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SHORT COMMUNICATION

BIFLAVONYLS OF THE ARAUCARIALES

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Abstract—TLC examination of complex mixtures of biflavonyls from the leaf extracts of *Araucaria bidwilli A. cookii, A. cunninghamii, Agathis alba* and *A. palmerstoni* has been carried out. Chromatographically homogeneous mixtures of natural biflavonyls including minor components have been identified after complete methylation. Attention is drawn to aspects of chemotaxonomical significance.

THE IDENTIFICATION of the parent biflavonyls and their partial methyl ethers except isomeric mixtures, found to occur in leaf extracts of *Podocarpus gracillior*[1] and *Zamia floridiana*,[2] has already been reported. The Araucariales presents a special problem due to the co-occurrence of complex mixtures of biflavonyls belonging to all the series known to date. It has, however, only been possible to separate mono-, and dimethyl ethers of agathisflavone[3–5] and tetramethyl ethers of amentoflavone[6] and cupressuflavone[7] as single entities from such mixtures by using TLC on silica gel with benzene–pyridine–formic acid.[11] The remaining constituents of the mixtures, although structurally heterogeneous, were chromatographically inseparable. The recent report of Natrajan *et al.*[8] for the separation from Cupressaceae of biflavonyl mixtures and their full methyl ethers by TLC led us to use their procedure for the examination of biflavonyls from the Araucariales. The two solvent systems (a) toluene–pyridine–acetic acid (10:1:1) and (20:1:1) (b) toluene–dimethylformamide–acetic acid (10:1:1) which were most satisfactory for Natrajan and his colleagues failed to separate the obstinate mixtures, and even those components which had earlier been easily and cleanly separated and fully authenticated. The same difficulty was encountered in detecting and separating their fully methylated derivatives. Two dimensional TLC in a number of solvent systems was also found to be unsatisfactory. The significant feature of the present work is that not only closely spaced bands but also chromatographically homogeneous mixtures after methylation (including even minor constituents) can be unequivocally detected by characteristic fluorescence in UV light. These include (a) amentoflavone, cupressuflavone, agathisflavone; (b) hinokiflavone, monomethyl ethers of amentoflavone and cupressuflavone; (c) monomethyl ether of hinokiflavone, dimethyl ethers of

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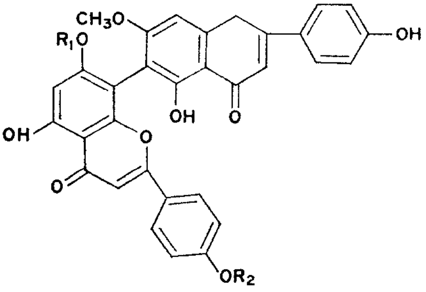
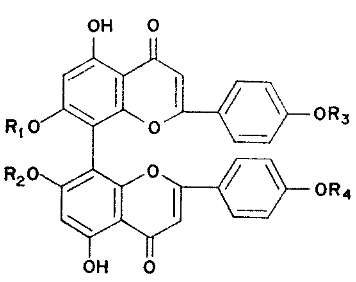
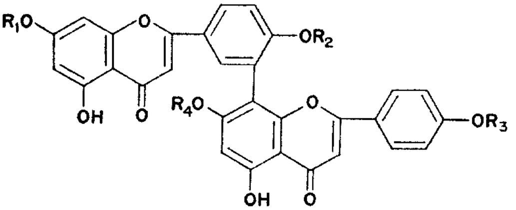


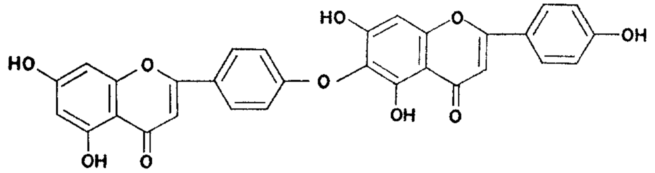
(a) $R_1 = R_2 = H$
(e) $R_1 = CH_3$; $R_2 = H$
(f) $R_1 = H$; $R_2 = CH_3$



(b) $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$
(g) $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$
(j) $R_1 = R_2 = R_3 = R_4 = CH_3$



