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BIFLAVONES FROM THE LEAVES OF *ARAUCARIA BIDWILLII* HOOKER AND *AGATHIS ALBA* FOXWORTHY (ARAUCARIACEAE)

N. U. KHAN, M. ILYAS, W. RAHMAN, T. MASHIMA, M. OKIGAWA and N. KAWANO
Department of Chemistry, Aligarh Muslim University, Aligarh, India and Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki, Japan

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Abstract– Six biflavones have been isolated from the leaf extracts of *Araucaria bidwillii* Hooker and *Agathis alba* Foxworthy; 7-O-methylcupressuflavone (**2a**) and 7,7''-di-O-methylagathisflavone (**1c**) are new compounds. The others are 7-O-methylagathisflavone (**1a**), 4''',7-di-O-methylagathisflavone (**1b**), 7,7''-di-O-methylcupressuflavone (**2b**) and bilobetin (**3**). In addition, the presence of several other biflavones has been indicated by TLC examination (Table 3).

SINCE the first report of the isolation and characterization of 7-O-methylagathisflavone (**1a**) and 4''',7-di-O-methylagathisflavone (**1b**) from *Agathis palmerstonii*[1] as members of a new series of naturally occurring completely aromatic optically active biflavones, the order Araucariales has become the focus of attention for investigations in this field. In the present paper we wish to report the isolation and characterization of biflavones from the leaves of *Araucaria bidwillii* Hooker and *Agathis alba* Foxworthy.

Purified phenolic extracts obtained from the dried and powdered leaves of *Araucaria bidwillii* were separated into six fractions by prep TLC and termed as Band I (R_f, 0·16), II (0·27), III (0·37), IV (0·43), V (0·54) and VI (0·61). Although Band I pigment was chromatographically homogeneous with an R_f value the same as that of agathisflavone or cupressuflavone, on methylation it gave three spots on TLC corresponding to hexamethyl ethers of agathisflavone, cupressuflavone and amentoflavone, which compounds are clearly detectable by TLC of the fully methylated mixture.[2] Band I pigment is therefore a mixture of agathisflavone, cupressuflavone and amentoflavone, although in quantities too small for isolation of each component for identification.

Band II gave a homogeneous pigment of m.p. 310°, which was methylated to give an optically active hexa-O-methylagathisflavone, m.p. 160–162°, $[\alpha]_D^{34}$ −56·5°, identified as **1a** by comparison of the NMR spectrum of its pentaacetate, m.p. 165–166° with that of an authentic sample (WA-II)[1]. Band III pigment was considered from its R_f value to be a monomethyl ether of cupressuflavone. However, methylation of the pigment followed by TLC examination disclosed that it was a mixture of monomethyl ethers of amentoflavone and cupressuflavone with a trace of hinokiflavone. This is in accordance with recent findings[2] that mixtures of (a) hinokiflavone and monomethyl ethers of amentoflavone and cupressuflavone, and (b) monomethyl ether of hinokiflavone and dimethyl ethers of amentoflavone and cupressuflavone, although undetectable as such are clearly distinguishable and effectively separable by TLC at the fully methylated stage.

Therefore, the pigment from Band III was subjected to counter current distribution

to give two components, bilobetin (3)[3] and a new compound, $C_{31}H_{20}O_{10}$, m.p. 186–190°, which was characterized as 7-O-methylcupressuflavone (**2a**). Band IV gave another new compound, $C_{32}H_{22}O_{10}$, m.p. 310°, for which the structure of 7,7″-di-O-methylagathisflavone (**1c**) was assigned. The pigment obtained from Band V was found to be a mixture of dimethyl ethers of cupressuflavone and amentoflavone by similar TLC examinations as described for the Band III pigment. Acetylation of the pigment from Band V and repeated recrystallizations gave a pure compound, m.p. 275–280° shown to be 7,7″-di-O-methylcupressuflavone (**2b**) tetraacetate.

Compound **2b** has previously been isolated from *Araucaria cunninghamii* and *A. cookii* although the 8,8″-biacacetin structure was initially proposed for it.[4–6] The NMR signals of the acetate are in accord with the structure of 8,8″-bigenkwanin (**2b**) tetraacetate (below) (Table 2) and finally it was identified by direct comparison with a synthetic sample sent from Seshadri,[7] which was obtained by partial demethylation of hexa-O-methylcupressuflavone followed by acetylation.

