A New Oxyprenyl Coumarin and Highly Methylated Flavones from the Exudate of *Ozothamnus lycopodioides* (Asteraceae)

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Z. Naturforsch. **55c**, 1–4 (2000); received November 5, 1999

Ozothamnus lycopodioides, Asteraceae, Leaf and Stem Exudate, Flavones, New Oxyprenyl Coumarin

A new oxyprenyl coumarin was isolated from the lipophilic exudate of *Ozothamnus lycopodioides.* Its structure was established as 7-(3,3′-dimethylallyloxy)-5-hydroxy-6-methoxycoumarin from its UV, MS and NMR spectral data, especially two dimensional experiments. In addition to six earlier reported flavonols, we found four highly substituted flavones, including two rare methylenedioxyflavones.

The genus *Ozothamnus* (Asteraceae) comprises some 50 species, native to Australia, New Zealand and New Caledonia (Bremer, 1994). A previous paper dealt with rare flavonoids occurring in the leaf and stem exudates of two *Odixia* and eleven *Ozothamnus* species from Australia. *O. lycopodioides* was reported to exhibit six flavonols, namely herbacetin-3,7,8,4′-tetramethyl ether, quercetin-3 methyl ether, quercetin-3,7-dimethyl ether, quercetagetin-3,6-dimethyl ether, gossypetin-3,7,8-trimethyl ether and gossypetin-3,7,8,3′,4′-pentamethyl ether (Wollenweber *et al.*, 1997). In addition, we now identified four flavones and a new oxyprenylated coumarin.

Material and Methods

Branches of *Ozothamnus lycopodioides* were collected at Black Bridge Gulli, Tasmania (42° 34′S 147° 49′E) on 26. Jan. 1989. A voucher (Davis 1259) is kept at the Herbarium Australiense in Canberra, Australia (CANB). Air-dried plant material was briefly rinsed with acetone to dissolve the lipophilic exudate. The concentrated solution was then subjected to column chromatography on Sephadex LH-20, eluted with methanol, to separate the phenolic fractions from the predominant terpenoids. Fractions containing flavonoid aglycones were concentrated and the residue (3.1 g) was further chromatographed on "flash" silica-gel using the binary mixture ethyl acetate – hexane (3:1 v/v), furnishing **1** (165 mg), **2** (52 mg), **3** (210 mg), **4** (360 mg) and **5** (23 mg).

M.p.'s are uncorrected. ^{1}H NMR: 300 MHz, TMS as int. Standard. ^{13}C NMR : 75.5 MHz, solvent as internal standard. 2D NMR experiments were performed using standard Bruker pulse sequences. MS: EI (70 eV), VG AutoSpec. IR: KBr, Bruker Vector 22. UV: MeOH, Hewlett Packard 8453.

5,6,7,8-tetramethoxy-3′,4′-methylendioxyflavone (**1**). Colourless crystals (MeOH), mp. 169–170 °C. TLC (SiO_2 60): Rf 0.55 (AcOEt-hexane, 3:1 v/v). IR, UV and ^{1}H NMR as literature (Ngo Le-Van *et al.*, 1979). ^{13}C NMR ($CDCl_3$), δ ppm: 61.50 (CH_3), 61.66 (CH_3), 61.89 (CH_3), 62.10 (CH_3), 101.79 (CH_2), 105.89 (CH), 106.92 (CH), 108.65 (CH), 114.70 (C), 120.94 (CH), 125.36 (C), 137.94 (C), 143.98 (C), 147.50 (C), 148.20 (C), 148.34 (C), 150.35 (C), 151.29 (C), 160.64 (C), 177.06 (C).

5,6,7,8,5′-pentamethoxy-3′,4′-methylendioxyflavone (**2**). Yellow crystals (MeOH), mp 185–187 °C. TLC (SiO_2 60): Rf 0.50 (AcOEt-hexane, 3:1 v/v). IR, UV, ^{1}H NMR as literature (Ngo Le-Van *et al.*, 1979). ^{13}C NMR ($CDCl_3$), δ ppm: 56.70 (CH_3), 61.63 (CH_3), 61.78 (CH_3), 61.94 (CH_3), 62.22 (CH_3), 61.63 (CH_3), 61.78 (CH_3), 61.94 (CH_3), 62.22 (CH_3), 100.43 (CH), 102.27 (CH_2), 106.53 (CH), 107.31 (CH), 114.82 (C), 125.89 (C), 137.99 (C), 138.27 (C), 143.88 (C), 144.13 (C),

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147.61 (C), 148.36 (C), 149.54 (C), 151.46 (C), 160.61 (C), 177.20 (C).

