Biflavones from the Leaves of *Araucaria rulei* F. Muell. and a Survey on Biflavanoids of the Araucaria Genus

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Eleven biflavones have been isolated from the leaves of *Araucaria rulei* and identified by NMR spectroscopy and via the permethylated and peracetylated products. The biflavones found until now in the *Araucaria* genus are summarized in a table.

The phenolic extractives of the dried and powdered leaves of *A. rulei* were separated by column chromatography on silica gel followed by TLC on silica gel to give eight components, termed as Band I to Band VIII (see Table I). Although Band I pigment was chromatographically homogeneous with an R_F value the same as that of cupressuflavone, amentoflavone, agathisflavone or robustaflavone, on methylation it gave four spots on TLC (Band IX to XII), which were identified as hexamethyl ethers of amentoflavone (**3b**), cupressuflavone (**2b**), agathisflavone (**1b**) and robustaflavone (**4b**) by comparison of NMR spectra with that of authentic samples [1–3].

Band II gave a homogeneous pigment (m.p. 315 °C) which was methylated to give hexa-O-methylagathisflavone (**1a**) (m.p. 165 °C). It was identified as 7-O-methylagathisflavone (**1c**) by comparison of the NMR spectrum of its pentaacetate, m.p. 166 °C with that of an authentic sample [2]. Band III was considered from its R_F value to be a monomethyl ether of amentoflavone or cupressuflavone. However, methylation followed by TLC examination showed it was a mixture of monomethyl ethers of amentoflavone, cupressuflavone and robustaflavone. These were not separated due to small amount. Band IV gave a pigment m.p. 308 °C and was identified as 4‴,7-di-O-methyl-agathisflavone (**1d**) by comparison of NMR spectrum of its tetraacetate, m.p. 175 °C, with an authentic sample [2]. Band V gave an optically active pigment (m.p. 285 °C) which on methylation gave hexa-O-methylcupressuflavone (**2b**) (m.p. 295 °C), identified as 7,7″-di-O-methylcupressuflavone (**2c**) by comparison of the NMR spectrum of its acetate, m.p. 275 °C, with an authentic sample [2]. Although Band VI pigment was chromatographically homogeneous, however, on methylation followed by TLC it gave two components. On fractional crystallization Band VI gave a major compound m.p. 275 °C, whose acetate, m.p. 245 °C, was identified as 7,7″,4‴-tri-O-methylcupressuflavone (**2d**) triacetate (m.p. and NMR). The minor compound, m.p. 295 °C, gave an acetate

Table I. R_F values of biflavones of *Araucaria rulei* and fully methylated derivatives.

Band	Compound	Solvent system BPF [a]	BPF [b]
I	Amentoflavone (**3a**)	0.17	0.27
	Cupressuflavone (**2a**)	0.17	0.27
	Agathisflavone (**1a**)	0.17	0.27
	Robustaflavone (**4a**)	0.17	0.27
II	7-O-methylagathisflavone (**1c**)	0.27	0.33
III	Monomethyl ethers of amentoflavone, cupressuflavone and robustaflavone	0.34	0.42
IV	4‴,7-Di-O-methylagathisflavone (**1d**)	0.44	0.55
V	7,7″-Di-O-methylcupressuflavone (**2c**)	0.54	0.64
VI	7,7″,4‴-Tri-O-methylcupressuflavone (**2d**),	0.61	0.71
	Sciadopitysin (**3c**)	0.61	0.71
VII	7,7″,4′,4‴-Tetra-O-methylamentoflavone (**3d**)	0.76	0.79
VIII	7,7″,4′,4‴-Tetra-O-methylcupressuflavone (**2e**)	0.79	0.86
	Fully methylated derivatives		
IX	Amentoflavone hexamethyl ether (**3b**)	0.40	0.26
X	Cupressuflavone hexamethyl ether (**2b**)	0.43	0.32
XI	Agathisflavone hexamethyl ether (**1b**)	0.45	0.36
XII	Robustaflavone hexamethyl ether (**4b**)	0.50	0.55

a = C_6H_6–Py–HCO_2H (36:9:5).
b = C_6H_6–Py–HCO_2H (20:5:1).
Both the solvents distinguish between the fully methylated derivatives of all the series of biflavones.

