-A of the biflavone seemed to be involved in the interflavonoid linkage. In particular the values showed that I-3' is linked to either II-6 or II-8. The observation that in biflavones, having aromatic substi-
### Table 1
Chemical shifts of protons of JBIMI and amentoflavone hexamethyl ether.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>JBIMI</th>
<th>Amentoflavone hexamethyl ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-I-8</td>
<td>6.44 (1H, d, J=3Hz)</td>
<td>6.46 (1H, d, J=3Hz)</td>
</tr>
<tr>
<td>H-I-6</td>
<td>6.33 (1H, d, J=3Hz)</td>
<td>6.34 (1H, d, J=3Hz)</td>
</tr>
<tr>
<td>H-II-6</td>
<td>6.61 (1H, s)</td>
<td>6.63 (1H, s)</td>
</tr>
<tr>
<td>H-I-3, II-3</td>
<td>6.52, 6.54 (1H each, s)</td>
<td>6.52, 6.58 (1H each, s)</td>
</tr>
<tr>
<td>H-I-6'</td>
<td>7.94 (1H, q, J₁=9Hz, J₂=3Hz)</td>
<td>7.90 (1H, q, J₁=9Hz, J₂=3Hz)</td>
</tr>
<tr>
<td>H-I-2'</td>
<td>7.84 (1H, d, J=9Hz)</td>
<td>7.83 (1H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-I-5'</td>
<td>7.10 (1H, d, J=9Hz)</td>
<td>7.10 (1H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-II-2', 6'</td>
<td>7.38 (2H, d, J=9Hz)</td>
<td>7.39 (1H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-II-3', 5'</td>
<td>6.75 (2H, d, J=9Hz)</td>
<td>6.73 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>OMe-II-5</td>
<td>4.06 (3H, s)</td>
<td>5.95 (3H, s)</td>
</tr>
<tr>
<td>OMe-I-5, I-7</td>
<td>3.92, 3.86 (3H each, s)</td>
<td>3.88, 3.86 (3H each, s)</td>
</tr>
<tr>
<td>OMe-II-7, I-6', II-4'</td>
<td>3.82, 3.80</td>
<td>3.80, 3.75, 3.73 (3H each, s)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, q = quartet, spectrum run in CDCl₃ at 100 MHz, TMS as internal standard (δ-scale).
tuents at I-8, the I-5-OMe group generally appeared below δ 5.016 (Table-2) led to believe that substituents (flavone unit) in JBIMI was located at II-8 and not at II-6. Further all methoxy groups on change of solvent from deuterochloroform to benzene moved upfield, as in cupressuflavone hexamethyl ether, showing that every methoxy group had at least one ortho proton, therefore, a II-8 rather than II-6 linkage was established.

On the basis of careful study of the splitting pattern of the 'H-NMR spectrum signals of JBIMI and amentoflavone hexamethyl ether, the structure of JBIMI was, therefore, assigned as I-4', II-4', I-5, II-5, I-7, II-7- Hexa-0-methyl [I-3', II-8] biflavone (XVIII).

TABLE - 2

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biflavonoids</th>
<th>I-5-OMe (δ)</th>
<th>II-5-OMe (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Amentoflavone hexamethyl ether</td>
<td>3.87</td>
<td>4.06</td>
</tr>
<tr>
<td>3.</td>
<td>Agathisflavone hexamethyl ether</td>
<td>3.59</td>
<td>4.05</td>
</tr>
<tr>
<td>4.</td>
<td>I-4'-O-II-8 biflavone</td>
<td>4.00</td>
<td>4.08</td>
</tr>
<tr>
<td>5.</td>
<td>JBIMI</td>
<td>3.92</td>
<td>4.06</td>
</tr>
</tbody>
</table>
JBIMII (m.p. 296-98°) was found to be cupressuflavone hexamethyl ether on TLC examination and comparison with authentic sample (m.p., m.m.p., \( R_f \) values characteristic fluorescence in UV light and 'H-NMR studies). The results of 'H-NMR studies are given in Table-3.

