Phytochemistry, 1978, Vol 17, pp 987–990 © Pergamon Press Ltd Printed in England. 0031-9422/78/0501-0987 $02.00/0

BIFLAVONES FROM THE LEAVES OF *ARAUCARIA EXCELSA*

NAJMA ILYAS*, M. ILYAS*, W. RAHMAN*, M. OKIGAWA† and N. KAWANO†

*Department of Chemistry, Aligarh Muslim University, Aligarh, India;
†Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki, Japan

(*Received* 13 *June* 1977)

Key Word Index—*Araucaria*; Araucariaceae; biflavones; new methyl ethers; amentoflavone; cupressuflavone; agathisflavone.

Abstract—Eight biflavones have been isolated from the leaf extracts of *Araucaria excelsa*. 7″-*O*-Methylamentoflavone, 7,7″-di-*O*-methylamentoflavone, 4′ or 4‴, 7-di-*O*-methylcupressuflavone, 7,7″,4‴-tri-*O*-methyl agathisflavone and 7,4′,7″-tri-*O*-methylamentoflavone are new compounds and are being reported for the first time. The others are 7,7″-di-*O*-methylagathisflavone,7,4′,7″,4‴-tetra-*O*-methylamentoflavone and 7,4′,7″,4‴-tetra-*O*-methylcupressuflavone. Mass and NMR spectral studies are used for structural elucidation. In addition, the presence of several other biflavones has been indicated by TLC examination of methylated products.

INTRODUCTION

Our investigations on the leaf extracts of *Agathis robusta* [1, 2] *A. alba* [3, 4], *Araucaria cookii* [5–7], *A. cunninghamii* [5–9] and *A. bidwilli* [4, 8, 9] have revealed that all these five species of the Araucariaceae contain members of the amentoflavone, hinokiflavone, cupressuflavone and agathisflavone groups of compounds, the last being unique to the family. The biflavone pattern of the family is therefore the most complex yet known. The optical activity as shown by some of these compounds, [1, 4–6, 8, 9] an observation not reported earlier in biflavones, is another notable feature of the family. In the present communication we report the results of our investigations on the biflavones from the leaf extracts of *Araucaria excelsa* Lamb. The complexity and distinctiveness of the biflavone pattern of the family is reinforced by the present investigations. The distribution of biflavones in *Araucaria* species is shown in Table 1.

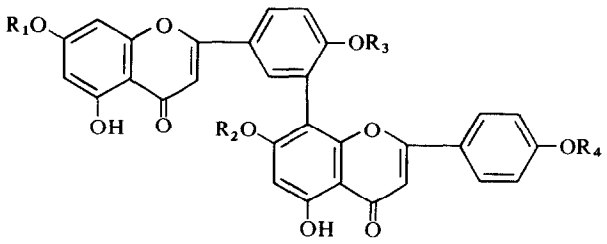
Table 1. Distribution of biflavonoids in *Araucaria* species

Biflavonoid	*Araucaria* spp*. A	B	C	D
Agathisflavone			(+)	(+)
Amentoflavone			(+)	(+)
Cupressuflavone			(+)	(+)
7-*O*-Methylagathisflavone		+	+	
Mono-*O*-Methylagathisflavone				(+)
7″-*O*-Methylamentoflavone	+	+		+
Bilobetin			+	
7-*O*-Methylcupressuflavone			+	
Hinokiflavone	+	(+)	(+)	
Mono-*O*-Methylcupressuflavone	(+)	(+)		
7,4‴-Di-*O*-Methylagathisflavone	+	+		
7,7″-Di-*O*-Methylagathisflavone			+	+
7,4′-Di-*O*-Methylamentoflavone	+	+		
7,7″-Di-*O*-Methylamentoflavone				+
7,7″-Di-*O*-Methylcupressuflavone	+	+	+	
7,4′ or 4‴-Di-*O*-Methylcupressuflavone				+
Di-*O*-Methylamentoflavone			(+)	
Mono-*O*-Methylhinokiflavone	(+)	(+)		
7,7″,4‴-Tri-*O*-Methylagathisflavone				+
Kayaflavone	+	+		
Sciadopitysin	+			
7,4′,7″-Tri-*O*-Methylamentoflavone				+
7,4′,7″-Tri-*O*-Methylcupressuflavone	+	+		
Tri-*O*-Methylamentoflavone			(+)	
Tri-*O*-Methylcupressuflavone			(+)	(+)
7,4′,7″,4‴-Tetra-*O*-Methylamentoflavone	+	+		+
7,4′,7″,4‴-Tetra-*O*-Methylcupressuflavone	+	+		+

