EXPERIMENTAL
EXPERIMENTAL

All m.ps. were measured on a kofler block and are uncorrected. Analytical and preparative TLC were performed on silica gel G (BDH), silica gel G (Stahl, Merck) or silica gel NCL (Poona) using benzene-pyridine-formic acid (BPF), 36:9:5, and dichloroethane-ethylacetate-acetic acid (DEA), 8:1:1 as developers. The $^1$H-NMR spectra were run on a Varian A-60 and JEOL 4H-100 spectrometers with TMS as the internal standard and the mass spectra were taken on an AEI MS30 instrument at an ionization energy of 70 eV. The $^{13}$C-NMR spectra were recorded on a JEOL F5 100 NMR spectrometer. All reagents used were of 'ANALAR' grade.

The following five plants have been investigated in detail for the extraction and isolation of flavanoids and biflavanoids.

1. **Lycopodium clavatum** Linn. (Lycopodiaceae)
2. **Callitris glauca** R.Br. (Cupressaceae)
3. **Garuga pinnata** Roxb. (Burseraceae)
4. **Cunninghamia lanceolata** Hook. (Taxodiaceae)
5. **Khus insignis** Hook. f. (Anacardiaceae)
Extraction of the leaves of *Lycopodium clavatum* Linn. (Lycopodiaceae)

Leaves of *Lycopodium clavatum* Linn. (2 Kg) procured from Dow Hill (Kurseong), Darjeeling, INDIA, were completely exhausted with hot acetone. The combined acetone extracts were concentrated under diminished pressure. The dark viscous concentrate was extracted successively with light petrol, benzene and chloroform until the solvent in each case was almost colourless. The brownish gummy mass was then refluxed with ethylacetate for 20 hrs and filtered. The filtrate was evaporated and dried under reduced pressure to give a dark brown residue (10 g) which responded to usual flavanoid colour tests.

**Purification of the extracts by chromatographic method**

A well stirred suspension of silica gel (200 g) in petroleum ether (40-60°C) was poured into a column (250 cm long and 50 mm in diameter). When adsorbent was well settled, the excess of petroleum ether was allowed to pass through the column. The dark brown solid (10 g) was dissolved in acetone (25 ml) and was adsorbed on silica gel (15 g) in a china dish. The excess solvent was evaporated until a dry residue obtained. This adsorbed silica gel was transferred to the column. Elution was performed with the following solvents successively, petroleum ether, benzene and benzene-ethylacetate (9:1, 8:2, 7:3, 6:4 and
The benzene-ethylacetate fractions (7:3, 6:4 and 5:5) gave positive colour tests for flavanoids. These three fractions were combined and purified further by TLC (silica gel) using benzene-pyridine-formic acid (BPF, 36:9:5) to give a yellow mass (35C mg). This showed a single spot on TLC, labelled as LCI.

Apigenin-4'-O-(2",6"-di-O-trans-p-coumaroyl)-D-glucoside (LCI) (LXIX)

LCI was obtained as yellowish powder from ethylacetate-petrol (300 mg), m.p. 254°; \( [\alpha]_D^{25} = -119^0 \) (c, 0.13 in pyridine); \( R_f \) 0.15 (BPF); \( C_{39}H_{32}O_{14} \) (724.65) found: C = 64.75, H = 4.68, calcd: C = 64.64, H = 4.45; UV \( (\text{MeOH}) \lambda = 212, 232 \) (sh), 271 (sh), 300 (sh), 314 nm; \( (\text{MeOH} + \text{NaOAc}) \) 277, 365 nm; \( (\text{MeOH} + \text{AlCl}_3) \) 300, 318, 384 nm.

IR (KBr): \( \nu = 3340 \) (OH), 1685, 1650 \( (\text{CO}_2 \text{H}) \), 1600 (C=O), 1218, 1150, 1075 (C=O), 826 (Ar) cm\(^{-1}\).

NMR \( (^1H, \text{DMSO-}d_6 + \text{TFA-}d \) TMS int.): values on \( \delta \) scale

- 8.01 ppm (d, J=9 Hz, H-2', 6'); 7.70, 7.62 (d, J=16 Hz, H-7''', 7'''); 7.59 (d, J=8 Hz, H-2'''', 6'''', 2''', 6'''''); 7.18 (d, J=9 Hz, H-3', 5'); 6.88 (s, H-3); 6.81, 6.82 (d, J=8 Hz, H-3''', 5''', 3''''', 5'''''); 6.65 (d, J=16 Hz, H-8'''); 6.49 (d, J=2 Hz, H-8); 6.44 (d, J=16 Hz, H-8'''); 6.25 (d, J=2 Hz, H-6); 5.49 (d, J=7 Hz, H-1'''); 5.09 (t, J=7 Hz, H-2'''); 4.40 (m, H-6'', 6'''); 3.3-3.9 (m, H-3'', 4'', 5''); 12.9 (s, OH-5).
NMR($^{13}$C, DMSO-$d_6$, TMS int.): values on $\delta$ scale

Sugar moiety: 63.4 ppm (C-6"'), 70.1 (C-4"'), 73.0 (C-3")$^a$, 73.7 (C-5")$^a$, 74.0 (C-2"'), 97.2 (C-1"').

Aglycone moiety: 94.0 ppm (C-8), 99.0 (C-6), 103.7 (C-3), 104.0 (C-10), 116.5 (C-3', C-5'), 124.4 (C-1'), 128.1 (C-2', C-6'), 157.1 (C-9), 159.1 (C-4'), 161.2 (C-5), 162.6 (C-7), 164.1 (C-2), 181.4 (C-4).

p-Coumarate moiety: 113.8 ppm (C-8"'), 114.8 (C-8''''), 115.7 (C-3'''', C-5'''', C-3''''', C-5'''''), 124.9 (C-1''', C-1''''), 130.2 (C-2''', C-6''', C-2'''''', C-6'''''), 144.9 (C-7'''', C-7'''''), 159.7 (C-4''', C-4'''''), 165.5 (C-9'''), 166.2 (C-9''''').

a,b,c assignment reversible.

LCI permethyl ether (LCIM) (LXIXa): According to the method of Brimacombe$^{139}$, a dried sample of LCI (2 mg) was dissolved in DMF (1 ml) dry solvent (water free). It was methylated by adding sodium hydride-powder (4 mg) and methyl iodide (2 ml) under N$_2$-atmosphere at room temperature. After one hour, the reaction mixture was extracted two times with 10 ml ether, washed with water several times and dried. It was subjected to mass spectrometry$^{140}$.