'H-NMR spectrum of JBIMII suggested that the molecule and an axis of symmetry. H-I-3, II-3 and H-I-6, II-6 were distinguished, the former (\( \delta \) 6.59) appearing at the characteristic position for H-I-3 of a flavone. There was a clearcut \( A_2B_2 \) pattern of protons associated with rings I-B and II-B, indicating the presence of methoxy groups at I-4' and II-4' positions. Two methoxy groups had value \( \delta \) below 5.00 (Ca=4.01). These were the I-5 and II-5 methoxy groups which were present in
### Table - 3

Chemical shift of protons of JBIMII

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-I-2',6',II-2',6'</td>
<td>4</td>
<td>7.29 (d, J=9Hz)</td>
</tr>
<tr>
<td>H-I-3',5',II-3',5'</td>
<td>4</td>
<td>6.78 (d, J=9Hz)</td>
</tr>
<tr>
<td>H-I-3, II-3</td>
<td>2</td>
<td>6.59 (s)</td>
</tr>
<tr>
<td>H-I-6, II-6</td>
<td>2</td>
<td>6.57 (s)</td>
</tr>
<tr>
<td>OMe-I-5, II-5</td>
<td>6</td>
<td>4.08 (s)</td>
</tr>
<tr>
<td>OMe-I-4', II-4'</td>
<td>6</td>
<td>3.78 (s)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet; spectrum run in CDCl₃ at 100 MHz, TMS as internal standard (δ - scale)

two different monoflavonoid units of the biflavone. This value (below δ 5.00) is the characteristic feature of such groups in I-8, II-8 linked biflavones or an 8-linked monoflavonoid unit of biflavone (Table-2). There was a singlet representing two protons around δ 6.57. This was assigned to aromatic protons at H-I-6 and II-6. To meet the requirements of symmetry and 'H-NMR spectrum, the two possible structures for the fraction JBIMII may be represented by (XIX) and (XX).

The final decision between C-I-8, II-8 and C-I-6, II-6 linkage was taken by benzene induced solvent shift studies of methoxy resonances. The shift in methoxy resonance as a result of change in solvent from deuterochloroform to benzene are shown in Table - 4.
Table - 4

<table>
<thead>
<tr>
<th>Position</th>
<th>Signals in CDCl₃ (Hz)</th>
<th>Signals in C₆H₆ (Hz)</th>
<th>Shift (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-5</td>
<td>412</td>
<td>356</td>
<td>+58</td>
</tr>
<tr>
<td>C-4'</td>
<td>386</td>
<td>329</td>
<td>+57</td>
</tr>
<tr>
<td>C-7</td>
<td>371</td>
<td>302</td>
<td>+67</td>
</tr>
</tbody>
</table>

Thus all the methoxy group shifted upfield as expected for a C-1-8, II-8 linkage.

The fragmentation pattern i.e. ions at m/z 622, 592, 576, 245, 132, and 135 in the mass spectrum (chart - VI) also support the structure as I-8, II-8 linked biflavone.

JBIMII was, therefore, assigned the structure as I-4', II-4', I-5, II-5, I-7, II-7- hexa-O-methyl [I-8, II-8] biflavone

JBII (parent) m.p. 345°
JBIIA (acetate) m.p. 239-41°
JBIIM (methyl ether) m.p. 269-71°