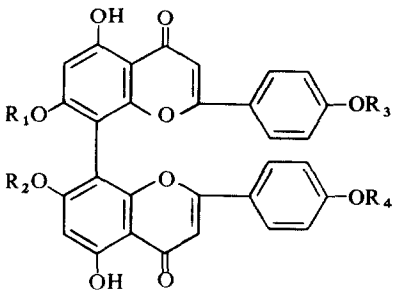
*A = *Araucaria cookii*, B = *A. cunninghamii*, C = *A. bidwilli*, D = *A. excelsa*, + = characterized (+) = detected.

RESULTS

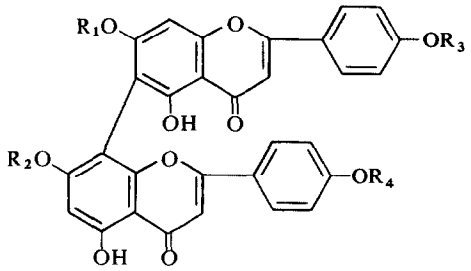
The phenolic extractives of the powdered leaves by solvent fractionation, column chromatography (magnesium silicate, Woelm and Si gel followed by PLC on Si gel, using BPF [10] yielded nine chromatographically homogenous fractions they were labelled AE_1–AE_9. The usual colour reactions and UV spectra in ethanol indicated they were all flavonoids. AE_1 and AE_2 were minor constituents and were detected by methylation followed by TLC examination and characteristic fluorescence in UV light [10] as mixtures of (a) amentoflavone (**1a**), cupressuflavone (**2a**) and agathisflavone (**3a**) and (b) the monomethyl ether of agathisflavone AE_3 and AE_4 were characterized as new compounds 7″-*O*-methylamentoflavone (**1b**) and 7,7″-di-*O*-methylagathisflavone (**3b**) respectively. **1b** was earlier reported as sotetsuflavone [11, 12]. The fraction AE_5, although homogenous chromatographically, separated into two components AE_5X and AE_5Y by CCD separation between ethylmethylketone and borate buffer (pH 9.8) and characterized as 7,7″-di-*O*-methylamentoflavone (**1d**) and 4′ or 4‴,7-di-*O*-methylcupressuflavone (**2b**) respectively, two new naturally occurring compounds. AE_6 was characterized as 7,7″,4‴-tri-*O*-methylagathisflavone (**3c**) a new biflavone methyl ether. AE_7 by methylation followed by TLC examination indicated the presence of tri-*O*-methylamentoflavone and tri-*O*-methylcupressuflavone. The fraction AE_7 was, therefore, subjected to CCD between ethylmethylketone and borate buffer (pH 9.8) and separated into two components AE_7X and AE_7Y. AE_7X was characterized as 7,4′,7″-tri-*O*-methylamentoflavone (**1c**), a new biflavone. AE_7Y being a minor component could not be identified. AE_8 and AE_9 were characterized as 7,4′,7″,4‴-tetra-*O*-methylamentoflavone (**1e**) and 7,4′,7″,4‴-tetra-*O*-methylcupressuflavone (**2c**) respectively.



(**1a**) $R_1 = R_2 = R_3 = R_4 = H$
(**1b**) $R_1 = R_3 = R_4 = H$, $R_2 = Me$
(**1c**) $R_4 = H$, $R_1 = R_2 = R_3 = Me$
(**1d**) $R_3 = R_4 = H$, $R_1 = R_2 = Me$
(**1e**) $R_1 = R_2 = R_3 = R_4 = Me$



(**2a**) $R_1 = R_2 = R_3 = R_4 = H$
(**2b**) $R_2 = R_3$ or $R_4 = H$, $R_1 = R_3$ or $R_4 = Me$
(**2c**) $R_1 = R_2 = R_3 = R_4 = Me$



(**3a**) $R_1 = R_2 = R_3 = R_4 = H$
(**3b**) $R_3 = R_4 = H$, $R_1 = R_2 = Me$
(**3c**) $R_3 = H$, $R_1 = R_2 = R_4 = Me$

* When we isolated this compound from *Araucaria* plants Dr H. Geiger kindly supplied a sample of reported sotetsuflavone for comparison. However, the NMR studies revealed that the sample from him was not sotetsuflavone but 7-*O*-methylamentoflavone (=sequoiaflavone).