Table II. Distribution of biflavones in five *Araucaria* species.

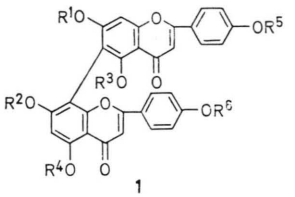
R_F values of bands	Biflavones	A	B	C	D	E	Remarks
I (0.17)	Ag			+	+	+[a]	a = agathisflavone
	Am			+	+	+[b]	b = amentoflavone
	Cu			+	+	+[c]	c = cupressuflavone
	Ro					+[d]	d = robustaflavone
II (0.27)	M-Ag		+[e]	+[e]	+	+[e]	e = 7-O-methylagathisflavone
III (0.34)	M-Am	+[f]	+	+[g]	+[f]	+	f = 7″-O-methylamentoflavone
	M-Cu	+	+	+[h]	+		g = bilobetin
	M-Ro					+	h = 7-O-methylcupressuflavone
	Hi	+[i]		+			i = hinokiflavone
IV (0.44)	D-Ag	+[j]	+[j]	+[j]	+[j]	+[k]	j = 7,7″-di-O-methylagathisflavone k = 4‴,7-di-O-methylagathisflavone
V (0.54)	D-Am	+[l]	+[l]	+	+[m]		l = 4′,7″-di-O-methylamentoflavone
	D-Cu	+[n]	+[n]	+[n]	+[o]	+[n]	m = 7,7″-di-O-methylamentoflavone n = 7,7″-di-O-methylcupressuflavone o = 7,4′- or 4‴-di-O-methylcupressuflavone
VI (0.58)	T-Ag				+[p]		p = 7,7″,4‴-tri-O-methylagathisflavone
VII (0.61)	T-Am	+[q]	+[r]	+	+[s]	+[r]	q = kayaflavone
	T-Cu	+[t]	+[t]	+	+	+[u]	r = sciadopitysin s = 7,7″,4′-tri-O-methylamentoflavone t = 7,7″,4′-tri-O-methylcupressuflavone u = 7,7″,4‴-tri-O-methylcupressuflavone
VIII (0.76)	Te-Am	+[v]	+[v]		+[v]	+[v]	v = 7,7″,4′,4‴-tetra-O-methylamentoflavone
IX (0.79)	Te-Cu	+[w]	+[w]		+[w]	+[w]	w = 7,7″,4′,4‴-tetra-O-methylcupressuflavone

A, *Araucaria cookii* [1, 2]; B, *A. cunninghamii* [4]; C, *A. bidwilli* [2]; D, *A. excelsa* [7]; E, *A. rulei*; Ag, agathisflavone; Am, amentoflavone; Cu, cupressuflavone; Ro, robustaflavone; Hi, hinokiflavone; M, mono; D, di; T, tri; Te, tetra; +, detected; +, with superscript, fully characterized.

m.p. 260 °C and was identified as sciadopitysin (**3c**) triacetate (m.p. and NMR) [5]. Band VII m.p. 275 °C on methylation gave hexa-O-methyl-amentoflavone (**3b**) and was identified as 7,7″,4′,4‴-tetra-O-methylamentoflavone (**3d**) by comparison of the NMR spectrum of its diacetate with that of an authentic sample [6]. Band VIII m.p. 160 °C was identified as 7,7″,4′,4‴-tetra-O-methylcupressuflavone (**2e**) by comparison of the NMR spectrum of its diacetate with an authentic sample [1].

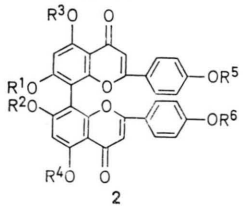
It is interesting to note that tetra-O-methylamentoflavone and all the partial methyl ethers of cupressuflavone isolated from *A. rulei* show optical rotation due to the restricted rotation through C – C bond of the interflavonyl linkage. They differ all in their values more or less from the previously reported substances. This is valid specially for 7,7″-di-O-methylcupressuflavone (found +199.5 °C, reported +37.5° and 60.0 °C).