TLC examination of JBII and its methyl ether JBIIM indicated it to be hinokiflavone²⁴, supported by PMR studies of its acetate (JBIIA) and methyl ether (JBIIM). The results of PMR studies of JBIIA and JBIIM are recorded in Table - 5.
The 'H-NMR spectra of JBIIA (Fig. 2) and JBIIM showed signals for five methoxy and five acetoxy groups respectively. The appearance of two sets of \( A_2B_2 \) protons in JBIIM and \( A_2B_2 \) of only one set shifting downfield, in the acetate suggested the presence of a free hydroxyl group at 4'-position of one of the B-ring and the implication of the corresponding position of the other B-ring in C-O-C linkage in JBIIA. The linkage could not be through I-C-3 or II-C-3 as there were almost two invariant protons at \( \delta 6.59 \) and \( \delta 6.65 \) in JBIIA. Moreover this linkage could lead to two meta-coupled pairs associated with ring I-A and II-A while only one such pair was observed. The presence of two meta-coupled doublets at \( \delta 6.85 \) and \( \delta 7.45 \) and a singlet at \( \delta 7.30 \) suggested that the linkage is either through C-6 or C-8 of ring-A of one of the flavone unit. On the basis of above observations, JBIIA may be represented by (XXI) or (XXII).

The final decision between I-4'-0-II-6 (XXI) and I-4'-0-II-8 (XXII) linkage was taken by benzene induced solvent shift studies of methoxy resonance of JBIIM. On change of solvent from CDCl\(_3\) to C\(_6\)H\(_6\), methoxy shifts were observed at 34Hz, 54Hz, 57Hz, 64Hz, 1Hz. These shifts were within the range for four unhindered methoxy groups and one hindered methoxy group. It was reasonable to assume that hindered methoxy group was the one at II-5 of structure (XXI) flanked by ether linkage on one side and carbonyl group on the other.
(XXI)

(XXII)
Table - 5
Chemical shift of protons of JBIIA and JBIIM

<table>
<thead>
<tr>
<th>Assignment</th>
<th>JBIIA</th>
<th>JBIIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-I-6</td>
<td>6.85 (1H, d, J=2.5Hz)</td>
<td>6.34 (1H, d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-I-8</td>
<td>7.45 (1H, d, J=2.5Hz)</td>
<td>6.52 (1H, d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-II-8</td>
<td>7.30 (1H, s)</td>
<td>6.56 (1H, s)</td>
</tr>
<tr>
<td>H-I-3</td>
<td>6.59* (1H, s)</td>
<td>6.58 (1H, s)</td>
</tr>
<tr>
<td>H-II-3</td>
<td>6.65* (1H, s)</td>
<td>6.60* (1H, s)</td>
</tr>
<tr>
<td>H-I-2',6'</td>
<td>7.95 (2H, d, J=9Hz)</td>
<td>7.96 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-II-2',6'</td>
<td>7.86 (2H, d, J=9Hz)</td>
<td>7.86 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-I-3',5'</td>
<td>7.03 (2H, d, J=9Hz)</td>
<td>6.96 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-II-3',5'</td>
<td>7.33 (2H, d, J=9Hz)</td>
<td>7.04 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>I-5, II-5, I-7</td>
<td>2.44, 2.35, 2.25</td>
<td>3.94 - 3.85</td>
</tr>
<tr>
<td>II-7, II-4'</td>
<td>2.12 (15H, s, 5xOAc)</td>
<td>(15H, s, 5xOMe)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, spectrum run in CDCl₃ at 100 MHz and TMS as internal standard (δ scale)
* Alternative assignment is possible

JBII, was thus assigned the structure II-4', I-5, II-5, I-7, II-7, pentahydroxy [I-4'-0-II-6] biflavone (XXI).

JBIII

R_f value, fluorescence in UV light, mass and 'H-NMR spectrum of JBIII methyl ether (JBIIMA) were found identical in all respects with those of authentic sample of hinokiflavone pentamethyl ether.