EXPERIMENTAL

All mps are uncorr. NMR spectra were recorded on a JEOL PS-100 instrument. TLC analysis was performed on Si gel (NCL, Poona) using C_6H_6–Py–HCOOH (BPF) (36:9:5) [10] as the developing solvent system.

Biflavones from the leaf extracts of Araucaria excelsa *Lamb.* Dried and powdered leaves (2 kg) collected at the Government Garden, Ooty, India after exhaustive extraction with petrol 40–60°) were extracted several times with boiling Me_2CO. The combined Me_2CO extracts on removal of solvent gave a dark green viscous mass which was extracted successively by refluxing with petrol, C_6H_6 and $CHCl_3$. The residue was then treated with boiling H_2O. The insoluble mass was dissolved in EtOH and the solvent evapd to give a brown residue (4 g) which responded to the usual flavonoid colour tests. The mixture sepd by PLC on Si gel (NCL, Poona) using BPF as the developing solvent system into 9 chromatographically homogeneous components which were labelled as AE_1–AE_9

Compound **1b**. TLC examination of AE_3 showed that it may be amentoflavone monomethyl ether. NMR spectrum of AE_3-acetate and its comparison with amentoflavone hexamethyl and hexacetate (Table 2) confirmed its structure as 7″-*O*-methylamentoflavone (**1b**). This was first reported as sotetsuflavone isolated from *Cycas revoluta* Thumb. (Japanese name, Sotestsu) [12]. However, reinvestigation of this plant showed that sotetsuflavone is a mixture, a major part of which is amentoflavone and related compounds [13] Another report on the isolation of sotetsuflavone from *Metasequoia glyptostroboides* [14] is also doubtful; this compound appears to be sequoiaflavone*. Accordingly, this isolation of **1b** is the first report from a natural source and we suggest 7″-*O*-methylamentoflavone be used instead of the trivial name (sotetsuflavone) to avoid confusion.

Compound **1d**. The R_f values, fluorescence in UV, MS and NMR of the methyl ether (AE_5XM) obtained from AE_5X were identical with those of the authentic sample of amentoflavone hexamethyl ether. The MS of AE_5X acetate (AE_5XA) (*m/e* 734 M^+) indicated it to be a dimethoxy-tetraacetylamentoflavone. Although the methyl ether of AE_5X was identical in all respects with the authentic amentoflavone hexamethyl ether, the parent compound and its acetate (AE_5XA) were not comparable with any of the known dimethyl ethers of amentoflavone. The NMR data of AE_5XA and the relevant members of the series are given in Table 2. The new dimethyl ether of amentoflavone is assigned the structure **1d** by comparison of the NMR spectrum of its acetate with the spectra of the acetates of sequoiaflavone and 7″-*O*-methylamentoflavone (AE_3).

Table 2 Chemical shifts of protons (τ scale)

Compounds	8	6	6″	2′,6′	5′	2″,6″	3‴,5‴	3,3″	4′,4‴	7,7″	55″
AE_5-acetate (**1b**)	2.74 (1H, *d*, *J* = 3Hz)	3.16 (1H, *d*, *J* = 3Hz)	3 23 (1H, *s*)	1 99 (1H,*d*, *J* = 3Hz) 1.99 (1H, *q*, *J* = 3 and 8 Hz)	2 53 (1H, *d*, *J* = 8Hz)	2.48 (2H, *d*, *J* = 8Hz)	2 95 (2H, *d*, *J* = 8Hz)	3.33,3.41 (*s*, 1H each)	7 62 7 68 (6H)	7 95(3H) 6 09(3H)	7 44,7 5 (6H)
Amentoflavone hexaacetate	2 73	3.13	2 97	1.94 1 99	2.48	2 5	2.92	3.3,3.32	7 67,7 72	7.89,7 93	7 5,7 59
Amentoflavone hexamethyl ether	3 52	3.66	3.38	2.16 2.1	2.88	2 62	3 24	3.42,3 48	6 25,6.27	6 12,6 18	5.94,6.08
AE_7XA	3.19	3.44	3 25	2.14 2.11	2 88	2.57	2.98	3 44(2H, *s*)	6 23,7 75	6 13,6 17	7 51,7 58
AE_5X-acetate	3.22	3.4	3 25	2.03 2.08	2 58	2 5	2 97	3 47	8 01.7,75	6.14,6 17	7 51,7 59
Bilobetin pentaacetate	2 75	3.19	3 05	2.13 2.08	2.84	2 53	2 95	3 36	6 22,7.79	7 73,7 98	7 56,7 6
Sciadopitysin triacetate	3 22	3 44	3 04	2 09 (*m*,2H)	2.85	2 61	3 22	3 22,3 43 (*s*, 1 H each)	6 27,6 17	6 22,7.97	7 54,7 59
Kayaflavone triacetate	2 73	3 22	3.2	2 1 2 04	2 86	2.51	3 22	3 43 (2H, *s*)	6 21,6 24	7.96,6 24	7.54,7.58
Sequoiaflavone acetate	3 22	3 42	3.01	1.97 2 06	2 57	2.52	2.98	3 36	7 96,7 87	6 28,7 9	7 54,7 49