Band VI pigment was a minor constituent corresponding to a trimethyl ether of amentoflavone or cupressuflavone. Methylation of the pigment followed by TLC examination showed the presence of trimethyl ethers of both cupressuflavone and amentoflavone.

The structures of the two new compounds, **1c** and **2a** were assigned as follows; **1c** gave an optically active hexamethyl ether, m.p. 160–162° (racemic form,[8] m.p. 242°), $[\alpha]_D^{34}$ −56°, identical with that obtained from **1a** (Band II). R_f values, fluorescence in UV light, and NMR spectra of the two methyl ethers were identical to those of an authentic sample (WA-III)[1] of agathisflavone hexamethyl ether. **1c** gave an acetate, m.p. 169–170°, which was optically active, $[\alpha]_D^{34}$ −12·50° ($CHCl_3$). The NMR signals of the acetate are listed in Table 1 together with those of related compounds. Two sets of A_2B_2 type doublets (7·97, 7·30, 7·54 and 7·09 ppm) are almost the same as those for **1a** pentaacetate, m.p. 165–166° (7·97, 7·30, 7·51 and 7·11 ppm), suggesting that no methoxy group is present at positions 4′ and 4‴. This is also supported by

TABLE 1. NMR SPECTRAL DATA FOR AGATHISFLAVONE DERIVATIVES

Position	Ag. hexamethyl ether	1a-acetate	1b-acetate	1c-acetate
2′, 6′	7·92 (2H, d, J = 9 Hz)	7·97 d	7·95 d	7·97 d
3′,5′	7·06 (2H, d, J = 9 Hz)	7·30 d	7·29 d	7·30 d
2‴, 6‴	7·41 (2H, d, J = 9 Hz)	7·51 d	7·43 d	7·54 d
3‴, 5‴	6·82 (2H, d, J = 9 Hz)	7·11 d	6·81 d	7·09 d
8	6·94 (1H, s)	7·05 s	7·02 s	7·04 s
6″	6·70 (1H, s)	7·05 s	6·99 s	6·77 s
3	6·60 (1H, s)	6·69 s	6·64 s	6·69 s
3″	6·55 (1H, s)	6·64 s	6·54 s	6·58 s
4′	3·90 (3H, s)	(2·33)	(2·36)	(2·33)
4‴	3·76 (3H, s)	(2·24)	3·79	(2·24)
7	3·80 (3H, s)	3·81	3·82	3·81
7″	3·88 (3H, s)	(2·09)	(2·12)	3·83
5	3·61 (3H, s)	(2·14)	(2·17)	(2·12)
5″	4·07 (3H, s)	(2·45)	(2·47)	(2·47)

Numbers in parentheses show the chemical shifts of acetyl protons

the downfield shifts of the 2‴,6‴ (7·43 → 7·54 ppm) and 3‴,5‴ (6·81 → 7·09 ppm) protons from those of **1b** acetate because of 4‴-acetoxy group. On the other hand, the 6″ proton shows a considerable upfield shift (6·99 → 6·77 ppm), suggesting a 7″-methoxy group in **1c** instead of the 7″-acetoxy group of **1b** acetate. The two methoxy and four acetoxy proton signals are also in good accordance with the structure of **1c** tetraacetate (Table 1).

2a gave hexa-O-methylcupressuflavone on methylation and a pentaacetate on acetylation, $C_{41}H_{30}O_{15}$, m.p. 147–150°, NMR signals of which are listed in Table 2 together with those of related compounds. When compared with the corresponding

