Phytochemistry, Vol. 26, No. 7, pp. 1985–1987, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

BIFLAVONES FROM THE GENUS *PODOCARPUS*

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(*Received* 27 *August* 1986)

Key Word Index—*Podocarpus taxifolia*; Podocarpaceae; dihydroamentoflavone; podocarpusflavanone; heveaflavone; I-7,II-4′-dimethyl ether of amentoflavone.

Abstract—A new biflavonoid, podocarpusflavanone, has been isolated from *Podocarpus taxifolia* and identified by chemical and spectral data as I-4′,I-5,II-5-trihydroxy-II-4′,I-7,II-7-trimethoxy-I-2,3-dihydro-[I-3′,II-8]biflavone along with other biflavonoids. The distribution of biflavonyl pigments in eight *Podocarpus* species is outlined.

INTRODUCTION

The Podocarpaceae consists of seven genera of which the largest is *Podocarpus* (*ca* 100 species) and *Dacrydium* (23 species), mostly evergreen trees distributed in the southern region [1]. Apart from the report of Hodges [2] of the presence of amentoflavone tetramethyl ether as the main biflavone in the leaves of *Dacrydium cupressinum*, the only data available for the family is for eight species of *Podocarpus* (Table 1).

Cambie and James reported [3] the presence of amentoflavone in the leaves of several members of the Podocarpaceae but were unable to detect any derivative of hinokiflavone. Later Kawano and coworkers [4–6] reported hinokiflavone in addition to amentoflavone and its mono-, di- and trimethyl ethers in the leaves of *Podocarpus macrophylla* [7], *P. gracilor* [8], *P. taxifolia* [6] and *P. nerifolius* [9]. Podocarpusflavone A is present in every species of *Podocarpus* so far investigated, except *P. latifolius*. Thus the *Podocarpus* species contain a simple pattern of derivatives based on amentoflavone and hinokiflavone. The present report of dihydroamentoflavone is new (see Experimental) and is the first example of its isolation and characterization. The occurrence of biflavones in leaf material of *Podocarpus taxifolia* Kunth has been reported [6, 10], but the di- and trimethyl ethers of amentoflavone were not fully characterized. As part of a thorough study of this plant, we now report the isolation of podocarpusflavanone (trimethyl ether of dihydroamentoflavone), heveaflavone and the I-7,II-4′-dimethyl ether of amentoflavone.

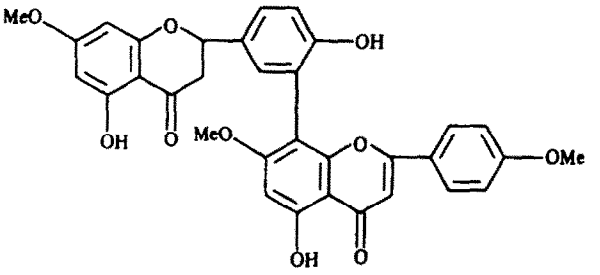
DISCUSSION

The new compound (**1**) besides the two known biflavones (2, 3) were isolated from the acetone soluble fraction of the leaves of *P. taxifolia*. Compounds **2** and **3** were identified as heveaflavone and II-4′,I-7di-*O*-methylamentoflavone. The main compound **1** (mp 236–238°), a microcrystalline, light brown powder from methanol, showed a UV spectrum with a maximum

Table 1. Distribution of biflavones in *Podocarpus* species

Bands	Biflavones	Species A	B	C	D	E	F	G	H	Remarks
I	Am		+	+a	+a	+a	+a		+a	(a) amentoflavone
II	Hi	+b				+b	+b			(b) hinokiflavone
III	M-Am	+c	+c	+c	+c	+c	+c	+c		(c) podocarpusflavone-A
						+d			+d	(d) bilobetin
						+e				(e) sequoiaflavone
III	Di-Am	+f			+f	+				(f) podocarpusflavone-B
			+g	+g	+g				+g	(g) isoginkgetin
	M-Hi	+h								(h) neocryptomerin
IV	Tri-Am	+i				+	+	+i		(i) sciadopitysin
				+j					+j	(j) kayaflavone
V	Tetra-Am					+k				(k) tetra-*O*-methyl-amentoflavone

