A *C*-Methylbiflavone from *Cephalotaxus harringtonia* K. Koch

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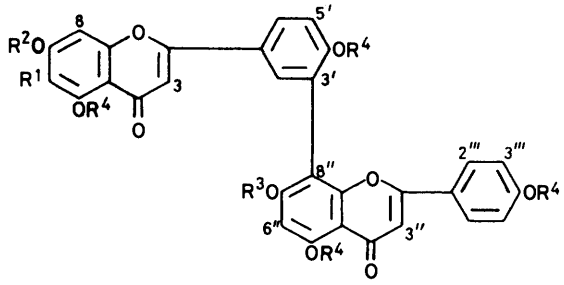
6-*C*-Methyl-7-*O*-methylamentoflavone (1) isolated from the leaves of *Cephalotaxus harringtonia* K. Koch (Cephalotaxaceae) has been identified on the basis of the spectral data of its hexamethyl ether (1a) and its penta-acetate (1b). The location of the *C*-methyl group was first deduced from ^{1}H n.m.r. studies with a lanthanide shift reagent and confirmed by a synthesis of the hexamethyl ether (1a).

FOUR biflavones, sciadopitysin, ginkgetin, sequoiaflavone, and amentoflavone, have been isolated from the leaf extracts of *Cephalotaxus drupacea* Sieb. and Zucc.[1] We now report a new *C*-methylbiflavone from the leaves of *C. harringtonia* K. Koch (Cephalotaxaceae) which was extracted from the fresh leaves with hot acetone. Besides the amentoflavone and its methyl ethers, a new compound, 6-*C*-methyl-7-*O*-methylamentoflavone (1), $C_{32}H_{22}O_{10}$, m.p. 340 °C, was isolated.

On methylation with dimethyl sulphate and potassium carbonate, the flavone (1) gave a methyl ether (1a), m.p.



(1) $R^1 = R^2 = Me$, $R^3 = R^4 = H$
a; $R^3 = R^4 = Me$
b; $R^3 = R^4 = Ac$
c; $R^3 = R^4 = CD_3$
(2) $R^1 = H$, $R^2 = R^3 = R^4 = Me$
(3) $R^1 = H$, $R^2 = Me$, $R^3 = R^4 = Ac$
(4) $R^1 = H$, $R^3 = Me$, $R^2 = R^4 = Ac$

234—236 °C, M^+ 636.2007 (Calc. for $C_{37}H_{32}O_{10}$, 636.1996). The ^{1}H n.m.r. spectral data of (1a) showed the presence of six methoxy and one *C*-methyl groups (see Table 1). Furthermore, the aromatic proton signals of (1a) were very similar to those of hexa-*O*-methylamentoflavone (2) except that there was a singlet at δ 6.67 instead of two *meta*-coupled doublets of (2) due to H-6 and -8 (δ 6.34 and 6.48), indicating a structure of 6- or 8-*C*-methylamentoflavone hexamethyl ether for (1a).

On acetylation, (1) gave a penta-acetate (1b), m.p. 250—252 °C, whose ^{1}H n.m.r. spectrum showed the presence of one methoxy, one *C*-methyl and five acetoxy-groups (see Table 2). The ^{1}H n.m.r. spectral data of (1b) were compared with those of two related compounds, sequoiaflavone penta-acetate (3) and 7″-*O*-methylamentoflavone penta-acetate (4)[2] (Table 2). The H-5′ and H-3‴ and -5‴ signals of (1b) suggested the presence of 4′- and 4‴-acetoxy-groups and the two

TABLE 1

^{1}H N.m.r. data (δ values) of compounds (1a) and (2) (*J* values in Hz)

Protons		(1a)	(2)
MeO-5	3 H, s	3.87	3.93
-5″		4.08	4.06
-7		3.87	3.89
-7″		3.92	3.93
-4′		3.79	3.76
-4‴		3.78	3.74
Me-6	3 H, s	2.18	
H-3	1 H, s	(6.60)	(6.59)
-3″	1 H, s	(6.67)	(6.53)
-6″	1 H, s	6.54	6.64
-6			6.34 (1 H, d)
-8		6.67 (1 H, s)	6.48 (1 H, d)
-2′	1 H, d, *J* 2	7.90	7.84
-6′	1 H, q, *J* 2 and 9	7.95	7.94
-2‴, 6‴	2 H, d, *J* 9	7.40	7.37
-5′	1 H, d, *J* 9	7.15	7.13
-3‴, 5‴	2 H, d, *J* 9	6.78	6.77