TABLE 2. NMR SPECTRAL DATA FOR COMPOUNDS RELATED TO CUPRESSUFLAVONE

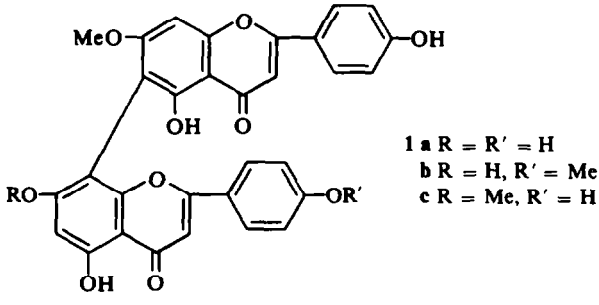
Assigned position	Acacetin diacetate	Genkwanin diacetate	Cupressufl. hexaacetate	**2a**-acetate	**2b**-acetate
3 (3″)	6·53 (1H, s)	6·51 (1H, s)	6·56 (2H, s)	6·51 (1H, s) 6·56 (1H, s)	6·55 (2H, s)
8	7·29 (1H, d) J = 2·5 Hz	6·81 (1H, d) J = 2·5 Hz	—	—	—
6 (6″)	6·79 (1H, d) J = 2·5 Hz	6·56 (1H, d) J = 2·5 Hz	7·06 (2H,2)	6·79 (1H, s) 7·09 (1H, s)	6·79 (2H, s)
3′, 5′ (3‴, 5‴)	6·96 (2H, d) J = 9 Hz	7·19 (2H, d) J = 9 Hz	7·01 (4H, d) J = 9 Hz	7·03 (4H, d) J = 9 Hz	7·04 (4H, d) J = 9 Hz
2′, 6′ (2‴, 6‴)	7·78 (2H, d) J = 9 Hz	7·83 (2H, d) J = 9 Hz	7·29 (4H, d) J = 9 Hz	7·33 (4H, d) J = 9 Hz	7·34 (4H, d) J = 9 Hz
4′ (4‴)	3·88 (3H, s)	2·32 (3H, S)	2·25 (6H, s)	2·27 (6H, s)	2·27 (6H, s)
7 (7″)	2·33 (3H, s)	3·89 (3H, s)	2·08 (6H, s)	3·85 (3H, s) 2·06 (3H, s)	3·86 (3H, s)
5 (5″)	2·43 (3H, s)	2·42 (3H, s)	2·48 (6H, s)	2·50 (6H, s)	2·51 (6H, s)

signals for the other compounds listed, the two sets of two singlets due to 3,3″ (6·51 and 6·56 ppm) and 6,6″ (6·79 and 7·09 ppm) protons of **2a** pentaacetate suggest the presence of 7-methoxy and 7″-acetoxy groups in this compound. Two 4H doublets of 3′,3‴,5′,5‴ (7·03 ppm) and 2′,2‴,6′,6‴ (7·33 ppm) protons suggest two acetoxy groups at 4′ and 4‴ positions when compared with the signals for cupressuflavone hexaacetate (7·01 and 7·29 ppm). The methoxy (3·85 ppm) and acetoxy (2·06, 2·27 and 2·50 ppm) signals are also compatible with the structure of **2a**. The chemical shifts of the 7-methoxy protons of cupressuflavone derivatives (3·85 or 3·86 ppm) are almost the same as that for genkwanin acetate (3·89 ppm). However, the 7-acetoxy group appears at a higher field (2·08 or 2·06 ppm) than that for acacetin acetate (2·33 ppm). This may mean that, because of the slightly greater length of the acetoxy group than the methoxy group, the 7-acetoxy protons receive a stronger anisotropic effect from the flavone nucleus oriented perpendicularly to the one to which it is attached.

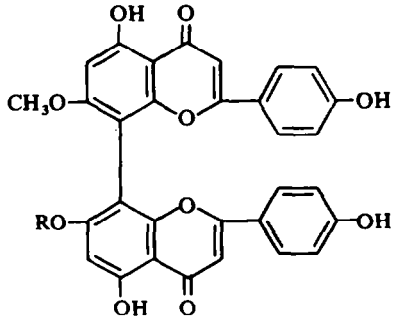
These findings support the 8–8″ bigenkwanin structure and not the biacacetin structure for **2b**. For further confirmation of the 4′ and 4‴ hydroxy groups in **2a** and **2b**, methylation of these compounds with deuterized diazomethane[9] was performed to afford a hexamethyl ether of cupressuflavone. Mass spectra of both the methylated compounds showed peaks of *m/e* 138 and 135, due to side phenyl fragments $^{+}O{\equiv}C{-}C_6H_4{-}OCD_3$ and $[CH{\equiv}C{-}C_6H_4{-}OCD_3]^{+}$, indicating deuteromethylation of 4′ and 4‴ hydroxy groups.