Key: Am = Amentoflavone; Hi = hinokiflavone; M = mono; A = *Podocarpus macrophylla*; B = *P. nagi*; C = *P. gracilor*; D = *P. nerifolius*; E = *P. taxifolia*; F = *P. elongata*; G = *P. uranii*; H = *P. latifolius*. + = detected; + with letters = fully characterized.



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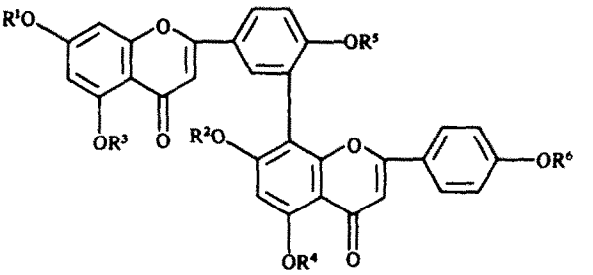
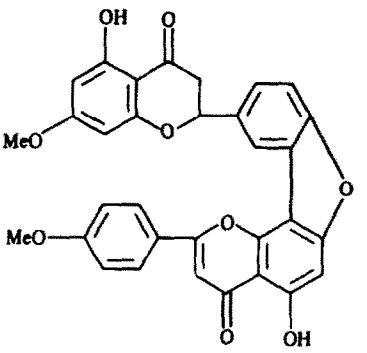


2 $R^1 = R^2 = R^6 = Me$; $R^3 = R^4 = R^5 = H$
3 $R^1 = R^6 = Me$; $R^2 = R^3 = R^4 = R^5 = H$



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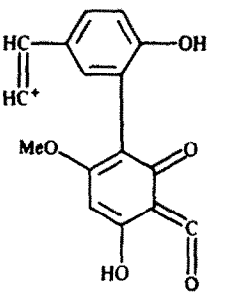

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at 285 and inflexion at 330 nm, characteristic of a flavone. Its mass spectrum showed a molecular ion at $[M]^+$ 582 (100 %). The peak at *m/z* 135 (67 %), indicated that the rings E and F were not involved in the interflavonoid linkage. The peak at *m/z* 550 (15%), which was formed by the loss of 32 mass units, was due to a condensation product (4) in which the *ortho* methoxyl and hydroxyl groups to the biphenyl linkage cyclize to the furan ring. The ion (5) at *m/z* 283 (9 %) was formed after RDA fragmentation. There were four more ions at *m/z* 415 (18%), 403 (45%), 390 (34%) and 140 (10%) corresponding to fragments indicative of a C–C linked flavanone-flavone with three phenolic hydroxy and three methoxy groups.

The ^{1}H NMR spectrum of the acetate of 1 in $CDCl_3$ showed the presence of three acetoxyls (δ2.02, 2.42 and 2.50, each a 3H, *s*) and three methoxyls (δ3.78, 3.89 and 3.96 each a 3H, *s*). The C-2-I and C-3-I protons resonating between δ2.70–2.90 and δ5.10–4.35. A doublet for 2H (H-3',5'-II) at δ6.70 clearly suggested OMe-4' and doublet for 2H (H-5'-I) at δ6.87 confirmed OAc-4'-I. The signals due to H-6, 8-I and H-6-II indicated the presence of OMe-7-I and 7-II groups and were characteristic of a B–D-ring linkage. This was confirmed by the presence of seven side

phenyl protons resonating between δ6.70 and δ7.58. The structure of **1** (I-3',II-8 naringenin-apigenin) was confirmed by its dehydrogenation to heveaflavone and subsequent methylation to the hexamethyl ether of amentoflavone.