MS: m/e 808 M$^+$ (rel. int. 1%), 647 (2), 511 (8), 486 (1), 350 (4), 298 (100), 189(61), 187(19), 178(77), 161(145), 157(20), 155(25), 133(78), 127(23), 111(88), 101(120), 89(54), 71(95), 45(62).
Acid Hydrolysis of LCI: A methanolic solution of LCI was refluxed with 10% aqueous HCl for 2 hrs. TLC (silica gel) indicated the presence of apigenin, \( R_f \) 0.29, trans-p-coumaric acid, \( R_f \) 0.47 and glucose (GC of TMS derivative) using dichloroethane:ethyl acetate: acetic acid (8:1:1, solvent B).

Alkaline Methanolysis of LCI: A solution of LCI in absolute MeOH was treated with a catalytic amount of NaOMe solution (2%) and set aside overnight. The solution was neutralized and evaporated to a syrup. The residue was extracted with dry ether. The ether soluble portion on evaporation gave methyl trans-p-coumarate (LXVII) (LCIb), \( R_f \) 0.70 (Dichloroethane:ethyl acetate:acetic acid, 8:1:1) and the insoluble portion on concentration provided apigenin-4'-O-\( \beta \)-D-glucoside (LCIa) (LXVIIa), \( R_f \) 0.10 (Dichloroethane:ethylacetate:acetic acid, 8:1:1).

Methyl-p-coumarate (LCIb) (LXVII)

\[ \text{NMR} (\text{H, CDCl}_3, \text{TMS int.}): \text{values on } \delta \text{ scale} \]

- 7.70 ppm (d, J=16 Hz, H-\( \beta \)); 7.48 (d, J=9 Hz, H-2,6);
- 6.89 (d, J=9 Hz, H-3,5); 6.33 (d, J=16 Hz, H-\( \alpha \)); 5.40 (br, OH-4);
- 3.83 (s, OCH\( _3 \)).

\[ \text{IR(KBr)}: \nu = 3400 \text{ (OH)}, 1675 \text{ (C=O), 1620 (C=C) cm}^{-1}. \]

\[ \text{MS: m/e 178 M}^+ \text{ (rel. int. 53%), 147 (100), 119 (50), 91 (52), 69 (26), 55 (28).} \]
Apigenin-4′-O-β-D-glucoside (LXVIIa) (LCIa): UV (MeOH) \( \lambda = 270, 311 \) nm; (MeOH + NaOAc) \( \lambda = 278, 295 \) (sh), 360 nm; (MeOH + AlCl\(_3\)) \( \lambda = 258 \) (sh), 280, 300, 335, 382 nm.

Apigenin-4′-O-β-D-glucoside permethyl ether (LXVIIb): The permethyl ether was prepared according to the method of Brimacombe and worked up as usual.

MS: m/e 516 \(^+\) (rel. int. 9%), 298 (100), 269 (15), 213 (25), 187 (96), 155 (44), 127 (48), 111 (117), 101 (132), 89 (62), 75 (65), 73 (40), 71 (88), 45 (97).

Apigenin-5,7-di-O-methyl ether: Hydrolysis of the apigenin-4′-O-β-D-glucoside permethyl ether with acid resulted in the isolation of apigenin-5,7-di-O-methyl ether.

UV (MeOH) \( \lambda = 264, 324 \) nm; (MeOH + NaOMe) \( \lambda = 264, 375 \) nm; (MeOH + AlCl\(_3\)) \( \lambda = 264, 303, 328, 395 \) nm.

MS: m/e 298 \(^+\) (rel. int. 100%), 297 (55), 269 (35), 267 (20), 252 (27), 225 (11), 167 (8), 151 (6), 121 (10), 118 (13).

LCI hexa-O-acetate (LCIa) (LXIXb): A mixture of LCI (200 mg), pyridine (1 ml) and acetic anhydride (2 ml) was heated on a water bath for 2 hrs. The reaction mixture was cooled and poured on to crushed ice. The white solid was filtered off, washed with water and dried. The acetate crystallized as colourless cubes (120 mg) from CHCl\(_3\)-MeOH, m.p. 231°.
IR (KBr) $\nu = 1750, 1720$ (CO$_2$R), 1640 (C = O) cm$^{-1}$.

NMR ($^1$H, CDC$_3$, TMS int.): Values on $\delta$ Scale: 7.76 ppm (d, J=16 Hz, H-7$''$); 7.76 (d, J=9 Hz, H-2',6'); 7.71 (d, J=16 Hz, H-7$'''$); 7.56 (d, J=9 Hz, H-2'',6'',2''',6'''); 7.29 (d, J=2 Hz, H-8); 7.15, 7.12 (d, J=9 Hz, H-3',5',3''',5''',3''''',5'''''); 6.88 (d, J=2 Hz, H-6); 6.52 (s, H-3); 6.39 (d, J=16 Hz, H-8'',8'''''); 5.08-5.65 (m, H-1'',2'',3'',4''); 4.44 (m, H-6'',6'''); 4.11 (m, H-5'''); 2.45 (s, OAc-5); 2.34, 2.30 (s, OAc-7,4''',4'''''); 2.08, 2.03 (s, OAc-3''',4'').

MS: (El 70 eV, 2 KV, 300 $\mu$A, ST 250$^0$; DE 240$^0$, 10$^{-6}$T), m/e 623 (rel. int. 2%), 581 (1), 539 (1), 500 (1), 477 (4), 458 (3), 435 (3), 416 (6), 354 (7), 342 (6), 312 (23), 300 (24), 271 (24), 270 (100), 242 (14), 241 (13), 229 (2), 213 (4), 190 (6), 189 (50), 169 (5), 163 (30), 153 (24), 147 (75), 119 (25), 118 (27), 109 (8), 91 (20), 69 (28).
Extraction of flavones and biflavones from the leaves of *Callitris glauca* K. Br. (Cupressaceae)

*Callitris glauca* K. Br. (Cupressaceae) was procured from Forest Research Institute, Dehradun (U.P.), India. The dried and powdered leaves (2.5 Kg) were completely exhausted with hot acetone and the acetone extracts were concentrated first at atmospheric pressure and then under reduced pressure. A gummy dark green mass was obtained. This was treated with petroleum ether (60–80°) and benzene till the solvent in each case was almost colourless, to remove nonflavanoidic and resinous matter. The gummy mass was refluxed with ethylacetate for 20 hrs and filtered. The filtrate was evaporated to dryness and the residue treated with hot water. The water insoluble mass was dissolved in alcohol and dried under reduced pressure to give a dark green residue (12 g) which responded to the usual colour tests for flavanoids.