The family Araucariaceae includes two genera, namely *Agathis* (21 species) and *Araucaria* (14 species). Four *Araucaria* species have already been investigated for the biflavone content of their leaves [1, 2, 4, 7]. All of these species are known to contain biflavones belonging to (6-8″, 8-8″, 3′-8″ and 4′-O-6″) series. The isolation and characterization of robustaflavone as hexamethyl ether [3] from *A. rulei* is the first time that this biflavone (linkage 3′-6″) has been identified as a natural product from the genus Araucaria. Beside this other biflavone types (6-8″, 8-8″ and 3′-8″) have been previously reported. The distribution of biflavones in the genus Araucaria is summarized in Table II. The biflavone pattern of this genus is the most complex yet known.



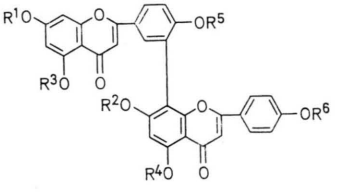
1

a $R^1=R^2=R^3=R^4=R^5=R^6=H$
b $R^1=R^2=R^3=R^4=R^5=R^6=Me$
c $R^1=Me$, $R^2=R^3=R^4=R^5=R^6=H$
d $R^1=R^6=Me$, $R^2=R^3=R^4=R^5=H$



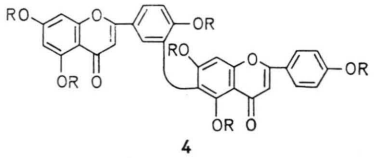
2

a $R^1=R^2=R^3=R^4=R^5=R^6=H$
b $R^1=R^2=R^3=R^4=R^5=R^6=Me$
c $R^1=R^2=Me$, $R^3=R^4=R^5=R^6=H$
d $R^1=R^2=R^6=Me$, $R^3=R^4=R^5=H$
e $R^1=R^2=R^5=R^6=Me$, $R^3=R^4=H$



3

a $R^1=R^2=R^3=R^4=R^5=R^6=H$
b $R^1=R^2=R^3=R^4=R^5=R^6=Me$
c $R^1=R^5=R^6=Me$, $R^3=R^4=R^5=H$
d $R^1=R^2=R^5=R^6=Me$, $R^3=R^4=H$



4

a R=H
b R=Me

The occurrence of highly methylated derivatives of cupressuflavone and amentoflavone is also a distinguishing feature of this genus. After surveying five species of *Araucaria*, no evidence could be obtained for the occurrence of biflavone glycosides or of biflavones based upon any other nucleus than apigenin. These observations may prove useful as chemotaxonomic markers.

Experimental

NMR spectra were recorded with a Varian A 60A instrument. Chemical shifts are expressed as ppm with TMS as internal standard for solutions in deuteriochloroform and DMSO-d_6. TLC and column chromatography were done on silica gel E. Merck. Solvent system C_6H_6-Py-HCO_2H, 36:9:5 was used for preparative TLC. All compounds were characterized by m.p., m.m.p., NMR, co-TLC and preparation of derivatives. Plant material was procured from Govt. Garden, Ooty, India.

Isolation: Dried and powdered leaves of *Araucaria rulei* (1 kg) were completely extracted with Me_2CO in a soxhlet. The acetone extract was concentrated under pressure to give a dark green gummy mass which was purified by extraction with petrol. The insoluble residue was treated with boiling water and the solution filtered. The insoluble mass was dissolved in MeOH and dried under diminished pressure to give greenish brown residue (4 g) which responded to the usual colour test for flavanoids. The residue was dissolved in Me_2CO and placed on a column of silica gel (E. Merck). The column was eluted successively with (a) petroleum ether, (b) benzene, (c) $CHCl_3$, and (d) EtOAc. Fractions b, c and d gave positive tests for flavanoids. These three fractions were combined (1.5 g), dissolved in dry Py and separated into the following eight fractions by preparative TLC: Band I (R_F 0.17), Band II (R_F 0.27), Band III (R_F 0.34), Band IV (R_F 0.44), Band V (R_F 0.54), Band VI (R_F 0.61), Band VII (R_F 0.76) and Band VIII (R_F 0.79). These were extracted separately with Me_2CO.