TABLE 2

^{1}H N.m.r. data (δ values) of compounds (1b), (3), and (4) (*J* values in Hz)

Protons		(1b)	(3)	(4)
MeO-7	3 H, s	3.87	3.80	
-7″				3.87
AcO-7	3 H, s			2.30
-4‴		2.27	2.22	2.25
-5		2.49	2.39	(2.43)
-5″		2.49	2.44	(2.48)
-7″		(2.09)	(2.01)	
-4′		(2.09)	(2.05)	1.98
Me-6	3 H, s	(2.05)		
H-3	1 H, s	6.65	(6.63)	(6.57)
-3″		6.65	(6.64)	(6.64)
-6″		(7.00)	6.99	6.75
-8		(6.76)		
-6	1 H, d, *J* 2		6.57	6.82
-8			6.78	7.25
-3‴, 5‴	2 H, d, *J* 9	7.03	7.03	7.04
-2‴, 6‴		7.50	7.49	7.49
-5′	1 H, d, *J* 9	7.44	7.44	7.44
-2′	1 H, d, *J* 2	8.06	8.03	7.98
-6′	1 H, q, *J* 2 and 9	7.94	7.94	7.95

singlets at δ 6.76 and 7.00 assigned to H-6″ and H-8 of (1b) suggested the presence of 7-methoxy- and 7″-acetoxy-groups. The presence of a 7-methoxy-group

was also supported by ^{1}H n.m.r. spectral studies of a trideuteriomethylation [3] product (1c). There was a sharp singlet at δ 3.87 assigned to MeO-7, whose *S*-value [Eu(fod)$_3$] was 0.22 p.p.m. [reported *S*-values [4] of MeO-7 and -7″ of hexa-*O*-methylamentoflavone (2) are 0.36 and 1.06 p.p.m., respectively].

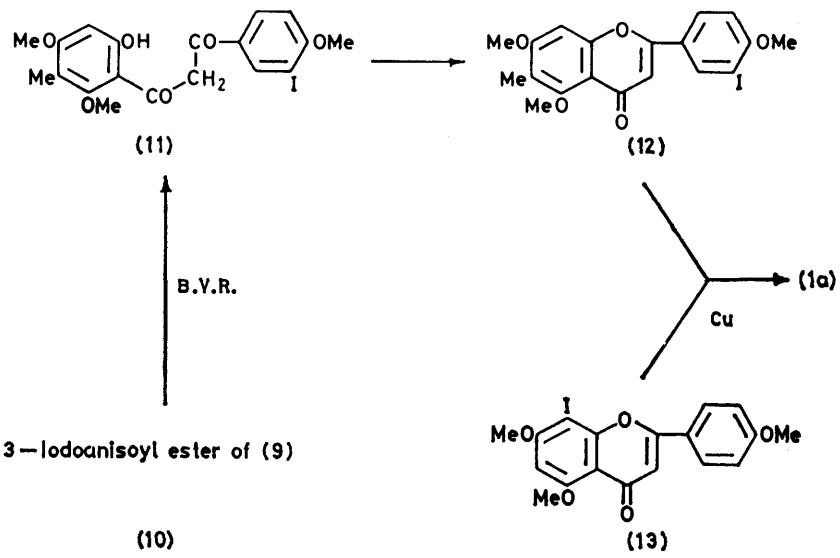
TABLE 3

S-values [Eu(fod)$_3$] of *C*-methylapigenin trimethyl ethers and (1a)

Protons	6-C-Me-Ap	8-C-Me-Ap	(1a)
MeO-5	13.06	17.08	5.76
-5″			9.36
-7	1.04	2.18	0.22
-7″			1.20
-4′	−0.10	−0.10	0.02
-4‴			−0.20
Me-6	5.04		2.20
-8		0.80	
H-3	−0.50	−0.76	(−0.20) (−0.10)
-3″			
-6″			4.98
-6		8.26	
-8	1.70		0.56
-2′	−0.16	−0.24	0.24
-6′			−0.04
-3′	−0.14	−0.12	
-5′			0.06
-2‴, 6‴			−0.16
-3‴, 5‴			−0.16

The location of the *C*-methyl group of (1a) was first deduced from the ^{1}H n.m.r. *S*-values induced by the Eu(fod)$_3$ reagent [4] (see Table 3). In a preliminary experiment on 6- and 8-*C*-methylapigenin trimethyl ethers [5] the *S*-values of the CMe signals of the two compounds were so different (5.04 and 0.80 p.p.m.) that the 6- and 8-CMe groups were readily assigned. The *S*-value (2.20 p.p.m.) of the 6-CMe signal of (1a) was much smaller than that (5.04 p.p.m.) of 6-*C*-methylapigenin trimethyl ether. However, in the case of amentoflavone the reagent had somewhat less effect on the flavone nucleus which bears the 6-CMe group than the other flavone nucleus. Therefore, the *S*-value (2.20 p.p.m.), a little less than half the value of 5.04 p.p.m., should be assigned to a 6-CMe signal. Accordingly, the 6-CMe structure (1a) was deduced for the hexamethyl ether.