Purification of water insoluble flavanoid mixture by column chromatography

The crude flavanoid mixture (12 g) was adsorbed on silica gel (15 g) and transferred over to a column of silica gel (225 g) set with petroleum ether (60–80°). The column was eluted with organic solvents in the increasing order of polarity. The results are given in Table-XXXIV.
The fraction obtained with benzene-ethylacetate, ethylacetate and acetone gave usual flavanoid colour tests. They were combined and the solvent distilled off to give yellowish brown residue (2.5 g).

**Separation of flavanoid mixture - preparative thin layer chromatography**

The glass plates (40x20 cm) were coated with a well stirred suspension of silica gel G (BDH) using thin layer spreader, (Toshniwal-India). The coated layer of silica gel was approximately 0.5 mm thick. After drying for two hrs at room
temperature, the plates were activated at 120° for one hour and preserved in a desiccator until required.

The complexity of the yellowish brown residue 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-ethylformate-formic acid (TEF; 5:4:1).
(c) Toluene-pyridine-acetic acid (TPA; 10:1:1).
(d) Benzene-ethylacetate-acetic acid (8:5:2).
(e) Chloroform-ethylacetate (1:1).

In solvent system (a), the flavanoid mixture showed four compact brown spots in UV light. They were labelled as CGI, R_f 0.05; CGII, R_f 0.18; CGIII, R_f 0.37 and CGIV, R_f 0.54. The differences in R_f values were so marked as to make it the developing system of choice for preparative thin layer chromatography. This solvent system was used for all the subsequent separations for the flavanoids.

The yellowish brown residue was dissolved in pyridine and the obtained solution (5%) was applied to plates (40x20 cm) with the help of mechanical applicator (Desaga, Heidelberg) 2 cm. from the lower edge of the plates. The plates mounted on stainless steel frames were placed in a Desaga glass chamber (45x22x25 cm) containing 500 ml of the developing solvent
(benzene-pyridine-formic acid, 36:9:5). When the solvent front travelled 15 cm from the starting line, the development was interrupted and plates were dried at room temperature. The positions of the bands were marked in UV light. The marked pigment zones were scraped with the help of a spatulla and eluted in separate columns with dry acetone. The solvent was recovered till the eluents were reduced to 20-30 ml. The addition of water yielded yellow precipitate in each case. The precipitate was filtered, washed with water several times and dried. The homogeneity of the pigments was checked by TLC using five solvent systems already listed. The four pure components were obtained as CGI (50 mg), CGII (400 mg), CGIII (200 mg) and CGIV (30 mg).

The complexities of all the fractions CGI, CGII, CGIII and CGIV were studied by TLC examination of their fully methylated products.

3',4',5,7,8-Pentahydroxyflavone (Hypolaetin) (CGI)

It was crystallized from ethanol as yellow needles (20 mg), m.p. 300°, Rf 0.05 (BPF).

3',4',5,7,8-Pentamethoxyflavone (CGIM)

CGI (20 mg), dry acetone (100 ml), anhydrous potassium carbonate (0.5 g) and dimethyl sulphate (0.5 ml) were refluxed on water bath for about 30 hrs. Refluxing continued until it
gave a negative alc. FeCl$_3$ test. It was then filtered and the residue washed several times with hot acetone. The filtrate and washing were combined and evaporated to dryness. The yellow residue washed 2-3 times with petroleum ether and then taken in chloroform (50 ml) and washed several times with water. The chloroform solution dried over anhydrous sodium sulphate, concentrated and purified by column chromatography. It was crystallized from CHCl$_3$-MeOH to give 3',4',5,7,8-Pentamethoxyflavone as colourless plates (15 mg); m.p. 195$^\circ$ (lit. m.p. 192-193$^\circ$); $R_f$ 0.51 (BPF); $\lambda_{max}^{MeOH}$ 250, 275 and 340 nm.

NMR (CDCl$_3$): Values on $\tau$ Scale:

2.42 (1H, q, J=9.2 Hz, H-6'); 2.59 (1H, d, J=2 Hz, H-2'); 3.02 (1H, d, J=9 Hz, H-5'); 3.40 (1H, s, H-3); 3.57 (1H, s, H-6); 6.14 (15H, 50Me).

MS: Main peaks, $M^+$ 372 (64), m/e 357 (100), 344 (20), 328 (24), 299 (12), 210 (8), 195 (16), 172 (10), 167 (32), 165 (12), 162 (8), 149 (32).

CGII: It was found to be the mixture of amentoflavone and other two minor components by TLC examination of CGII and its complete methyl ethers. The minor components were not identified.
I-4',II-4',I-5,II-5,1-7,II-7-Hexa-O-methyl[1-3',II-8]biflavone (CGII(0M))

A mixture of CGII (100 mg), anhydrous potassium carbonate (2 g) and dimethylsulphate (1 ml) in dry acetone (400 ml) was refluxed on a water bath for 10 hrs. The mixture on usual work up and purification by preparative TLC yielded a white solid which crystallized from CHCl₃-MeOH to give hexamethyl ether of amentoflavone as colourless cubes (40 mg); m.p. 226-227° (lit.153 m.p. 228°); Rₙ 0.40 (BPF).

NMR (CDCl₃): Values on T Scale

2.17 (1H, d, J=3 Hz, H-I-2'); 2.14 (1H, q, J₁=9 Hz, J₂=3 Hz, H-I-6'); 2.63 (2H, d, J=9 Hz, H-II-2',II-6'), 2.88 (1H, d, J=9 Hz, H-II-5'); 3.38 (1H, s, H-II-6), 3.26 (2H, d, J=9 Hz, H-II-3',5'); 3.54 (1H, d, J=3 Hz, H-I-8); 3.44, 3.49 (2H, s, H-I-3,II-3); 3.68 (1H, d, J=3 Hz, H-I-6); 6.26 (6H, s, I-4', II-4'); 6.08, 5.95 (6H, s, H-I-5,II-5); 6.17, 6.12 (6H, s, H-I-7, II-7).

CGII (200 mg) was subjected to CCD separation between ethylmethylketone and a borate buffer (pH 9.80). The main part was recovered (150 mg) and labelled as CGII(X).
I-4',II-4',I-5,II-5,I-7,II-7-Hexaacetoxy[1-3',II-8]biflavone (CGIIIXA)

A solution of CGIIX (60 mg) in Pyridine (0.5 ml) and acetic anhydride (1 ml) was heated on a water bath for 2 hrs. The mixture on usual workup and crystallization from CHCl₃-MeOH gave colourless cubes of amentoflavone hexaacetate, m.p. 241-42° (lit. m.p. 240°).