The 'H-NMR spectrum of JBIII acetate (JBIIM) (Fig.3) m.p. 196-98° indicated it to be a monomethyl ether of hinoki-
### Table 6

Chemical shift of protons of JBIIIA and JBIIIM

<table>
<thead>
<tr>
<th>Assignment</th>
<th>JBIIIA</th>
<th>JBIIIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-I-6</td>
<td>6.01 (1H, d, J=2.5Hz)</td>
<td>6.34 (1H, d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-I-8</td>
<td>7.29 (1H, d, J=2.5Hz)</td>
<td>6.52 (1H, d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-II-8</td>
<td>7.01 (1H, s)</td>
<td>6.56 (1H, s)</td>
</tr>
<tr>
<td>H-I-3</td>
<td>6.60 (1H, s)</td>
<td>6.58 (1H, s)</td>
</tr>
<tr>
<td>H-II-3</td>
<td>6.56 (1H, s)</td>
<td>6.60 (1H, s)</td>
</tr>
<tr>
<td>H-I-2',6'</td>
<td>7.89 (2H, d, J=9Hz)</td>
<td>7.96 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-II-2',6'</td>
<td>7.76 (2H, d, J=9Hz)</td>
<td>7.86 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-I-3',5'</td>
<td>7.01 (2H, d, J=9Hz)</td>
<td>6.96 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>H-II-3',5'</td>
<td>7.24 (2H, d, J=9Hz)</td>
<td>7.04 (2H, d, J=9Hz)</td>
</tr>
<tr>
<td>II-7</td>
<td>3.87 (3H, s, OMe)</td>
<td></td>
</tr>
<tr>
<td>I-5, II-5</td>
<td>2.29 (3H each, s, 2xOAc)</td>
<td>6.06 - 6.15 (3H each, s, 5xOMe)</td>
</tr>
<tr>
<td>I-7, II-4'</td>
<td>2.24, 2.37 (3H each, 2xOAc)</td>
<td></td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, spectrum run in CDCl$_3$ at 100 MHz and TMS as internal standard. (6 scale).
flavone as it showed signals for five methoxy and four acetoxy groups respectively. The 'H-NMR data of JBIIIM and JBIIIA are recorded in Table - 6.

NMR and Mass spectral studies of JBIIIA showed it to be a monomethoxy tetraacetoxy hinokiflavone. The possibility of methoxy group being at I-5 or II-5 was ruled out as in the FMR spectrum of the parent compound itself there were two hydrogen bonded hydroxyl groups at δ 13.15 and δ 12.90, expected for these positions. As H-II-8 was found invariant in JBIIIA and JBIIIM (Table - 6), the methoxy group was assigned to II-7 position.

The ions at m/z 520, 283, 299, 403 and 282 in the mass spectrum of JBIII (chart IX) further support the above assignment.

JBIII was, therefore, assigned the structure II-4', I-5, II-5, I-7- tetrahydroxy-II-7-O-methyl [I-4'-0-II-6] biflavone (XXIII) (Isocryptomerin).
Experimental
Extraction of biflavonoids from Juniperus bedfordiana (Cupressaceae)

Dried and powdered leaves (2 kg), procured from Forest Research Institute, Dehradun, were completely (four times) exhausted with petroleum ether (40-60°). The petrol exhausted leaves were successively refluxed with ethyl acetate and acetone. The combined ethyl acetate and acetone extracts were concentrated first at atmospheric pressure and then under reduced pressure on a water bath. The dark viscous mass obtained, was refluxed with petroleum ether (40-60°) and benzene successively till the solvent in each case was almost colourless. A brown semi-solid mass was left behind.

Purification of the semi-solid mass by column chromatography:

A well stirred suspension of silica gel (150 gm) in dry petroleum ether (40-60°) was poured into a column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess petrol was allowed to pass through the column. The semi-solid mass (24 gms) adsorbed on silica gel was added to the column. The column was successively eluted with petrol, benzene and varying ratio of benzene-ethyl acetate (9:1, 8:2, 7:3, 6:4, 1:1, 2:3, 1:4). The last three fractions gave usual flavonoid colour tests. They were combined together and the solvent distilled off to give brown solid mass (8 gms).

Separation of biflavonoid mixture by preparative layer chromatography (PLC).