However, in some cases, such as hinokiflavone [6] and saharanflavone,[7] unequivocal syntheses decided their final structures correcting the formerly deduced structures. Accordingly, a compound of the deduced structure (1a) was synthesized by a route (see Scheme 1), similar to Nakazawa's synthetic route to (2).[8] In the synthesis of (1a) 6-*C*-methyl-3′-iodoapigenin trimethyl ether (12) was used instead of 3′-iodoapigenin trimethyl ether in the Ullmann reaction reported by Nakazawa. 5-Methyl-2-hydroxy-4,6-dimethoxyacetophenone (9) [9] was an important intermediate for the synthesis of the compound (12). There were two possible routes for obtaining the intermediate (9): A, *C*-methylation [10] of phloroacetophenone followed by partial methylation [11] of the phenol groups, and B, *C*-methylation [12] of 3,5-dimethoxyphenol (5) followed by acetylation.[9] Because of the difficulty of partial methylation route B was better than A. The intermediate (9) was prepared from (5) according to the method reported by Jain *et al.*[9,12] (Scheme 2). Although the two formylation products [(6) and (7)] were obtained in nearly equal amounts the former (6) was less soluble in ethyl acetate than the latter (7). Reduction of (6) followed by acetylation gave (9), which was treated with 3-iodo-*p*-anisoyl chloride [8] to

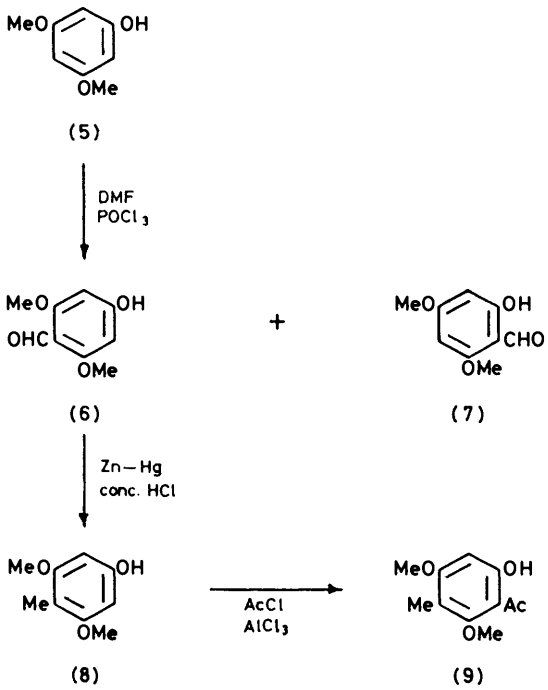


SCHEME 1

give an ester (10). The Baker–Venkataraman rearrangement (B.V.R.) product of (10) was cyclized to give compound (12), which was finally condensed with (13) in an Ullmann reaction without solvent [13] to give (1a), identical with the methyl ether of the natural biflavone (1) in all spectral data and mixed m.p. determination.

On the basis of the above results, the structure (1) was established as the first example of naturally occurring

biflavone bearing a *C*-methyl group on a flavone nucleus. The possibility of useful application of the shift reagent $Eu(fod)_3$ for determining the location of a *C*-methyl group at either the 6- or 8-position of flavone compounds has also been established.



SCHEME 2

EXPERIMENTAL

^{1}H N.m.r. spectra were recorded with a JEOL PS-100 instrument using tetramethylsilane as internal reference in $CDCl_3$ solutions. Mass spectra were obtained from a JEOL JMS-01SG double-focus high resolution instrument. U.v. and i.r. spectra were recorded with a Hitachi 323 recording spectrometer and a JASCO IRA-2 grating infrared spectrophotometer respectively. Column chromatography was carried out with Mallinckrodt silicic acid (100 mesh) unless otherwise noted.