NMR (CDCl₃): Values on δ Scale

\[\begin{align*}
1.97 & (1H, d, J=3 Hz, H-I-2') \\
2.06 & (1H, q, J_1=9 Hz, J_2=3 Hz, H-I-6') \\
2.50 & (2H, d, J=9 Hz, H-II-2',II-6') \\
2.52 & (1H, d, J=9 Hz, H-I-5') \\
2.99 & (1H, s, H-II-6) \\
2.94 & (2H, d, J=9 Hz, H-II-3',II-5') \\
2.74 & (1H, d, J=3 Hz, H-I-8) \\
3.23 & (2H, s, H-I-3,II-3) \\
3.18 & (1H, d, J=3 Hz, H-I-6) \\
7.76, 7.96 & (6H, s, I-4',II-4') \\
7.53, 7.57 & (6H, s, H-I-5,II-5) \\
7.70, 7.20 & (6H, s, H-I-7,II-7).
\end{align*}\]

CGIII: The fraction CGIII was found to be the mixture of monomethyl ether of amentoflavone and hinokiflavone (minor) by TLC examination of CGIII and its completely methylated products (Rf value and characteristic shade in UV light). CGIII on acetylation and then fractional crystallization gave an acetate CGIIIIA which was analysed as I-7-O-methylamentoflavone penta-acetate.
1,4',11-4',1-5,11-5,11-7-Pentaacetoxy-1-7-O-methyl[1-3',11-8]
biflavone (CGIII A)

CGIII (30 mg), pyridine (0.3 ml) and acetic anhydride (0.5 ml) were refluxed on a water bath for 2 hrs. The reaction mixture was cooled to room temperature and poured on to crushed ice. The solid product was filtered, washed with water and dried. On repeated crystallization from CHCl₃-MeOH, it gave colourless cubes (20 mg), m.p. 244° (lit. m.p. 245°).

NMR (CDCl₃): Values on γ Scale

1.96 (1H, d, J=3 Hz, H-I-2'); 2.04 (1H, q, J₁=9 Hz, J₂=3 Hz, H-I-6'); 2.48 (2H, d, J=9 Hz, H-II-2',11-6'), 2.55 (1H, d, J=9 Hz, H-I-5'); 3.00 (1H, s, H-II-6); 2.92 (2H, d, J=9 Hz, H-II-3',11-5'); 3.22 (1H, d, J=3 Hz, H-I-8); 3.35, 3.36 (1H, each s, H-I-3,11-3); 3.41 (1H, d, J=3 Hz, H-I-6); 7.70, 7.76 (6H, s, H-I-4',II-4'); 7.52, 7.58 (6H, s, H-I-5,II-5); 6.16, 7.96 (6H, s, H-I-7,11-7).

CGIV: The minor fraction CGIV was methylated using dimethyl sulphate and potassium carbonate in dry acetone. TLC examination of the methylated product showed one spot in UV light, corresponding to hexamethyl ether of amentoflavone. Thus CGIV was found to be dimethyl ether of amentoflavone (Rₜ value and shade in UV light).
Water soluble fraction

The water soluble portion was extracted with ethylacetate and the solvent evaporated. The residue gave two spots labelled as CgI and CgII, checked on TLC polyamide (Woelm) using ethylmethylketone-toluene-acetic acid-methanol-water (80:10:2:5:6) as the developing solvent system.

Glycoside from water soluble fraction of Callitris glauca R.Br.

The ethylacetate extract of Callitris glauca R.Br. was treated with hot water. The water soluble fraction was extracted with ethylacetate. The process was repeated three times till the aqueous solution was almost colourless. The ethylacetate extracts were combined and the solvent evaporated. The semi solid mass left behind was marked as Cg.

Chromatographic examination of Cg

The methanolic solution of Cg was subjected to chromatographic analysis on Whatmann no.1 filter paper employing both the ascending and the descending techniques. The following solvent systems were used.

The chromatograms were run for 12 hrs. After drying at room temperature the chromatograms on examination under UV light revealed only one spot.

Thin layer chromatographic plates (5x20 cm) of 0.5 mm thickness were prepared by the usual methods using polyamide (Woelm). The spot of Cg in methanol was applied to the starting line and the plates were run with the solvent systems as follows.


The plates were run to the distance of 15 cm removed from the tank and dried. On examination under UV light the chromatogram showed the presence of two bands in solvent system number 2. They were labelled as CgI, \( R_f \) 0.83 (major) and CgII, \( R_f \) 0.65 (minor).

**Separation of Cg by column chromatography**

The semi solid mass Cg was dissolved in methanol, adsorbed on silica gel and transferred over to a column of
silica gel set with petroleum ether (60-80°). The elution was performed successively with petrol (60-80°), benzene, chloroform, benzene-ethylacetate (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80) and ethylacetate. The benzene-ethylacetate fraction (40:60) gave a single spot (CgI) on TLC polyamide (Woelm). The second fraction obtained with the same solvent system (30:70) gave a minor component as yellow solid mass (CgII). The fractions thus separated were tested for homogeneity by TLC on polyamide (Woelm) using the same solvent systems as before. Both the fractions gave the tests for flavanoid. CgII being a minor component was not identified.

**Kaempferol-5-O-rhamnoside (CgI)**

The fraction CgI was crystallized from ethylacetate to give yellow crystals, (50 mg), m.p. 198°, Rₐ 0.83 (ETALI) on polyamide (Woelm).

**Kaempferol (CgII)**

An alcoholic solution of CgI (10 mg) was heated with 8% aqueous hydrochloric acid on water bath. The heating was continued for one hr to ensure complete hydrolysis. After leaving for 1 hr at room temperature, the yellow solid (CgII), thus separated out was filtered, washed well with water and dried. It was crystallized from methanol as yellow needles (5 mg), m.p. 275-278°, Rₐ 0.54 (BPF).
Chromatographic Identification of Sugar

The filtrate was passed through polyamide column to remove aglycone matter. The filtrate was concentrated on watch glass to a syrup. The sugar was identified by paper chromatography on Whatmann no.1 filter paper using ethylacetate:pyridine:water (12:5:4) with authentic sugar. The spraying reagent was prepared by dissolving 1 g of aniline and 1.66 g of phthalic acid in 100 ml butanol saturated with water and sprayed the filter paper. The chromatogram on drying at 100-105° showed the presence of rhamnose only.

5-O-rhamnosyl-3,7,4'-trimethoxyflavone (CgIM)

A mixture of CgI (40 mg), anhydrous potassium carbonate (1 g) and dimethyl sulphate (0.5 ml) in dry acetone (150 ml) was refluxed on a water bath for 10 hrs. The mixture on usual work-up yielded yellow solid which crystallized from CHCl₃-MeOH to give (CgIM) as yellow needles (35 mg), m.p. 120°.