Spectra run in $CDCl_3$ at 100 MHz, TMS as int. = τ 10.0

Compound **2b** Fraction AE_5Y on complete methylation followed by TLC was found to be cupressuflavone hexamethyl ether (R_f, mp and fluorescence in UV). The MS and NMR of the methyl ether (AE_5YM) were also comparable in all respects with those of the authentic sample of cupressuflavone hexamethyl ether. The MS of AE_5Y-acetate (AE_5YA), (*m/e* 734, M^+) indicated it to be a dimethoxy-tetraacetoxycupressuflavone. Although the methyl ether of AE_5Y was identical with the authentic cupressuflavone hexamethyl ether, the parent compound and its acetate were not comparable with the known dimethylethers of cupressuflavone. The NMR data of AE_5YA and the other members of cupressuflavone series are given in Table 3. The new dimethyl ether of cupressuflavone, is therefore, assigned the structure **2b** by comparison of the NMR data of its acetate (AE_5YA) with those of acetates of 7-*O*-methylcupressuflavone, 7,7″-di-*O*-methylcupressuflavone and 7,4′,7″, 4‴,-tetra-*O*-methylcupressuflavone.

Compound **3c**. The parent compound AE_6 mp 300°, M^+, 580 gave an acetate (AE_6A) mp 185°, M^+, 706 and a methyl ether (AE_6M) mp 242°, M^+, 622, (AE_6M) and was identified by mp, mmp, R_f, fluorescence in UV, MS, and NMR as hexa-*O*-methylagathisflavone. TLC examination of AE_6 and MS of its acetate (*m/e*, 706 M^+) indicated AE_6 as tri-*O*-methylagathisflavone. The NMR data of AE_6 acetate (AE_6A) and other related members of the series are recorded in Table 4. By comparison of the NMR data of AE_6A with those of the acetates of 7,7″-di-*O*-methylagathisflavone and 7,4′-di-*O*-methylagathisflavone, the new compound has been assigned the structure **3c**. The chemical shifts of 3′,5′ protons (τ 2.72) in AE_6A are comparable to the corresponding protons 7,7″-di-*O*-methylagathisflavone (τ 2.72). The value (τ 3.18) assigned to 3‴,5‴ protons is also comparable to 3‴,5‴ protons of 7,4‴-di-*O*-methylagathisflavone but shows, as expected, an upfield shift in contrast to the corresponding protons of 7,7″-di-*O*-methyl-agathisflavone (τ 2.94).

Compound **1c**. TLC of AE_7X and MS of its acetate (*m/e* 706 M^+) indicated that AE_7X was tri-*O*-methylamentoflavone. The NMR spectral data of the acetate (AE_7XA) and some other members of the series are recorded in Table 2. Comparison of the NMR spectrum of acetate of AE_7X with the spectra of acetates of (a) 7,7″-di-*O*-methylamentoflavone for rings I-A, II-A and II-B and (b) bilobetin for ring I-B supports structure **1c** for the new tri-*O*-methylamentoflavone. Further support for structure **1c** is provided by comparing the NMR spectra of AE_7XA with the acetate of the known tri-*O*-methyl ethers of amentoflavone.