Isolation.—Fresh leaves (10 kg) of *Cephalotaxus harringtonia* K. Koch, procured from Forest Research Institute, Dehradum, India, were extracted with hot acetone and the solvent was distilled off. The dark green concentrate was dried under reduced pressure and then treated with light petroleum (b.p. 40—60 °C), benzene, chloroform, and hot water. The insoluble residue was refluxed with ethyl acetate for 8 h and the filtrate was concentrated to give a dark coloured residue, which was chromatographed on silica gel eluting with light petroleum, benzene, chloroform and benzene–ethyl acetate mixtures (1 : 1 and then 1 : 2). The last two fractions were positive for usual colour tests on flavonoid compounds and combined to give an yellowish brown residue (5.0 g), a mixture of crude biflavones. It was subjected to preparative t.l.c. using a solvent system, benzene–pyridine–formic acid (36 : 9 : 5) to give the following four bands: (i) CHI, characterized as amentoflavone, (ii) CHII, which on methylation gave a mixture of hexa-*O*-methylamentoflavone (2) and a new biflavone methyl ether. The fraction CHII was therefore subjected to repeated preparative t.l.c. on silica gel to give the following two components: CHIIA (7-*O*-methylamentoflavone = sequoiaflavone) and CHIIB (200 mg) (6-*C*-methyl-7-*O*-methylamentoflavone), (iii) CHIII, a mixture of methyl ethers of amentoflavone, and (iv) CHIV, tetra-*O*-methylamentoflavone.

6-C-*Methylhexa*-O-*methylamentoflavone* (1a).—Methylation of CHIIB with dimethyl sulphate and potassium carbonate in acetone under reflux for 5 h gave pale yellow needles from MeOH, m.p. 234—236 °C, $\lambda_{max.}$ (EtOH) (log ε) 269 (4.60) and 326 nm (4.66); $\nu_{max.}$ (KBr) 2 920, 1 646, 1 594, and 1 330 cm^{-1}; ^{1}H n.m.r. data are given in Tables 1 and 3 (Found: C, 70.05; H, 5.1. $C_{37}H_{32}O_{10}$ requires C, 69.80; H, 5.07%).

6-C-*Methyl*-7-O-*methylamentoflavone Penta-acetate* (1b).—Acetylation of CHIIB with acetic anhydride and anhydrous sodium acetate at 120 °C for 1 h gave a solid, which was recrystallized from EtOAc to form needles, m.p. 250—252 °C, $\lambda_{max.}$ (EtOH) (log ε) 264 (4.58) and 313 nm (4.64); $\nu_{max.}$ (KBr) 1 764, 1 635, 1 606, and 1 363 cm^{-1}; ^{1}H n.m.r. data are given in Table 2.

Deuteriomethylation of CHIIB.—CHIIB (20 mg) in dioxan (20 ml) and D_2O (0.1 ml) was mixed with a deuteriated diazomethane solution [3] (20 ml). After 2 days the solvent was evaporated off and the residue was purified by preparative t.l.c. on silica gel with $CHCl_3$ containing 2% MeOH to give pale yellow crystals (1c) (9 mg) from MeOH, m.p. 230—234 °C.

4-*Hydroxy*-2,6-*dimethoxybenzaldehyde* (6), 2-*Hydroxy*-4,6-*dimethoxybenzaldehyde* (7), 4-*Hydroxy*-2,6-*dimethoxytoluene* (8), *and* 2-*Hydroxy*-5-*methyl*-4,6-*dimethoxyacetophenone* (9).—These compounds were prepared according to the reported method.[9,12] Compound (6) showed δ 3.83 (6 H, s), 6.07 (2 H, s), 9.58 (1 H, s), and 10.61 (1 H, s, OH); compound (7) δ 3.76, 3.78 (3 H, s, each), 5.85, 5.96 (1 H, d, each), 10.20 (1 H, s), and 12.22 (OH); compound (8) δ 1.96 (C-Me), 3.73 (6 H, s), and 6.02 (2 H, s); and compound (9) δ 2.04 (C-Me), 2.64 (Ac), 3.69, 3.80 (OMe, each), 6.20 (1 H, s), and 13.16 (OH).