3,7,4'-Trimethoxy-5-hydroxyflavone (CgIMa)

An alcoholic solution of CgIM (35 mg) was refluxed with 8% aqueous hydrochloric acid on water bath for two hrs. After usual work up, yellow solid residue was obtained which crystallized from CHCl₃-MeOH to give (CgIMa) yellow cubes (30 mg), m.p. 140-143° (lit.152 m.p. 144-147°).
3,7,4'-Trimethoxy-5-acetoxyflavone (CgIMaA)

A mixture of CgIMa (25 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was heated on a water bath for 2 hrs. The reaction mixture on usual work up and crystallization from CHCl₃-MeOH gave colourless cubes CgIMaA, m.p. 135-136°.

NMR (CDCl₃): Values on T Scale

1.76 (2H, d, J=9 Hz, H-2',6'); 2.82 (2H, d, J=9 Hz, H-3',5'); 3.14 (1H, d, J=2.5 Hz, H-8); 3.40 (1H, d, J=2.5 Hz, H-6); 7.54 (3H, 5-OAc); 6.10, 6.14 (9H, 3,7,4'-OMe).
A biflavone from the leaves of *Guruga pinnata* Roxb. (Burseraceae)

Dried and powdered leaves of *Guruga pinnata* Roxb. (1.5 Kg) were completely exhausted with boiling acetone. The combined acetone extracts were concentrated to give a dark viscous mass. This was refluxed successively with petroleum ether (60-80°), benzene, chloroform and ethylacetate till the solvent in each case was almost colourless. The ethylacetate fraction was treated with hot water and the insoluble portion was dissolved in ethanol and dried under reduced pressure. A dark brown residue (4 g) thus obtained responded to the usual colour tests for flavanoids.

The crude dark brown residue (4 g) was dissolved in dry acetone and the solution added to a column containing silica gel as adsorbent in petroleum ether. After development of the column, it was eluted with organic solvents in the increasing order of polarity, i.e. petroleum ether (60-80°), benzene, chloroform and ethylacetate-benzene (1:9, 2:8 and 3:7). The fraction obtained with ethylacetate-benzene (3:7) gave the usual flavanoid colour tests. This was further purified by preparative TLC (silica gel) using benzene-pyridine-formic acid (36:9:5) as the developing solvent system. The yellow solid (0.2 g) obtained, gave a single spot on TLC and labelled as GPI, $R_f$ 0.18, m.p. > 320°.
Methylation (GPI)

GPI (80 mg) was methylated using dimethyl sulphate (0.5 ml) and potassium carbonate (2 g) in 250 ml of dry acetone. The methylated product on TLC examination showed the presence of amentoflavone hexamethyl ether ($R_f$ values and characteristic fluorescence in UV light). This was purified by preparative thin layer chromatography and labelled as GPIM.

*I-4',II-4',I-5,II-5,I-7,II-7-Hexa-C-methyl[I-3',II-8]biflavone (GPIM)*

It was crystallized as colourless needles (50 mg) from CHCl₃-MeOH; m.p. 181-182° (lit.²¹ m.p. 170-171°), $R_f$ 0.40.

NMR (CDCl₃): Values on $\tau$ Scale

2.18 (1H, d, $J=3$ Hz, H-I-2'); 2.06 (1H, q, $J_1=3$ Hz, $J_2=8.5$ Hz, H-I-6'); 2.64 (2H, d, $J=8.5$ Hz, H-II-2',6'); 2.91 (1H, d, $J=8.5$ Hz, H-I-5'); 3.26 (2H, d, $J=8.5$ Hz, H-II-3',5'); 3.54 (1H, d, $J=3$ Hz, H-I-8); 3.68 (1H, d, $J=3$ Hz, H-I-6); 3.38 (1H, s, H-II-6); 3.44, 3.50 (1H each, s, H-I-3,II-3); 5.94, 6.08, 6.11, 6.18, 6.25, 6.28 (18H, 6OMe).
A solution of GPI (80 mg) in pyridine (0.5 ml) and acetic anhydride (1 ml) was heated on a water bath for 2 hrs. The mixture on usual work up and crystallization from CHCl₃-MeOH gave colourless needles of amentoflavone hexaacetate (60 mg) m.p. 242-243° (lit.¹¹ m.p. 235°).

NMR (CDCl₃): Values on τ Scale

1.99 (1H, d, J=3 Hz, H-I-2'); 2.05 (1H, q, J₁=3 Hz, J₂=8.5 Hz, H-I-6'); 2.52 (2H, d, J=8.5 Hz, H-II-2',6'); 2.56 (1H, d, J=8.5 Hz, H-I-5'); 2.96 (2H, d, J=8.5 Hz, H-II-3',5'); 2.77 (1H, d, J=2.5 Hz, H-I-8); 3.10 (1H, d, J=2.5 Hz, H-I-6); 3.01 (1H, s, H-II-6); 3.30, 3.35 (1H each, s, H-I-3,II-3); 7.54, 7.60, 7.72, 7.77, 7.95, 7.98 (18H, 60Ac).
Extraction of biflavonoids from the leaves of *Cunninghamia lanceolata* Hook. (Taxodiaceae)

Dried and powdered leaves (1.5 Kg) of *Cunninghamia lanceolata* Hook. procured from Darjeeling (W.B., India) were completely exhausted with petroleum ether (40-60°) and then with boiling acetone. From the acetone extract, on recovery of the solvent, a gummy dark green mass was obtained. This was refluxed with petroleum ether (40-60°), benzene and chloroform till the solvent in each case was almost colourless. The residue left behind was then treated with boiling water. The insoluble dark brown gummy mass was refluxed with ethyl acetate for 8-10 hrs and filtered off. The filtrate was concentrated to give a dark brown solid (2 g). It was further purified by column chromatography (silica gel). The column was eluted successively with petroleum ether (40-60°), benzene, chloroform and benzene-ethyl acetate (1:1 and 1:2). The last two fractions were combined and solvent was distilled off. A yellowish brown solid (1 g) thus obtained on TLC examination (BPF; 36:9:5) showed the presence of five compact brown spots in UV light. It was, therefore, subjected to preparative TLC (silica gel, E. Merck) and the five bands were separated and labelled as CLI (R_f 0.17, 150 mg); CLII (R_f 0.37, 150 mg); CLIII (R_f 0.50, 40 mg); CLIV (R_f 0.54, 120 mg) and CLV (R_f 0.61, 30 mg).
**CLI**: CLI (120 mg) on methylation using dimethylsulphate and potassium carbonate in dry acetone gave two methyl ethers which were worked up as described earlier. They were separated by PLC (silica gel) and marked as CLIMI, 80 mg and CLIMII, 25 mg. TLC examination of CLI and its complete methyl ethers (CLIMI and CLIMII), showed it to be a mixture of amentoflavone and robustaflavone.