5,6,7,8,3',4'-hexamethoxyflavone (**3**). Yellow crystals, mp. 130–131 °C (MeOH). TLC (SiO_2 60): Rf 0.31 (AcOEt-hexane 3:1 v/v). IR, UV, ^1H NMR as literature (Ngo Le-Van *et al.*, 1979). ^{13}C NMR (CDCl3), δ ppm: 55.64 (CH_3), 55.78 (CH_3), 61.35 (CH_3), 61.50 (CH_3), 61.65 (CH_3), 61.94 (CH_3), 106.50 (CH), 108.24 (CH), 110.97 (CH), 114.52 (C), 119.65 (C), 126.50 (C), 137.71 (C), 143.76 (C), 147.39 (C), 148.06 (C), 148.97 (C), 151.11 (C), 151.63 (C), 160.69 (C), 176.95 (C).

5,6,7,8,3',4',5'-heptamethoxyflavone (**4**). Yellow crystals, mp 104–105 °C. TLC (SiO_2 60): Rf 0.40 (AcOEt-hexane 3:1 v/v). IR, UV, ^1H NMR as literature (Ngo Le-Van *et al.*, 1979). ^{13}C NMR ($CDCl_3$), δ ppm: 56.02 (2xCH_3), 60.81 (CH_3), 61.31 (CH_3), 61.49 (CH_3), 61.60 (CH_3), 61.71 (CH_3), 62.05 (CH_3), 103.04 (2xCH), 107.41 (CH), 114.63 (C), 126.48 (C), 137.80 (C), 140.85 (C), 143.96 (C), 147.52 (C), 148.21 (C), 151.35 (C), 153.38 (2xC), 160.55 (C), 177.07 (C).

7-(3,3'-dimethylallyloxi)-5-hydroxy-6-methoxy-coumarin (**5**). Yellow crystals, mp 143–145 °C. TLC (SiO_2 60): Rf 0.82 (AcOEt-hexane 3:1 v/v). UV (MeOH) λ_{max} nm (log ε): 210 (4.47), 325 (4.10); IR ν_{max} cm^{-1}: 3298 (OH), 1703 (C=O), 1624 (C=C). MS (70 eV) *m/z* (rel. int.), 276 [M+] (9), 208 (100), 193 (70), 165 (8), 137 (7), 95 (12), 69 (75). For ^1H NMR ($CDCl_3$ and C_6D_6) and ^{13}C NMR data see Table I.

Results and Discussion

The lipophilic exudate produced by aerial parts of *Ozothamnus lycopodioides* was shown previously to contain six flavonol aglycones. From remaining fractions we now isolated the methylenedioxyflavones linderoflavone B (lucidin dimethyl ether) (**1**), eupalestin (**2**), nobiletin (**3**), and 5'-methoxynobiletin (**4**). Their structures were assigned by comparison with spectral data described in literature (**1**: Lee *et al.*, 1965; **2**: Ngo Le-Van *et al.*, 1979; **3**: Tseng, 1938; **4**: Ngo Le-Van *et al.*, 1979).

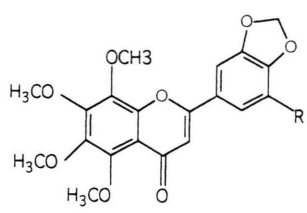
Compound **5** was obtained as a yellow solid. The EI mass spectrum of **5** showed a weak M^+ ion at *m/z* 276, which by accurate mass measurent corresponded to a molecular formula of $C_{15}H_{16}O_5$. Both, ^1H NMR and ^{13}C NMR spectra, contained too many signals to fit the above mentioned formula.