Agathis alba Foxworthy is well known as a source of Indian copal and widespread in Malaysian and Polynesian regions. Phenolic extracts obtained from the leaves of this plant showed quite similar spots on TLC to those for *Araucaria bidwillii* extract, suggesting similar constituents. Solvent fractionations followed by chromatographic separation on silicic acid afforded four biflavones, **1a**, **1b**, **2a** and **2b**, the former two being major. By similar treatment and prep TLC as described for *Araucaria bidwillii* the purified phenolic extracts obtained from this plant gave six similar bands and the presence of **1a**, **2a**, **1b** and **2b** in Band II, III, IV, and V respectively was confirmed by isolation and characterization of each compound. The other minor constituents of each band were detected by TLC examinations, on similar grounds as above. The components found in *Agathis alba* are listed in Table 3.

In conclusion, biflavone constituents of these two plants are very similar to each other except for the difference that *Agathis alba* contains **1b** while *Araucaria bidwillii* contains **1c**. It is noteworthy that these two plants contain all four kinds of biflavone skeleton, amentoflavone, hinokiflavone, cupressuflavone and agathisflavone.



1 a R = R′ = H
b R = H, R′ = Me
c R = Me, R′ = H



2 a R = H
b R = Me

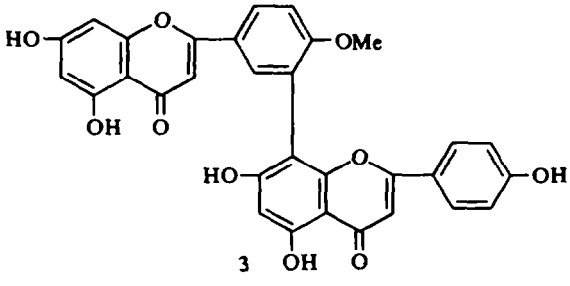


TABLE 3. BIFLAVONES OF *Araucaria bidwillii* AND *Agathis alba*

Band	*Araucaria bidiwillii*	*Agathis alba*
I	(agathisflavone) (cupressuflavone) (amentoflavone)	(agathisflavone) (cupressuflavone) (amentoflavone)
II	7-O-methylagathisflavone (**1a**)	7-O-methylagathisflavone (**1a**)
III	bilobetin (**3**) 7-O-methylcupressuflavone (**2a**) (hinokiflavone)	7-O-methylcupressuflavone (**2a**) (amentofl. monomethyl ether) (hinokiflavone)
IV	7,7″-di-O-methylagathisfl. (**1c**)	4‴,7-di-O-methylagathisfl. (**1b**)
V	7,7″-di-O-methylcupressufl. (**2b**) (amentofl. dimethyl ether)	7,7″-di-O-methylcupressufl. (**2b**) (amentofl. dimethyl ether)
VI	(cupressufl. trimethyl ether) (amentofl. trimethyl ether)	(cupressufl. trimethyl ether) (amentofl. trimethyl ether)

EXPERIMENTAL

All m.ps are uncorrected. NMR spectra were recorded on a JEOL 4H-100 or Hitachi H-60 instrument. Chemical shifts are expressed as ppm with TMS as internal standard. Mass spectra (MS) were determined on a JEOL JMS-01SG double focus high resolution mass spectrometer with a direct inlet system and operated at an ionization energy of 75 eV. IR spectra were taken with a Nihon-Bunko DS-301 spectrometer. TLC analysis was performed on silica gel G according to Stahl (Merck) or silica gel NCL-Poona using three solvent systems; benzene–pyridine–ethyl formate-dioxane, 5:1:2:2 (BPED), benzene–pyridine-formic acid, 36:9:5 (BPF) and toluene–ethyl formate–formic acid, 5:4:1 (TEFF).

Extraction of the leaves of Araucaria bidwillii *Hooker.* Dried and powdered leaves (2 Kg) collected at Sipore, West Bengal, India were completely extracted with petroleum ether (b.p. 40–60°). The treated leaves were dried and again extracted with boiling acetone until the extract was almost colourless. The combined acetone extracts were concentrated at atmospheric pressure to give a dark viscous mass. This was extracted successively with refluxing petroleum ether, benzene, and $CHCl_3$ until the solvent in each case was almost colourless. The residue was then treated with boiling water. The insoluble mass was dissolved in EtOH and dried under reduced pressure. Solid green extracts (5 g) were obtained which responded to the usual colour test for flavonoids.