**I-4',II-4',I-5,II-5,I-7,II-7-Hexa-C-methyl[I-3',II-8]biflavone (CLIMI)**

It was crystallized from CHCl₃-MeOH as colourless needles (50 mg), m.p. 226-227°.

**NMR (CDCl₃): Values on T Scale**

3.54 (d, 1H, J=2.5 Hz, H-I-8); 3.68 (d, 1H, J=2.5 Hz, H-I-6); 3.37 (s, 1H, H-II-6); 3.50, 3.43 (s, 1H each H-I-3,II-3); 2.06 (q, 1H, J₁= 3 Hz, J₂=9 Hz, H-I-6'); 2.15 (d, 1H, J=3 Hz, H-I-2'); 2.89 (d, 1H, J=9 Hz, H-I-5'); 2.62 (d, 2H, J=9 Hz, H-II-2',6'); 3.27 (d, 2H, J=9 Hz, H-II-3',5'); 5.94 (3H, OMe-II-5); 6.08 (3H, OMe-I-5); 6.10, 6.18 (6H, OMe-I-7,II-7); 6.20, 6.24 (6H, OMe-I-4',II-4').
I-4',11-4',1-5,11-5,1-7,11-7-Hexa-O-methyl[1-3',11-6]biflavone (CLIMII)

The minor band CLIMII on crystallization from CHCl₃-MeOH gave colourless needles (15 mg) m.p. 305-308°.

NMR (CDCl₃): Values on T Scale

2.98 (d, 2H, J=9 Hz, H-11-3',5'); 2.91 (d, 1H, J=9 Hz, H-I-5'), 2.31 (d, 2H, J=9 Hz, H-II-2',6'); 2.19 (d, 1H, J=2.5 Hz, H-I-2'); 2.13 (q, 1H, J₁=2.5 Hz, J₂=9 Hz, H-I-6'); 3.42 (d, 1H, J=2.5 Hz, H-I-8); 3.12 (s, 1H, H-II-8); 3.35 (s, 2H, H-I-3,II-3); 3.65 (d, 1H, J=2.5 Hz, H-I-6); 6.12, 6.14, 6.12, 6.18, 6.07, 6.39 (s, 3H each, OMe-II-4',1-4',1-7,II-7,1-5,II-5 respectively).

CLII: CLII was methylated with Me₂SO₄/K₂CO₃ in dry acetone and was found to be the mixture of hinokiflavone and monomethyl ether of amentoflavone by TLC examination of CLII and its completely methylated products. The methyl ethers were separated by preparative TLC to give pure methyl ethers CLIIIX and CLIIIMII.

CLII (150 mg) when subjected to CCD separation between ethylmethyl ketone and borate buffer (pH 9.6) yielded two components CLIIIX (80 mg) and CLIIY (35 mg).
I-4',II-4',I-5,II-5,II-7-Pentaacetoxy-I-7-O-methyl[I-3',II-8] biflavone (CLIIIXA)

CLIIIX (40 mg) was acetylated with pyridine and acetic anhydride and worked up as usual. It was crystallized from CHCl₃-MeOH (30 mg) m.p. 243°.

NMR (CDCl₃): Values on γ Scale

1.96 (d, 1H, J=3 Hz, H-I-2'); 2.06 (q, 1H, J₁=9 Hz, J₂=3 Hz, H-I-6'); 2.50 (d, 2H, J=9 Hz, H-II-2',II-6'); 2.54 (d, 1H, J=9 Hz, H-I-5'); 3.00 (s, 1H, H-II-6); 2.94 (d, 2H, J=9 Hz, H-II-3',II-5'); 3.22 (d, 1H, J=3 Hz, H-I-8); 3.34, 3.36 (s, 1H each, H-I-3,II-3); 3.41 (d, 1H, J=3 Hz, H-I-6); 7.92, 7.96 (s, 6H, H-I-4',II-4'); 7.52, 7.56 (s, 6H, H-I-5,II-5); 6.16, 7.74 (s, 6H, H-I-7,II-7).

II-4',I-5,II-5,I-7,II-7-Penta-O-methyl[I-4'-O-II-6]biflavone (CLIIIMII)

It was crystallized from CHCl₃-MeOH as colourless needles (20 mg), m.p. 260-261°.

NMR (CDCl₃): Values on γ Scale

3.66 (d, 1H, J=2.5 Hz, H-I-6); 3.46 (d, 1H, J=2.5 Hz, H-I-8); 3.44 (s, 1H, H-II-8); 3.42* (s, 1H, H-I-3); 3.40* (s, 1H,
H-II-3); 2.04 (d, 2H, J=8.5 Hz, H-I-2',I-6'); 2.14 (d, 2H, J=8.5 Hz, H-II-2',I-6'); 3.04 (d, 2H, J=8.5 Hz, H-I-3',I-5'); 2.94 (d, 2H, J=8.5 Hz, H-II-3',I-5'); 6.06-6.12 (s, 15H, OMe-II-4',I-5,I-5,I-7,I-7).

*Alternative assignment is possible.

Il-4',l-5,Il-5,I-7,II-7-Pentaacetoxy [I-4'-0-II-6]biflavone
(CLIIYA)

CLIIY (30 mg), pyridine (0.5 ml) and acetic anhydride (1 ml) was refluxed on a water bath for 2 hrs. The reaction mixture was poured on to crushed ice and left over night. The solid was filtered, washed with water and dried.

It was crystallized from CHCl$_3$-MeOH as colourless cubes (25 mg), m.p. 236-240°.

NMR (CDCl$_3$): Values on T Scale

3.08 (d, 1H, J=2 Hz, H-I-6); 2.49 (d, 1H, J=2 Hz, H-I-8); 2.80 (s, 1H, H-II-8); 3.24* (s, 1H, H-I-3); 3.34* (s, 1H, H-II-3); 2.00 (d, 2H, J=9.5 Hz, H-I-2',6'); 2.04 (d, 2H, J=9.5 Hz, H-II-2',6'); 3.75 (d, 4H, J=9.5 Hz, HI-3',5' and II-3',5'); 7.57, 7.66, 7.75, 7.88, 7.89 (s, 3H each, 5-OAc).