In the ^1H NMR spectrum of **5** (recorded in $CDCl_3$), the doublets at δ 6.19 and 7.96 (J = 9.6 Hz) could be attributed to H-3 and H-4 and the low-field nature of the chemical shift of H-4 suggested the presence of an oxygenated group at C-5 (Murray *et al.*, 1982). A multiplet at δ 5.46 (1H), a doublet at δ 4.60 (2H, J = 6.6 Hz) and two sin-

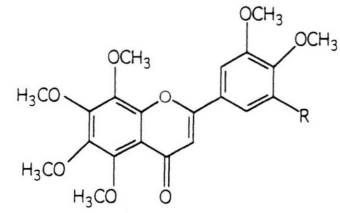
Table I. ^1H and ^{13}C NMR assignments and ^1H–^{13}C long-range correlations of **5** by HMBC, and ^1H and ^{13}C NMR spectral data of **6**.

	5					**6**	
Position	^1H ($CDCl_3$)	^1H (C_6D_6)	Δ*	^{13}C ($CDCl_3$)	Cross-peaks in HMBC spectrum	^1H ($CDCl_3$)	^{13}C ($CDCl_3$)
2				161.59			161.3
3	6.19 (d, 9.6)	5.73 (d, 9.6)	0.46	111.38	102.41 (10), 161.59 (2)	6.23 (d)	111.6
4	7.96 (d, 9.6)	7.36 (d, 9.6)	0.60	138.71	93.25 (8), 145.69 (5), 151.57 (9), 161.59 (2)	7.97 (d)	138.8
5				145.69			146.1
6				131.81			131.8
7				154.84			154.7
8	6.42 (s)	6.00 (s)	0.42	93.25	102.41 (10), 131.80 (6), 151.57 (9), 154.84 (7)	6.43 (s)	93.0
9				151.57			151.6
10				102.41			103.2
1'	4.60 (br d, 6.6)	3.91 (br d, 6.6)	0.69	65.93	118.61 (2'), 139.02 (3'), 154.84 (7)	4.35 (dd)	70.5
2'	5.46 (m)	5.13 (m)	0.33	118.61	18.29, 25.74, 65.93 (1')	4.10 (dd)	75.8
3'				139.02			71.9
CH_3-3'	1.75 (d, 0.8)	1.21 (br s)		18.29	18.29, 25.74,	1.35 (s)	25.1
	1.79 (d, 1.0)	1.35 (br s)		25.74	118.61(2') 139.02 (3')	1.39 (s)	26.3
CH_3-O	3.88 (s)	3.28 (s)	0.60	61.20	131.81 (6)	4.07 (s)	61.5

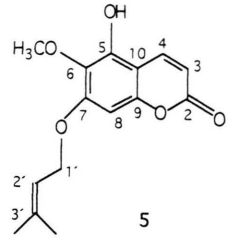
* : $\Delta = \delta_{CDCl3} - \delta_{C6D6}$.



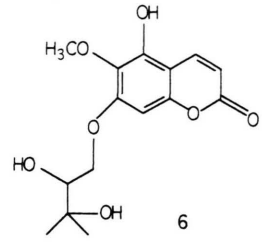
1: R = H
2: R = OCH_3



3: R = H
4: R = OCH_3



5



6

glets at δ 1.75 and 1.79 (3H each) indicated the presence of a 3,3′-dimethylallyloxy side-chain, which was confirmed by the ^{13}C NMR spectrum. The methoxyl singlet at δ 3.88 and the presence of a singlet at δ 6.42 in the ^{1}H NMR spectrum showed that compound **5** is a coumarin with three oxygenated substituents in the aromatic region.

The assignment of the hydroxy, methoxy and dimethylallyloxy moieties was carried out using the bidimensional experiments: HMBC and NOESY spectra. The HMBC spectrum showed the following connectivities: a) the protons of the methoxyl group at δ 3.88 showed ^{3}J to δ 131.81; b) the protons of the methylene group of the prenylated moietie at δ 4.60 showed ^{3}J coupling to carbon of the aromatic ring at δ 154.84 and to carbon of the olefinic group at δ 139.02; c) H-4 at δ 7.96 showing ^{3}J couplings to carbons of the aromatic nucleus resonating at δ 145.69 and δ 151.57, and to carbon at δ 161.59 (CO). This allows the assignment of the C-5 hydroxy substituent and confirms that the chemical shift of C-5 may be either δ 145.69 or δ 151.57. Additionally, the HMBC spectrum showed ^{3}J couplings between the proton H-3 at δ 6.19 and the carbon of the aromatic ring at δ 102.41; also the aromatic proton at δ 6.41 showed ^{3}J coupling to carbon at δ 102.41, indicating that the proton of the aromatic nucleus is at the position C-6 or C-8.