Purification of the extracts by prep TLC. The extracts (4 g) were dissolved in dry acetone (50 ml) and placed on a column (150 cm in length and 50 mm in diameter) of magnesium silicate (Woelm, 140 g) with petroleum ether. Elution was performed by the following solvents successively, giving the material shown in parentheses: (i) petroleum ether (green oil), (ii) benzene (green gummy mass), (iii) $CHCl_3$ (green oil), (iv) EtOAc (yellow solid, 1 g), (v) acetone (yellow solid, 0·1 g), (vi) EtOAc saturated with water (yellow solid, 1 g) and (vii) EtOH (brown wax). Fractions 4, 5 and 6 gave positive colour tests for flavonoids. These three fractions were combined (2 g), dissolved in dry pyridine (50 ml) and separated into the following six fractions by preparative TLC (NCL, BPF): Band I (R_f 0·16, 15 mg), Band II (R_f 0·27, 150 mg), Band III (R_f 0·37, 150 mg), Band IV (R_f 0·43, 160 mg), Band V (R_f 0·54, 250 mg) and Band VI (R_f 0·61, 15 mg).

7-O-*Methylagathisflavone* (**1a**). Band II on extraction with acetone gave a yellow pigment of m.p. 310°, UV_{max} (EtOH) 276 nm (log ε 4·34), 338 (4·34); IR (KBr) 3400 cm^{-1} (OH), 1630 (CO). This compound (60 mg) was acetylated with Ac_2O and NaOAc and recrystallized from EtOAc to give colourless prisms (35 mg), m.p. 165–166° (lit.[1] m.p. 165–168°); NMR ($CDCl_3$) 3·81 ppm (OMe), 2·09, 2·14, 2·24, 2·33, 2·45 (five OAc), 6·64, 6·69 (1H, s each), 7·05 (2H, s), 7·11, 7·30, 7·51, 7·97 (2H, d each, J = 9 Hz). **1a**-methyl ether: m.p. 160–162°, $[\alpha]_D^{24}$ −56·5°, no m.p. depression on admixture with hexa-O-methylagathisflavone. R_f values, fluorescence in UV light, and IR and NMR spectral data also showed good accordance with the authentic sample.[1]

Bilobetin (4-O-*methylamentoflavone*) (**3**). The pigment (150 mg) obtained from Band III was subjected to counter current distribution between ethyl methyl ketone and a borate buffer of pH 9·20. After 80 transfers it was separated into two components, III-A and III-B. III-A was obtained from tubes No. 1–17 and recrystallized from MeOH as yellow crystals (25 mg), the acetone of which, m.p. 183–184° was identified with bilobetin pentaacetate by m.m.p. and spectral data (IR and NMR).

7-O-*Methylcupressuflavone* (**2a**). III-B was obtained from tubes No. 21–55 and gave yellow minute prisms (100 mg) from C_6H_6–MeOH, m.p. 186–190°, UV_{max} (EtOH) 270 nm (log ε 4·10), 335 (3·84); IR (KBr) 3400 cm^{-1} (OH), 1635 (CO), 1595, 1505, 1435, 1415, 1365, 1275, 1233, 1198, 1170, 1220, 1105, 1060, 1015, 910, 834. (Calc. for $C_{31}H_{20}O_{10} \cdot 2H_2O$: C, 63·26; H, 4·11. Found: C, 62·99; H, 4·38%). **2a**-methyl ether: m.p. 299°, no m.p. depression on admixture with hexa-O-methylcupressuflavone. IR and NMR spectra also showed good accordance with the authentic sample. **2a**-acetate: colourless prisms from EtOAc, m.p. 147–150°, M^+ calc. for $C_{41}H_{30}O_{15}$: 762·158. Found: 762·159, NMR ($CDCl_3$) 2·06 (OAc), 2·27, 2·50 (6H, s each, four OAc), 3·85 (OMe), 6·51, 6·56, 6·79, 7·09 (1H, s, each), 7·03, 7·33 (4H, d each, J = 9 Hz).