*Alternative assignment is possible.
CLIII: CLIII was found to be a monomethyl ether of hinokiflavone by TLC examination of CLIII and its complete methyl ether. CLIII (40 mg) on derivatization gave an acetate (CLIIIA) as usual.

II-4',I-5,II-5,II-7-Tetraacetoxy-II-7-O-methyl[II-4'-O-II-6]
biflavone (CLIIIA)

It was crystallized from CHCl₃-MeOH as colourless needles (30 mg), m.p. 213-214°.

NMR (CDCl₃): Values on T Scale

3.18 (d, 1H, J=2.5 Hz, H-I-6); 2.70 (d, 1H, J=2.5 Hz, H-I-8); 2.98 (s, 1H, H-II-8); 3.40 (s, 1H, H-I-3); 3.44 (s, 1H, H-II-3); 2.10 (d, 2H, J=9 Hz, H-I-2',6'); 2.24 (d, 2H, J=9 Hz, H-II-2',6'); 2.99 (d, 2H, J=9 Hz, H-I-3',5'); 2.76 (d, 2H, J=9 Hz, H-II-3',5'); 6.13 (s, 3H, OMe-II-7); 7.70 (6H, OAc-I-5, II-5); 7.76, 7.63 (s, 3H each, OAc-I-7,II-4').

CLIV: CLIV was methylated using dimethyl sulphate and potassium carbonate in dry acetone. The methylated mixture by TLC examination showed the presence of amentoflavone hexamethyl ether and apigenin trimethyl ether (Rf values and characteristic fluorescence in UV light).

The CCD separation of CLIV (100 mg) between ethyl methyl ketone and borate buffer (pH 9.5) gave the following two
fractions, CLIVX and CLIVY. CLIVX and CLIVY were characterized as I-7,II-7-di-O-methylamentoflavone and apigenin by NMR studies of their acetate respectively.

I-4',II-4',I-5,II-5-Tetraacetoxy-1-7,-II-7-di-O-methyl[1-3',II-8]biflavone (CLIVXA)

CLIVX (30 mg) was acetylated with pyridine (0.5 ml) and acetic anhydride (1 ml). After usual work up, the acetate (CLIVXA) was crystallized from CHCl₃-MeOH as colourless needles (20 mg).

NMR (CDCl₃): Values on T Scale

2.02, 2.08 (d, q, 2H, H-I-2',I-6'); 2.56 (d, 1H, H-I-5'); 2.50 (d, 2H, H-II-2',II-6'); 2.96 (d, 2H, H-II-3',II-5'); 3.20 (d, 1H, H-I-8); 3.40 (d, 1H, H-I-6); 3.24 (s, 1H, H-II-6); 3.45 (s, 1H each, H-I-3, II-3); 8.01, 7.74 (s, 3H each, OAc-I-4',II-4'); 7.50, 7.59 (s, 3H each, OAc-I-5,II-5); 6.14, 6.17 (s, 3H each, OMe-I-7,II-7).

5,7,4'-Triacetoxyflavone (CLIVYA)

CLIVY (25 mg) was heated with pyridine (0.5 ml) and acetic anhydride (1 ml) on water bath for 2 hrs, worked up as usual and crystallized from CHCl₃-MeOH (15 mg) as colourless needles, m.p. 185-186°.
NMR (CDCl$_3$): Values on $\gamma$ Scale

3.42 (s, 1H, H-3); 3.20 (d, 1H, J=2.5 Hz, H-6); 2.80 (d, 1H, J=2.5 Hz, H-8); 2.75 (d, 2H, J=9 Hz, H-3',5'); 2.14 (d, 2H, J=9 Hz, H-2',6'); 7.68 (s, 6H, OAc-4',7); 7.58 (s, 3H, OAc-5).

I-5,II-5,II-7-Triacetoxy-I-4',II-4',II-7-tri-O-methyl[I-3',II-8]

biflavone (CLVA)

CLV was found to be trimethyl ether of amentoflavone by TLC examination of CLV and its complete methyl ether. CLV (20 mg) on derivatization gave an acetate (CLVA) which was crystallized from CHCl$_3$-MeOH (14 mg), m.p. 190-192°.

NMR (CDCl$_3$): Values on $\gamma$ Scale

2.10, 2.15 (d, q, 2H, J$_1$=9 Hz, J$_2$=3 Hz, H-I-2',I-6'); 2.88 (d, 1H, J=9 Hz, H-I-5'); 2.66 (d, 2H, J=9 Hz, H-II-2',6'); 3.22 (d, 2H, J=9 Hz, H-II-3',5'); 3.50 (s, 1H, H-I-3); 3.40 (s, 1H, H-II-3); 2.73 (d, 1H, J=2.5 Hz, H-I-8); 3.22 (d, 1H, J=2.5 Hz, H-I-6); 3.20 (s, 1H, H-II-6); 3.27 (s, 3H, OMe-I-4'); 6.40 (s, 3H, OMe-II-4'); 7.76 (s, 3H, OAc-I-7); (6.20) (s, 3H, OMe-II-7); 7.53 (s, 3H, OAc-I-5); 7.42 (s, 3H, OAc-II-5).
Extraction of flavanones from the leaves of *Rhus insigne* Hook. f. (Anacardiaceae)

*Rhus insigne* Hook. f. (Anacardiaceae) was procured from Kurseong, Darjeeling (W. Bengal; INDIA). The dried and powdered leaves (1 Kg) were completely exhausted with petroleum ether (40-60°) and then with boiling acetone. From the acetone extract, on recovery of the solvent, a gummy dark green mass was obtained. It was treated with different solvents as described earlier and gave a dark brown solid which was further purified by column chromatography (silica gel) eluting successively with pet. ether (40-60°), benzene, chloroform and benzene-ethylacetate (9:1, 8:2 and 7:3). The last three fractions were combined and solvent distilled off. A yellow solid mass (1 g) was obtained. TLC examination of the crude mixture in TEF (toluene:ethyl-formate:formic acid, 5:4:1) revealed four compact spots in UV light which were separated by column chromatography (silica gel) and labelled as RI-I (200 mg, R_f 0.60), RI-II (300 mg, R_f 0.64), RI-III (150 mg, R_f 0.70) and RI-IV (50 mg, R_f 0.88).

3,7,3',4'-Tetrahydroxyflavanone (RI-I)

The fraction RI-I was crystallized from CHCl_3-MeOH as fine needles, m.p. 227-230° (lit.¹⁵⁷ m.p. 228-229°).
UV absorption ($\lambda_{\text{max}}$, nm)

MeOH 276, 312 sh; NaOMe 250, 296 sh, 332 (dec.); AlCl$_3$ 233, 306, 347 sh; AlCl$_3$/HCl 232, 276, 306; NaOAc 254 sh, 283, 332; NaOAc/H$_3$BO$_3$ 279, 312 sh.