Using a thin layer spreader (Desaga, Heidelberg), glass plates
(40 x 20 cms) were coated with a well stirred suspension of silica
gel (50 gm in 95 ml of water) to give a layer approximately 0.5 mm
in thickness. After drying at room temperature, the plates were
activated at 110-20° for two hours.

The complexity of the biflavonoid mixture, obtained after
purification by column chromatography was examined by TLC using the
following solvent systems.

(a) Benzene - pyridine - formic acid (BPF, 36:9:5)
(b) Toluene - ethyl formate - formic acid (TEF, 5:4:1)
(c) Toluene - pyridine - acetic acid (TPA, 10:1:1)
(d) Benzene - ethyl acetate - acetic acid (BEA; 3:5:2)

In solvent system (a), the biflavonoid mixture showed three
clear spots in UV light. The marked difference in the Rf value in
BPF system made it the developing system of choice for quantitative
separation.

The brown mass (8 gm) was dissolved in pyridine and applied
to plates with the help of a mechanical applicator (Desaga
Heidelberg) 2 cm from the lower edge of the plates. The plates
mounted on a stainless steel frame were placed in a Desaga glass
chamber (45 x 22 x 22 cm) containing 500 ml of developing solvent.
When the solvent front had travelled 15 cm from the starting line,
the plates were taken out and dried at room temperature. The
position of the bands were marked in UV light and labelled as JBI,
JBII and JBIII. The marked pigment zones were scraped with the
help of a nickel spatula and eluted separately in soxhlet with dry acetone. The eluate in each case was distilled off to give an oily liquid which on addition of water yielded yellow precipitate. It was filtered, washed with water and dried. Homogeneity of the pigments was again checked by TLC using different solvent systems.

**JBI**

**Methylation (JBIM):** JBI (150 mg), anhydrous potassium carbonate (2 gm) dimethyl sulphate (1 ml) and dry acetone (300 ml) were refluxed on a water bath for 36 hours. The reaction mixture was filtered and the residue washed several times with hot acetone. The filtrate and washings were combined and evaporated to dryness. The yellow residue left behind was washed with hot petroleum ether to remove excess of dimethyl sulphate. It was then taken up in chloroform (100 ml) into a separating funnel and was washed several times with water, dried over anhydrous sodium sulphate and concentrated to give crude solid.

TLC examination of the methylated product JBIM showed it to be mixture of two methyl ethers. They were separated in pure form by preparative layer chromatography using BPF as the developing solvent and were labelled as JBIMI and JBIMII.

**I-4', II-4', I-5, II-5, I-7, II-7 - Hexa-O-methyl [I-3', II-8] biflavone (JBIMI):**

It was crystallized from CHCl₃-MeOH as colourless needles (45 mg), m.p. 210-12°.
$^1$H-NMR (CDCl$_3$): Values on $\delta$ scale:

6.44 (1H, d, H-I-8); 6.33 (1H, d, H-I-5); 6.61 (1H, s, H-II-6);
6.52, 6.54 (1H each, s, H-I-3, II-3); 7.94 (1H, q, H-I-6'); 7.84
(1H, d, H-I-2'); 7.10 (1H, d, H-I-5'), 7.38 (2H, d, H-II-2',6'),
6.75 (2H, d, H-II-3',5'); 4.06, 3.92, 3.86, 3.82, 3.80, 3.76 (3H
each, s, OMe-II-5, I-5, I-7, II-7, I-4', II-4').

I-4', II-4', I-5, II-5, I-7, II-7 - Hexa-O-methyl/[I-8, II-8]
biflavone (JBIMII):

JBIMII on crystallization from chloroform - methanol gave
colourless shining needles (40 mg), m.p. 296-98°.

$^1$H-NMR (CDCl$_3$): Values on $\delta$ scale:

7.29 (4H, d, H-I-2',6', II-2',6'); 6.78 (d, 4H, H-I-3',5',
II-3',5'); 6.59 (2H, s, H-I-3, II-3); 6.57 (2H, s, H-I-6, II-6);
4.08 (6H, s, OMe-I-5, II-5); 3.83 (6H, s, OMe-I-7, II-7); 3.78
(6H, s, I-4', II-4').