2-*Acetyl*-4-*methyl*-3,5-*dimethoxyphenyl* 3-*Iodo*-p-*anisate* (10).—A mixture of (9) (2.1 g), 3-iodo-*p*-anisoyl chloride (4.5 g), and pyridine (6 ml) was kept at 110 °C for 10 min and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ and washed with 10% HCl and then with water. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ and then with water. After drying over Na_2SO_4, the chloroform was evaporated off to give the *ester* (10), which was recrystallized from EtOAc to yield prisms (4.4 g, 94% yield), m.p. 162—163 °C, $\lambda_{max.}$ (EtOH) (log ε) 234 (4.61) and 266 nm (4.42); $\nu_{max.}$ (KBr) 2 960, 2 930, 1 733, 1 688, 1 592, 1 490, and 1 350 cm^{-1}; δ 2.13 (CMe), 2.48 (Ac), 3.69, 3.80, 3.93 (OMe, each), 6.48 (1 H, s), 6.86 (1 H, d, *J* 9 Hz), 8.12 (1 H, q), and 8.57 (1 H, d, *J* 2 Hz) (Found: C, 48.8; H, 4.0. $C_{19}H_{19}IO_6$ requires C, 48.53; H, 4.07%).

2-(3-*Iodo*-p-*anisoylacetyl*)-3,5-*dimethoxy*-4-*methylphenol* (11).—Compound (10) (1.40 g) and powdered KOH (0.50 g) dissolved in pyridine (5 ml) were stirred at 80 °C for 5 min. The cooled reaction mixture was decomposed with AcOH (5 ml), and MeOH (10 ml) was added. Yellow sandy crystals were filtered off, washed with methanol, and recrystallized from acetone to afford yellow prisms (0.94 g, 67% yield), m.p. 221—223 °C, $\lambda_{max.}$ (dioxan) (log ε) 256 (4.31), 286 (4.32), and 324 (4.31); $\lambda_{min.}$ (dioxan) 269 (4.24) and 310 nm (4.28); $\nu_{max.}$ (KBr) 1 695, 1 638, 1 608, 1 575, 1 490, and 1 330 cm^{-1}; δ 2.08, 3.70, 3.84, 3.94 (3 H, s, each),

6.26 (1 H, s), 6.85 (1 H, d, *J* 9 Hz), 7.32 (1 H, s), 7.92 (1 H, q), and 8.37 (1 H, d, *J* 2 Hz) (Found: C, 48.85; H, 3.95. $C_{19}H_{19}IO_6$ requires C, 48.53; H, 4.07%).

6-C-*Methyl*-3′-*iodo*-4′,5,7-*trimethoxyflavone* (12).—A hot solution of (11) (1.50 g) in AcOH (25 ml) was mixed with a mixture (4 ml) of concentrated H_2SO_4 and AcOH (1 : 4 w/w) and kept for 10 min. The mixture was poured into ice–water (100 ml) to give a white precipitate, which was filtered off, washed with water, dried, and recrystallized from dioxan. Almost colourless *needles* (1.17 g, 81%), m.p. 260—261 °C, were obtained, $\lambda_{max.}$ (dioxan) (log ε) 271 (4.23) and 316 (4.44); $\lambda_{min.}$ (dioxan) 282 nm (4.13); $\nu_{max.}$ (KBr) 2 940, 1 640, 1 600, 1 495, and 1 340 cm^{-1}; δ 2.14, 3.84 (3 H, s, each), 3.92 (6 H, s), 6.60, 6.72 (1 H, s, each), 6.85 (1 H, d, *J* 9 Hz), 7.82 (1 H, q), and 8.33 (1 H, d, *J* 2 Hz) (Found: C, 50.7; H, 3.65. $C_{19}H_{17}IO_5$ requires C, 50.46; H, 3.79%).

6-C-*Methylhexa*-O-*methylamentoflavone* (1a).—A mixture of (12) (0.30 g), 8-iodo-4′,5,7-trimethoxyflavone [8] (13) (0.30 g), and freshly prepared activated copper powder [14] (1.2 g) was kept at 225—230 °C in an oil-bath for 50 min. The dark brown reaction mixture was extracted in a Soxhlet apparatus with $CHCl_3$. The solvent was evaporated off and the residue was purified by column chromatography on silica gel. The first eluate with ethyl acetate contained unchanged flavone compounds [(12) and (13)]. The following eluate with chloroform containing 2% methanol was evaporated and purified by preparative t.l.c. to give pale yellow needles from MeOH (24 mg), identical (u.v., i.r., ^1H n.m.r., and mass spectra) with the methyl ether obtained from CHIIB, m.p. and mixed m.p. 234—236 °C.

The authors are grateful to Professor J. Chopin, University of Lyon, France, for supplying samples of *C*-methylapigenin and to Dr. K. C. Sahni, Forest Research Institute, Dehradun, India, for procurement of the plant materials.

[0/1423 *Received*, 16*th September*, 1980]

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