Detection of minor components by TLC. The fraction AE_1 (10 mg) was methylated with Me_2SO_4 and freshly fused K_2CO_3 in dry boiling Me_2CO and the product showed, by TLC (BPF) the presence of hexamethyl ethers of amentoflavone, cupressuflavone and agathisflavone (R_f and characteristic fluorescence in UV [10], thereby confirming the presence of

Table 3. Chemical shifts of protons (τ scale)

Compound	6,6″	3,3″	2′,6′ 2‴,6‴	3′,5′ 3‴,5‴	4′,4‴	5,5″	7,7″
AE_5YA	3.2 (1H, *s*)	3.48 (1H, *s*)	2.7 (4H, *d*)	2.92 (2H, *s*)	7.76 (6.22)	7.52 (6H)	(6.16) 7.89
	3.0 (1H, *s*)	3.52 (1H, *s*)		3.28 (2H, *s*)	(6H)		(6H)
7′-*O*-methylcupressuflavone	3.21 (1H, *s*)	3.49 (1H, *s*)	2.67 (4H, *d*)	2.97 (4H, *d*)	7.73 (6H)	7.5 (6H)	(6.15) 7.94
	2.91 (1H, *s*)	3.44 (1H, *s*)					(6H)
7′,7″-Di-*O*-methylcupressuflavone	3.2 (2H, *s*)	3.47 (2H, *s*)	2.69 (4H, *d*)	2.95 (4H, *d*)	7.71 (6H)	7.47 (6H)	(6.14) (6H)
7,4′,7″,4‴-Tetra-*O*-methylcupressuflavone	3.19 (2H, *s*)	3.45 (2H, *s*)	2.75 (4H, *d*)	3.22 (4H, *d*)	(6.22) (6H)	7.51 (6H)	(6.2) (6H)

Numbers in parentheses represent methoxy groups.

Table 4. Chemical shifts of protons (τ scale)

Compound	8	6″	3,3″	2′,6′	3′,5′	2‴,6‴	3‴,5‴	4′,4‴	5,5′	7.7″
AE_6A	2 97	3.28	3.33	2.05	2.72	2,57	3.18	7 88	7.67	6.19
	(H, *s*)	(H, *s*)	3.49	(2H, *d*)	(2H, *d*)	(2H, *d*)	(2H, *d*)	(6.26)	7.54	6 22
			(*s*, 1H each)					(6H)	(6H)	(6H)
7,7″-Di-*O*-methylagathisflavone	2 97	3.35	3.47	2.07	2.72	2.5	2 94	7.85	7.5	6.17
tetraacetate	(H, *s*)	(H, *s*)	3.4	(2H, *d*)	(2H, *d*)	(2H, *d*)	(2H, *d*)	7 73	7.64	6.15
			(*s*, 1H each)					(6H)	(6H)	(6H)
7,4′-Di-*O*-methylagathisflavone	3.01	3.02	3.38	2.08	2 73	2.6	3.19	7.86	7.67	(6.21)
tetraacetate	(H, *s*)	(H, *s*)	3.46	(2H, *d*)	(2H, *d*)	(2H, *d*)	(2H, *d*)	(6.24)	7.56	7 91
			(*s*, 1H each)					(6H)	(6H)	(6H)

Numbers in parentheses represent methoxy groups.

parent biflavones. Similarly the presence of mono-*O*-methylagathisflavone and tri-*O*-methylcupressuflavone was found in AE_2 and AE_7 resp.

Compounds **1e**, **2c** and **3b** were isolated from fraction AE_8, AE_9 and AE_4 resp. and identified by direct comparison with authentic samples (R_f, UV, IR, NMR, mp and mmp of their acetates and methyl ethers [1,6].

Compound **1b** crystallized as yellow needles (100 mg) from $CHCl_3$–MeOH mp 300°, R_f 0.37 (BPF), MW 552 (mass). On acetylation it gave from $CHCl_3$–EtOH colourless needles (40 mg), mp 195–197°, MW 762 (mass' λ_{max}^{EtOH} 272 and 323 nm (ε 47600 and 32600), ν_{max}^{KBr} 1780, 1655, 1610, 1500, 1430, 1370, 1285, 1195, 1125, 1020, 910 and 840 cm^{-1}. Methylation afforded the hexamethylbiflavone from $CHCl_3$–MeOH as colourless needles (30 mg), mp 212°, MW 622 (mass) NMR ($CDCl_3$) (τ scale): 6.25 (*s*, 6H, OMe-4′, 7); 6.27 (*s*, 3H. OMe-4‴); 6.13 (*s*, 3H, OMe-5); 5.94 (*s*, 3H, OMe-5″); 6.18 (*s*, 3H, OMe-7″).

Compounds from fraction AE_5. The CCD separation of AE_5 between ethyl methyl ketone and borate buffer (pH 9.8, 140 transfers) gave the following two fractions AE_5X (100 mg, 81–115 transfers) and AE_5Y (10 mg, mp < 300, ν_{max}^{KBr} 1640, 1350, 1245, 1090, 1020, 920 and 775 cm^{-1} (131–140 transfers). AE_5X (60 mg) on acetylation gave colourless needles, MW 734 (MS). AE_5Y similarly gave an acetate as colourless needles, MW 734 (MS).