Mass:

C$_{36}$H$_{30}$O$_{10}$ (622) m/z 622 M$^+$ (100 rel.int), 621 (38),
607 (8), 592 (18), 576 (4), 312 (7), 311 (14), 245 (11), 135 (26) and 132(14).

II-4', I-5, II-5, I-7, II-7 - penta-O-methyl [I-4'-0-II-6]
biflavone (JBIIM):

JBIIM (35 mg) was methylated as described earlier. On usual
work up, it gave colourless needles (25 mg) from CHCl$_3$-MeOH, m.p.
269-71°.
$^1$H-NMR (CDCl$_3$) : Values on δ scale:

6.34 (1H, d, H-I-6); 6.52 (1H, d, H-I-8); 6.56 (1H, s, H-II-8); 5.58* (1H, s, I-3); 6.60* (1H, s, H-II-3); 7.96 (2H, d, H-I-2', 6'); 7.86 (2H, d, H-II-2', 6'); 6.65* (1H, s, H-II-3'); 7.04 (2H, d, H-II-3', 5'); 3.94 – 3.85 (3H each; s, OMe – II-4', I-5, II-5, I-7, II-7).

* Alternative assignment is possible

Acetylation (JBIIA):

JBII (50 mg) was heated with pyridine (1.5 ml) and acetic anhydride (3 ml) on a water bath for 2 hours. It was then cooled to room temperature and poured onto crushed ice. The separated solid was filtered, washed well with water and dried.

II-4', I-5, II-5, I-7, II-7- pentaacetoxy [I-4'-0-II-6]

biflavone (JBIIA):

The fraction JBIIA on crystallization from chloroform - petroleum ether gave white needles (35 mg), m.p. 239-41°.

$^1$H-NMR (CDCl$_3$) : Values on δ scale :

6.85 (1H, d, H-I-6); 7.45 (1H, d, H-I-8); 7.30 (1H, s, H-II-8); 6.59* (1H, s, H-I-3); 6.65* (1H, s, H-II-3); 7.95 (2H, d, H-I-2', 6'); 7.86 (2H, d, H-II-2', 6'); 7.03 (2H, d, H-I-3', 5'); 7.33 (2H, d, H-II-3', 5'); 2.35, 2.44, 2.25, 2.12 (3H each, s, II-4', I-5, II-5, I-7, II-7).

* Alternative assignment is possible.
JBIII:

Methylation:

JBIII on methylation gave hinokiflavone pentamethyl ether (JBIIIM), m.p. 269-710.

Acetylation (JBIIIA): JBIII was acetylated by heating it with pyridine and acetic anhydride. The acetylated product (JBIIIA) gave colourless needles, m.p. 196-98°.

$^1$H-NMR (CDCl$_3$): Values on δ scale:

6.01 (1H, d, H-I-6); 7.29 (1H, d, H-I-8); 7.01 (1H, s, H-II-8); 6.60 (1H, s, H-I-3); 6.56 (1H, s, H-II-3); 7.89 (2H, d, H-I-2', 6'); 7.76 (2H, d, H-II-2', 6'); 7.01 (2H, d, H-I-3', 5'); 7.24 (2H, d, H-II-3', 5'); 3.87 (3H, s, OMe-II-7), 2.29 (6H, s, OAc-I-5), 2.24, 2.37 (3H each, s, OAc-I-7, II-4').
Bibliography


63. B. Jackson, H.D. Locksley, F. Schienmann and W.A. Walstenholme, Tetrahedron Letters, (a) 787 (1967) and (b) 3049, 4095 (1967).


83. V. Ashahiva and M. Inubuse, Ber., 61B, 1646 (1928).
LIST OF PUBLICATIONS

1. Chemical Investigation of Juniperus bedfordiana. 