7,7″-*Di*-O-*methylagathisflavone* (**1c**). Band IV gave yellow crystals, m.p. 310°, UV_{max} (EtOH) 276 nm (log ε 4·45), 336 (4·44), IR (KBr) 3410 cm^{-1} (OH), 1630 (CO), 1600, 1505, 1440, 1370, 1290, 1190, 1160, 1110, 1085, 1018, 908, 835. (Calc. for $C_{32}H_{22}O_{10} \cdot 2H_2O$: C, 63·78; H, 4·35. Found: C, 63·30; H, 4·12%). **1c**-methyl ether: m.p. and m.m.p. 160–162°, identical with the compound obtained by methylation of **1a**. **1c**-acetate: colourless prisms from EtOAc, m.p. 169–170°, $[\alpha]_D^{34}$ −12·50° ($CHCl_3$), NMR ($CDCl_3$) 2·12, 2·24, 2·33, 2·47 (four OAc), 3·81, 3·83 (two OMe), 6·58, 6·69, 6·77, 7·04 (1H, s each), 7·09, 7·30, 7·54, 7·97 (2H, d each, J = 9 Hz).

7,7″-*Di*-O-*methylcupressuflavone* (**2b**). The pigment obtained from Band V was methylated with MeI and K_2CO_3 in boiling dry acetone for 12 hr. The presence of hexamethyl ethers of amentoflavone and cupressuflavone in the methylated product was indicated by the R_f values and characteristic fluorescence in UV light on TLC (BPF) examination.[2] Prep TLC gave both components, m.p. 225°, R_f 0·40 and m.p. 299°, R_f 0·43, identified with hexa-O-methylamentoflavone and hexa-O-methylcupressuflavone respectively by m.m.p. and spectral data (IR and NMR). Acetylation of the pigment obtained from Band V and repeated recrystallizations from EtOH–$CHCl_3$ gave colourless needles, m.p. 275–280°, NMR ($CDCl_3$) 2·27, 2·51 (four OAc), 3·68 (two OMe), 6·55, 6·79 (2H, s each), 7·04, 7·34 (4H, d each, J = 9 Hz). They were identical (m.m.p. and NMR spectra) with an authentic sample of **2b**-acetate.[7]

Detection of minor components by TLC. The minor pigment obtained from Band I was methylated with MeI and freshly ignited K_2CO_3 in boiling acetone and the product showed on TLC (BPF and BPED) the presence of hexamethyl ethers of agathisflavone, cupressuflavone and amentoflavone (R_f values and characteristic fluorescence[2] in UV light). Similarly, the presence of hinokiflavone was found in Band III pigment and the presence of trimethyl ethers of amentoflavone and cupressuflavone in Band VI pigment.

Extraction of Agathis alba *Foxworthy.* Air-dried and powdered leaves (500 g) collected in Formosa were extracted with boiling MeOH three times to give greenish black extracts, which were treated with hot water repeatedly to remove water-soluble brownish substances and then refluxed with trichloroethylene (TCE). Insoluble residues were collected, washed with TCE until the washings were almost colourless, dissolved in 3% KOH aq and filtered. The filtrate was acidified to give a dark brown precipitate (7 g), which was refluxed with 50% EtOH (100 ml) for 2 hr. A brownish deposit which separated on cooling was treated with acetone and EtOAc to remove insoluble dark brown substances. A mixture of biflavones (3 g) was obtained as a yellowish brown solid.

Chromatographic separation. Repeated chromatography of the above mixture (1 g) on a column of silica gel (Mallinckrodt, 100 mesh) by eluting with C_6H_6 mixed with a gradually increased amount (1–6%) of MeOH gave the following fractions, which were checked by TLC (TEFF): A; cupressuflavone dimethyl ether (13 mg) eluted by 3% MeOH–C_6H_6, B; agathisflavone dimethyl ether (23 mg) eluted by 4% MeOH–C_6H_6, C; cupressuflavone monomethyl ether (18 mg) and D; agathisflavone monomethyl ether (25 mg) eluted by 5% MeOH–C_6H_6. Subsequent fractions eluted by 6% MeOH–C_6H_6 contained agathisflavone and cupressuflavone but in quantities too small to isolate.