The biflavones from AE_6 crystallized as yellow needles from $CHCl_3$–MeOH, mp 300°, R_f 0.613 (BPF), MW 580 (MS) and the acetate had mp 185°, λ_{max}^{EtOH} 274 and 327 nm (ε 29700 and 29700), ν_{max}^{KBr} 1710, 1640, 1600, 1500, 1435, 1360, 1195, 1170, 1110, 1010 and 830 cm^{-1}. MW 706 (MS). Methylation afforded as colourless needles mp 242°, MW 622 (MS), NMR ($CDCl_3$) (λ scale) 6.26 (*s*, 3H, OMe-4′); 6.22 (*s*, 3H, OMe-4‴); 6.14 (*s*, 3H, OMe-7); 6.12 (*s*, 3H, OMe-7″); 6.41 (*s*, 3H, OMe-5); 5.95 (*s*, 3H, OMe-5″).

Biflavone from fraction AE_7. The CCD separation of AE_7 (55 mg) between ethyl methyl ketone and borate buffer (pH 10.2, 92 transfers) gave the fraction AE_7X (40 mg, 81–91 transfers). The acetate had mp 209–210° λ_{max}^{EtOH} 237, 261 and 320 nm (ε 44400, 40,600 and 48,700), ν_{max}^{KBr} 1755, 1620, 1595, 1360, 1245, 1180, 1100, 1005, 880 and 825 cm^{-1} MW 706 (MS).

REFERENCES

1. Pelter, A., Warren, R., Usmani, J. N., Rizvi, R. H., Ilyas, M. and Rahman, W. (1969) *Experientia* **25**, 351.
2. Varshney, A. K., Rahman, W. Okigawa, M and Kawano, N. (1973) *Experientia* **29**, **784**.
3. Mashima, T., Okigawa, M., Kawano, N., Khan, N. U., Ilyas, M. and Rahman, W. (1970) *Tetrahedron Letters* **33**, 2937.
4. Khan, N. U., Ilyas, M., Rahman, W., Mashima, T. Okigawa, M. and Kawano, N (1972) *Tetrahedron* **28**, 5689
5. Ilyas, M., Usmani, J. N., Bhatnagar, S. P., Ilyas, M., Rahman, W. and Pelter, A. (1968 *Tetrahedron Letters* **31**, 5515.
6. Pelter, A., Warren, R., Ilyas, M., Usmani, J. N. Bhatnagar, S. P., Rizvi, R. H. and Rahman, W. (1969) *Experientia* **25**, 350.
7. Khan, N. U., Ansari, W. H., Usmani, J. N., Ilyas, M. and Rahman, W. (1971) *Phytochemistry* **10**, 2129.
8. Khan, N. U., Ansari, W. H., Rahman, W. Okigawa, M and Kawano, N. (1971) *Chem. Pharm. Bull. (Japan)* **19**, 1500.
9. Khan, N. U., Ilyas, M., Rahman, W., Okigawa, M. and Kawano, N. (1970) *Tetrahedron Letters* **33**, 2941.
10. Chexal, K. K., Handa, B. K., and Rahman, W. (1970) *J. Chromatog.* **48**, 484.
11. Chang, C. T., Chem. T. Ueng, T. S., Choong, S. T. and Chen, P. C. (1960) *J. Formosan Sci* **14**, 1.
12. Kariyone, T. and Sawada, T. (1958) Yakugaku Zasshi **78**, 1013, 1016; Kawano, N. and Yamada, M. (1960) *J. Am. Chem. Soc.* **82**, 1505; (1960) Yakugaku Zasshi **80**, 1576.
13. Handa, B. K., Chexal, K. K., Rahman, W., Okigawa, M. and Kawano, N. (1971) *Phytochemistry* **10**, 436; Geiger, H. and Pfleiderer, W. G. (1971) *Phytochemistry* **10**, 1936; Varshney, A. K., Mah, T., Khan, N. U., Rabman, W, Hwa, C. W., Okigawa, M. and Kawano, N. (1973) *Indian J Chem.* **11**, 1209.
14. Beckmann, S., Geiger, H. and Pfleiderer, W. G. (1971) *Phytochemistry* **10**, 2465.