EXPERIMENTAL

Mps are uncorr. Analytical and prep. TLC were performed on silica gel G (BDH) using C_6H_6–pyridine–HCO_2H (BPF, 36:9:5) as solvent [11].

Isolation procedures. Leaves (3 kg) of *Podocarpus taxifolia* Kunth procured from the Government Botanic Garden, Ooty, India were extracted with boiling petrol and acetone. The acetone concentrates was refluxed successively with petrol and benzene to remove non-flavanoid and resinous matter. The brown solid (5 g) thus obtained responded to the usual colour test for flavanoids and showed the presence of four major and a number of minor overlapping spots. The brown solid (5 g) was absorbed on silica gel (8.00 g) set with petrol (b.p. 40–60°). The column was eluted successively with benzene–EtOAc (9:1, 8:2) and monitored by TLC. Fractions (50 ml) were collected and following compounds were isolated from different pools of identical fractions, **2** (120 mg), **1** (220 mg) and **3** (115 mg).

I-4',I-5,II-5-*Triacetoxy*, II-4',I-7,II-7-*trimethoxy*-I-2, 3-*dihydro* [I-3',II-8] *biflavone* ($C_{33}H_{26}O_{10}$, $[M]^+$ 582); MS: (EI 70 eV, 4 kV, 100 μA, 250°; DI 10^{-6} T) *m/z* 582 $[M]^+$ (100 rel. int.), 550 (15), 416 (34), 403 (45), 385 (20), 390 (34), 291 (8), 283 (9), 253 (14), 193 (13), 167 (18), 140 (10), 138 (8), 135 (67), 95 (15), 78 (10), 69 (22), 57 (15), UV λ_{max}^{MeOH} nm: 285, 225 (sh), 330 (sh). ^{1}H NMR: ($CDCl_3$, TMS int., 100 MHz): δ2.02, 2.42 and 2.50 (each a 3H, singlet, OAc-I-4',I-5 and II-5); 2.70–2.90 (2H, *m*, I-3), 3.78, 3.96 and 3.89 (each 3H, *s*, OMe-I-4', I-7 and II-7); 5.10–5.35 (1H, *m*, I-2); 6.54 (1H, *d*, I-6); 6.58 (1H, *s*, II-3); 6.67 (1H, *s*, II-6); 6.70 (2H, *d*, II-3',5'); 6.72 (1H, *d*, I-8); 6.87 (1H, *d*, I-5'); 7.01–7.28 (2H, *d*, II-2',6'); 7.40–7.58 (2H, *m*, I-2',6'). The acetate crystallized from $CHCl_3$–petrol as needles, m.p 205–207°. Dehydrogenation of **1** was carried out by the method of ref [12]. The product was completely methylated to provide hexamethylamentoflavone, identified by R_f and in comparison with an authentic sample.

I-4',I-5,II-5-*Trihydroxy*-II-4',I-7,II-7-*trimethoxy*[I-3',II-8] *biflavone* **2** was characterized as heveaflavone by direct comparison with an authentic sample (R_f value, mp, mmp and fluorescence in UV light). It was further confirmed by ^{1}H NMR values of its acetate and comparison with an authentic heveaflavone triacetate.

I-4',I-5,II-5,II-7-*Tetrahydroxy*-II-4',I-7-*dimethoxy*[I-3',II-8]*biflavone*. **3** was characterized as II-4',I-7-dimethoxy amentoflavone by comparison with an authentic sample (R_fs, mp, mmp). It was further confirmed by ^{1}H NMR values of its acetate and comparison with an authentic II-4',I-7-dimethylamentoflavone tetra-acetate.

Acknowledgements—We are grateful to Prof. Dr. E. Wollenweber of Botanik Institute, Darmstadt, F.R.G. for spectral measurements. One of us (S.K.R.) thanks CSIR, New Delhi for the award of a fellowship.

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