4‴,7-*Di*-O-*methylagathisflavone* (**1b**). Fraction B was recrystallized from C_6H_6–MeOH (1:9) to give minute yellow crystals, m.p. 219–220°, UV_{max} (EtOH) 276 nm (log ε 4·51), 335 (4·50); IR (KBr) 3380 cm^{-1} (OH), 1625 (CO). **1b**-acetate: colourless needles, m.p. 238–243°, no m.p. depression on admixture with authentic sample (WAVIII[1]). NMR ($CDCl_3$) 2·12, 2·17, 2·36, 2·47 (four OAc), 3·79, 3·82 (two OMe), 6·54, 6·64, 6·99, 7·02 (1H, s each), 6·81, 7·29, 7·43, 7·95 (2H, d each, J = 9 Hz). These spectral data were the same with those of WA-VIII.[1]

7,7″-*Di*-O-*methylcupressuflavone* (**2b**), 7-O-*Methylcupressuflavone* (**2a**) and 7-O-*Methylagathisflavone* (**1a**). These compounds were similarly obtained from fractions A, C and D respectively and identified by direct comparison with those isolated from *Araucaria bidwillii* (R_f values, UV, IR and NMR spectral data and m.m.p. of their acetates).

Detection of other components in Agathis alba. Dried and powdered leaves of the plant were extracted

and treated similarly as described for *Araucaria bidwillii* to give six bands by TLC. The R_f values of each band were the same as above and the pigments were similarly obtained from each band. **1a**, **2a**, **1b** and **2b** were isolated and identified from Bands II, III, IV and V respectively. The other minor pigments of each band were detected by the same methods as used for *Araucaria bidwillii* to give the results shown in Table 3.

Methylation of **2a** *and* **2b** *with deuterized diazomethane.* Diazomethane prepared from nitrosometylurea (10 g) and 50% KOH aq was collected in ether solution (20 ml), which was mixed with D_2O (2 ml) and dioxane (5 ml) and left to stand for 2 hr. A solution of **2a** (5 mg) in dioxane (2 ml) was mixed with a drop of D_2O and then with the above ether solution (6 ml) and left to stand overnight. The solvent was distilled off under reduced pressure and the resulting methyl ether was used for high resolution mass analysis. Chamber temp. 182°; sample temp. 200°; *m/e* calc. for $C_{36}H_{15}O_{10}D_{15}$, $C_8H_4O_2D_3$ and $C_9H_5OD_3$: 637·278, 138·063 and 135·076 respectively. Found: 637·276, 138·064 and 135·073. **2b** (3 mg) was similarly methylated for mass analysis; *m/e* calc. for $C_{36}H_{18}O_{10}D_{12}$, $C_8H_4O_2D_3$ and $C_9H_5OD_3$: 634·259, 138·063 and 135·076 respectively. Found: 634·258, 138·064 and 135·077. Exchange efficiency was estimated as 70% by comparison of NMR signal intensity between methoxy and acetyl protons of *p*-methoxyacetophenone prepared by deuteriomethylation of *p*-hydroxyacetophenone with the same ether solution.

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REFERENCES

[1] A. Pelter, R. Warren, J. N. Usmani, R. H. Rizvi, M. Ilyas and W. Rahman, *Experientia* **25**, 351 (1969)
[2] K. K. Chexal, B. K. Handa and W. Rahman, *J. Chromatog.* **48**, 484 (1970)
[3] W. Baker, A. C. M. Finch, W. D. Ollis and K. W. Robinson, *J. Chem. Soc.* 1477 (1963)
[4] M. Ilyas, J. N. Usmani, S. P. Bhatnagar, M. Ilyas, W. Rahman and A. Pelter, *Tetrahedron Letters* **53**, 5515 (1968)
[5] T. Mashima, M. Okigawa, N. Kawano, N. U. Khan, M. Ilyas and W. Rahman, *Ibid.* **33**, 2937 (1970)
[6] N. U. Khan, M. Ilyas, W. Rahman, M. Okigawa and N. Kawano, *Ibid.* **33**, 2941 (1970)
[7] S. Natarajan, V. V. S. Murti and T. R. Seshadri, *Ind. J. Chem.* **8**, 113 (1970)
[8] A. Pelter, R. Warren, B. K. Handa, K. K. Chexal and W. Rahman, *Ibid.* **9**, 98 (1971)
[9] K. J. van der Merwe, P. S. Steyn and S. H. Eggers, *Tetrahedron Letters* 3923 (1964)