Acetylation of RI-I

On acetylation with Ac$_2$O/Pyridine and usual work up, it gave an acetate hI-IA which was crystallized from ethanol, m.p. 148° (lit.$^{158}$ m.p. 149°).

NMR (CDCl$_3$): Values on $\gamma$ Scale

3.35 (q, 1H, $J_1$=9 Hz, $J_2$=2.5 Hz, H-6); 3.15 (d, 1H, J=2.5 Hz, H-8); 2.05 (d, 1H, J=9 Hz, H-5); 2.60 (m, 3H, H-2',5', 6'); 4.25 (d, 1H, J=11.5 Hz, H-2); 4.70 (d, 1H, J=11.5 Hz, H-3); 7.95 (s, 3H, OAc-7); 7.72 (s, 9H, OAc-3,3',4').

3,5,7,3',4'-Pentahydroxyflavanone (RI-II)

It was crystallized from benzene ethylacetate as colourless needles (200 mg), $R_f$ 0.64 (TEF), m.p. 245° (lit.$^5$ m.p. 221-253°).
UV absorption ($\lambda_{\text{max}}, \text{nm}$)

MeOH 288, 325 sh; NaOMe 244 sh, 324 dec.; AlCl$_3$ 278 sh, 310, 373; AlCl$_3$/HCl 310, 373; NaOAc 287 sh, 325; NaOAc/H$_3$BO$_3$ 290, 335 sh.

**Acetylation of RI-II**

A solution of RI-II (50 mg) in Pyridine (0.5 ml) and acetic anhydride (1 ml) was heated on a water bath for 2 hrs. The mixture on usual work up, crystallized from CHCl$_3$-MeOH and gave colourless needles (RI-IIA) m.p. 145-149° (lit.$^{160}$ m.p. 147-148°).

**NMR (CDCl$_3$):** Values on $\gamma$ Scale

2.64 (m, 3H, H-2',5',6'); 3.30 (d, 1H, J=2.5 Hz, H-6); 3.08 (d, 1H, J=2.5 Hz, H-8); 4.24 (d, 1H, J=12 Hz, H-2); 4.65 (d, 1H, J=12 Hz, H-3); 7.61 (s, 3H, OAc-5); 7.71 (s, 9H, OAc-3,3',4'); 7.96 (s, 3H, OAc-7).

**5,7,3',4'-Tetrahydroxyflavanone (RI-III)**

It was crystallized from benzene-ethylacetate as yellow cubes (100 mg), $R_f$ 0.70, m.p. 264° (lit.$^{161}$ 265-266°).
UV absorption (λ\textsubscript{max}, nm)

MeOH 287, 322 sh; NaOMe 244, 322; AlCl\textsubscript{3} 308, 376; AlCl\textsubscript{3}/HCl 307, 371; NaOAc 287 sh, 323; NaOAc/H\textsubscript{3}BO\textsubscript{3} 287, 331 sh.

Acetylation of RI-III

It was acetylated with Ac\textsubscript{2}O/Pyridine and crystallized from CHCl\textsubscript{3}-MeOH as colourless needles, m.p. 140-145\degree (lit\textsuperscript{160} m.p. 141-142\degree).

NMR (CDCl\textsubscript{3}): Values on τ Scale

2.73 (m, 3H, H-2',5',6'); 3.36 (d, 1H, J=2.5 Hz, H-6); 3.10 (d, 1H, J=2.5 Hz, H-8); 4.55 (q, 1H, J\textsubscript{1}=12 Hz, J\textsubscript{2}=4 Hz, H-2); 6.77 (q, 1H, J\textsubscript{1}=12 Hz, J\textsubscript{2}=17 Hz, H-3\textsubscript{ax}); 7.08 (q, 1H, J\textsubscript{1}=4 Hz, J\textsubscript{2}=17 Hz, H-3\textsubscript{eq}); 7.63 (s, 3H, OAc-5); 7.73 (s, 9H, OAc-3,3',4').

Dehydrogenation\textsuperscript{162} and methylation of RI-III

A solution of RI-III (50 mg) in DMSO (1 ml), two drops of conc. H\textsubscript{2}SO\textsubscript{4} and a small crystal of iodine was heated at 120\degree for half an hour. The mixture was poured on to crushed ice and worked up as usual. It was crystallized from ethanol and gave yellow cubes RI-IIID (40 mg), m.p. 328\degree (lit\textsuperscript{5d} m.p. 330-331\degree). RI-IIID was methylated with Me\textsubscript{2}SO\textsubscript{4}/K\textsubscript{2}CO\textsubscript{3} in dry acetone. The
product on usual work up and crystallization from CHCl₃-MeOH, gave colourless needles RI-IIIDM (25 mg), m.p. 190° (lit. 192-193°).

**NMR (CDCl₃): Values on τ Scale**

2.50 (q, 1H, J₁=9Hz, J₂=3Hz, H-6'); 2.65 (d, 1H, J=3 Hz, H-2'); 3.05 (d, 1H, J=9 Hz, H-5'); 3.35 (d, 1H, J=3 Hz, H-8); 3.45 (s, 1H, H-3); 3.65 (d, 1H, J=3 Hz, H-6); 6.04, 6.08 (s, 6H, OMe-5,7); 6.12, 6.18 (s, 6H, OMe-3',4').

**5,7,4'-Trihydroxyflavanone (RI-IV)**

The fraction, RI-IV was crystallized as pale yellow needles from benzene-ethylacetate (25 mg), Rf 0.88 (TEF), m.p. 255-257°.

**UV absorption (λ_max, nm)**

MeOH 287, 324 sh; NaOMe 243, 321; AlCl₃ 310, 373; AlCl₃/HCl 309, 369, NaOAc 282 sh, 321; NaOAc/H₃BO₃ 288, 330 sh.

**NMR (CDCl₃): Values on τ Scale**

2.66 (d, 2H, J=9.5 Hz, H-2',6'); 3.15 (d, 2H, J=9.5 Hz, H-3',5'); 4.06 (s, br, 2H, H-6,8); 4.60 (q, 1H, J₁=12 Hz, J₂=4 Hz, H-2); 7.24-7.04 (m, 2H, J₁=12 Hz, J₂=4 Hz, J₃=17 Hz, H-3,3); -3.98 (s, 3H, OH-5,7,4').