The assignment of the positions of the oxyprenyl and methoxy groups was again based on NOESY experiment, this spectrum showing bidirectional connectivities between the aromatic proton at δ 6.41 and the protons of the methylene oxyprenylated at δ 4.60. It showed no correlation with the protons of the methoxyl group.

The study of HMBC and NOESY spectra allowed the assignment of two plausible structures for compound **5**: a) 7-(3,3′-dimethylallyloxy)-5-hydroxy-8-methoxy-coumarin or b) 7-(3,3′-dimethylallyloxy)-5-hydroxy-6-methoxy-coumarin. The final structural decision was made by means of a solvent shift method with $CDCl_3$ and benzene-d_6 as the solvents, and the 2D ^{1}H-^{1}H COSY experiment.

Solvent induced shifts in benzene-d_6 relative to $CDCl_3$ ($\Delta = \delta_{CDCl3} - \delta_{C6D6}$) have been measured and used to determine the position of methoxyl groups in coumarins (González *et al.*, 1973; Grigg *et al.*, 1966). Methoxyl groups located at C-8 show only minor changes ($\Delta\delta \equiv 0.1 - 0.2$ ppm) and are readily distinguished from other isomers (Dean *et al.*, 1978). In compound **5** this effect is large for the methoxy group ($\Delta\delta = 0.60$ ppm), similar to the effect of a methoxyl group in C-6 of capensin (García *et al.*, 1988); hence the methoxyy group has to be assigned to position C-6 of the coumarin **5**. Additionally, the 2D ^{1}H-^{1}H COSY spectrum

showed an interaction between H-4 (δ 7.96) and the aromatic proton at δ 6.42, this being assigned to H-8, since the literature reports a W-like coupling between H-4 and H-8 in coumarins (Murray *et al.*, 1982; Rashid *et al.*, 1992; Vilegas *et al.*, 1995).

All these data are compatible with the structure of 7-(3,3′-dimethylallyloxy)-5-hydroxy-6-methoxycoumarin **5**. The ^{1}H NMR and ^{13}C NMR spectral data of compound **5** agree with those previously described for compound **6** (Vilegas *et al.*, 1995), except for the signals corresponding to the prenyl group (Table I).

Linderoflavone B (**1**), first reported from *Lindera lucida* (Lee *et al.*, 1965), was later found in *Ageratum* (Wollenweber *et al.*, 1994) and in *Eupatorium* (Ngo Le-Van *et al.*, 1979). Eupalestin (**2**), first known from *Eupatorium coelestinum* (Ngo Le-Van *et al.*, 1979), was also found in *Ageratum* species (Wollenweber *et al.*, 1994). Nobiletin (**3**) is a well-known constituent of *Citrus* fruit peel (Tseng, 1938), but also known to occur e.g. in the Asteraceae *Ageratum conyzoides* (González *et al.*, 1991), *Eupatorium leucolepis* (Herz *et al.*, 1982) and *Viguiera rosei* (Wollenweber *et al.*, 1995). 5′-methoxynobiletin (**4**) was earlier reported from the Asteraceae *Ageratum conyzoides* (González *et al.*, 1991), *Ageratum tomentosum* (Vázquez *et al.*, 1988), *Conoclinium greggii* (Martínez-Vázquez *et al.*, 1993) and *Eupatorium coelestinum* (Ngo Le-Van *et al.*, 1979). To our knowledge this is the first time that methylenedioxyflavones are definitively reported as constituents of lipophilic exudates. We assume, however, that earlier authors did not care for the localization of these compounds, that they may also be accumulated externally on the plant species cited above (with the exception of *Citrus* peel, where they are localized in oil cavities).

Acknowledgements

The authors wish to thank Dr Chris Puttock (Canberra) for the plant material, and Mrs Marion Dörr (Darmstadt) for technical assistance.

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