(c) $R_2 = CH_3$; $R_1 = R_3 = R_4 = H$
(h) $R_2 = R_3 = R_4 = CH_3$; $R_1 = H$
(i) $R_1 = R_2 = R_3 = R_4 = CH_3$



(d)

amentoflavone and cupressuflavone; and (d) trimethyl ethers of amentoflavone and cupressuflavone. The fully methylated ethers, thus, may be separated by preparative TLC and characterized although the separation or even detection of individual components of the original mixtures is not possible. Some of the constituents from such mixtures have, however, been obtained in pure form by counter current methods.[1,4]

The present communication deals with the complex mixtures of biflavonyls in the leaf extracts of *Araucaria bidwilli*, *A. cookii*, *A. cunninghamii*, *Agathis alba* and *A. palmerstoni*. The biflavonyl constituents identified in each species are recorded in Table 1. The species examined show a novel feature of possessing biflavonyls belonging to all the series known so far. The presence of agathisflavone series seems to be characteristic of the order. *Araucaria bidwilli*, *Agathis alba* and *Agathis palmerstoni* are comparable in having biflavonyl mixtures from parent compounds to trimethyl ether while *Araucaria cookii* and *Araucaria cunninghamii* show the presence of biflavonyls from monomethyl-, to tetramethyl ethers. The last, but not the least, important characteristic of the order is the optical activity as shown by some members.[4,7] These observations may prove useful to chemotaxonomists.

TABLE 1. DISTRIBUTION OF BIFLAVONYLS IN ARAUCARIALES

Band	Biflavonyls*	Species† A	B	C	D	E	Remarks
I	Ag	+	+	+			
	Cu	+	+	+			
	Am	+	+	+			
II	M-Ag	$+^{a}$	$+^{a}$	$+^{a}$		+	a = 7-*O*-Methylagathisflavone[3–5]
III	M-Cu	$+^{b}$	+	$+^{b}$	+	+	b = 7-*O*-Methylcupressuflavone[4,5]
	M-Am	$+^{c}$	+	+	+	+	c = Bilobetin[5]
	Hi	+	+	+	$+^{d}$	+	d = Hinokiflavone[9]
IV	D-Ag	$+^{e}$	$+^{f}$	$+^{f}$	$+^{f}$	+	e = 7,7″-Di-*O*-methylagathisflavone[5]
							f = 7,4‴-Di-*O*-methylagathisflavone[3,4,9]
V	D-Cu	$+^{g}$	+	$+^{g}$	$+^{g}$	+	g = 7,7″-Di-*O*-methylcupressuflavone[4–6]
	D-Am	+	+	+	+	+	
	M-Hi		+		+	+	
VI	T-Cu	+	+	+	+	+	
	T-Am	+	+	+	$+^{h}$	$+^{h}$	h = Kayaflavone[10]
VII	Te-Am				$+^{i}$	$+^{i}$	i = 7,7″,4′,4‴-Tetra-*O*-methyl amentoflavone[6]
VIII	Te-Cu				$+^{j}$	$+^{j}$	j = 7,7″4′,4‴-Tetra-*O*-methyl cupressuflavone[7]

* Cu = Cupressuflavone; Am = Amentoflavone; Hi = Hinokiflavone; M = Mono; D = Di-; T = Tri-; Ag = Agathisflavone. Te = Tetramethyl ether; + = detected; + with superscript = fully characterized.

† A = *Araucaria bidwilli*; B = *Agathis palmerstoni*; C = *Agathis alba*; D = *Araucaria cookii* and E = *Araucaria cunninghamii.*

EXPERIMENTAL

Dried and powdered leaves (100 g) of each species after exhaustive extraction with light petrol (40–60°) were extracted with acetone. The concentrate, after solvent fractionation, was separated by column chromatography on magnesium silicate (Woelm) and fractions subjected to TLC examination. Both TLC and preparative TLC chromatography were on silica gel G (E. Merck) using BPF (benzene–pyridine–formic acid, 36:9:5)[11] unless otherwise stated. The complexities of all the chromatograms were revealed in UV light, but the spots of biflavonyls and their partial methyl ethers were also located by using $FeCl_3$–EtOH and diazotized sulphanilic acid as chromogenic reagents.[12] The methylation of pure components as well as chromatographically homogeneous mixtures was carried out using MeI–K_2CO_3 in dry acetone.

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