A mixture of Band I (125 mg), DMS (1.0 ml), dry Me_2CO (300 ml) and anhydrous K_2CO_3 (10 g) was refluxed on a steam bath for 12 h. The mixture

was filtered and the residue was washed with Me_2CO. The filtrate and washings were combined and the solvent was distilled off. The residue was washed several times with petrol, dissolved in $CHCl_3$ and separated into the following four Bands by preparative TLC. Band IX (R_F 0.40), Band X (R_F 0.43), Band XI (R_F 0.45) and Band XII (R_F 0.50). These fractions were extracted separately with $CHCl_3$ to give **3b**, **2b**, **1b**, and **4b** respectively.

Hexa-O-methylamentoflavone (**3b**) crystallized from $CHCl_3$-MeOH as colourless needles (30 mg), m.p. 210 °C.

Hexa-O-methylcupressuflavone (**2b**) gave colourless needles (30 mg), from $CHCl_3$-MeOH, m.p. 295 °C.

Hexa-O-methylagathisflavone (**1b**) crystallized from $CHCl_3$-MeOH as colourless needles (15 mg), m.p. 165 °C.

Hexa-O-methylrobustaflavone (**4b**) was crystallized from $CHCl_3$-hexane (12 mg), m.p. 302 to 305 °C.

7-O-Methylagathisflavone (**1c**): Band II on extraction with Me_2CO gave a yellow pigment which was crystallized from Py-MeOH to give needles (40 mg), m.p. 315 °C. A mixture of **1c** (35 mg), Py (1.5 ml) and Ac_2O (1 ml) was warmed on a steam bath for 2 h. The cooled mixture was poured onto crushed ice and the white precipitate was collected and crystallized from $CHCl_3$-EtOH to give colourless needles (20 mg), m.p. 166 °C.

4‴,7-Di-O-methylagathisflavone (**1d**) from Band IV gave yellow needles (30 mg) from Py-MeOH, m.p. 303 °C, **1d** (25 mg) was acetylated with Py and Ac_2O to give colourless needles (18 mg) from $CHCl_3$-MeOH, m.p. 175 °C.

7,7″-Di-O-methylcupressuflavone (**2c**) from Band V gave brownish yellow needles (100 mg) from Py-MeOH, m.p. 295 °C, $[\alpha]_D^{25}$ +199.5 °C. Acetylation of **2c** (50 mg) gave needles of tetraacetate (30 mg) from $CHCl_3$-MeOH, m.p. 280 °C.

The pigment obtained from Band VI was methylated with DMS and K_2CO_3 in boiling dry Me_2CO for 8 h. On fractional crystallization Band VI gave two components **2d** (major) and **3c** (minor).

7,7″,4‴-Tri-O-methylcupressuflavone (**2d**) crystallized (35 mg) from $CHCl_3$-MeOH, m.p. 275 °C, $[\alpha]_D^{25}$ +30.30 °C. Acetylation of **2d** (25 mg) gave colourless needles of triacetate (15 mg) from C_6H_6-hexane, m.p. 245 °C.

Sciadopitysin (**3c**) was crystallized (20 mg) from Py-MeOH, m.p. 295 °C. Acetylation of **3c** (20 mg) gave colourless needles of triacetate (10 mg) from $CHCl_3$-MeOH, m.p. 260 °C.

7,7″,4′,4‴-Tetra-O-methylamentoflavone (**3d**) from Band VII was crystallized as yellow needles (30 mg) from $CHCl_3$-MeOH, m.p. 275 °C, $[\alpha]_D^{25}$ + 60.5 °C. Acetylation of **3d** (20 mg) gave colourless needles of diacetate (15 mg) from $CHCl_3$-MeOH, m.p. 222 °C.

7,7″,4′,4‴-Tetra-O-methylcupressuflavone (**2e**) from Band VIII was crystallized as bright yellow needles (50 mg) from $CHCl_3$-MeOH, m.p. 160 °C, $[\alpha]_D^{25}$ +60.5 °C. Acetylation of **2e** (30 mg) gave colourless needles of diacetate (20 mg) from $CHCl_3$-MeOH, m.p. 158 °C.

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