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TWO NEW FLAVONES FROM *EUPATORIUM COELESTINUM*

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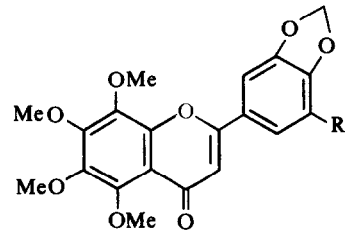
Key Word Index—*Eupatorium coelestinum*; Eupatorieae; Compositae; new flavones.

Abstract—Besides coumarin, nobiletin, lucidin dimethyl ether and 5,6,7,3′,4′-pentamethoxyflavone, two new highly oxygenated flavones were isolated from *Eupatorium coelestinum*. Their structures were determined by spectroscopic methods and alkaline degradations as 5,6,7,8,3′,4′,5′-heptamethoxyflavone and 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxyflavone.

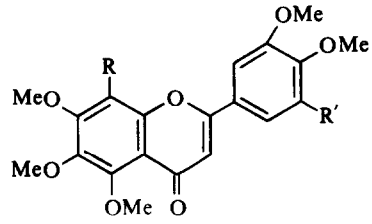
INTRODUCTION

In continuation of our search for tumor inhibitors from Compositae we have investigated *Eupatorium coelestinum*. Plants of this large genus are known to produce sesquiterpene lactones and flavones, many of which are cytotoxic or exhibit antineoplastic activity and several other types of natural products are also present [1].

We report herein the isolation and structure elucidation of two new highly oxygenated flavones, which we name eupalestin (**1**) and 5′-methoxynobiletin (**2**)† as well as coumarin, lucidin dimethyl ether **3** [2], nobiletin **4** [3] and 5,6,7,3′,4′-penta-*O*-methylflavone **5** [4] from *Eupatorium coelestinum*. This appears to be the first report of the characterization of lucidin dimethyl ether, nobiletin and 5,6,7,3′,4′-penta-*O*-methylflavone from a *Eupatorium* species. Nobiletin exhibits strong fungistatic activity toward *Deuterophoma tracheiphila*, which causes the 'mal secco' disease of citrus varieties [5]; it is noteworthy that the two new flavones possess similar oxygenation patterns as nobiletin.



1 R = OMe
3 R = H



2 R = R′ = OMe
4 R = OMe; R′ = H
5 R = R′ = H

RESULTS AND DISCUSSION

The aerial parts of *Eupatorium coelestinum* afforded in high yield coumarin and a mixture of several flavonoids which could be separated only after many recrystallizations and TLC. Three of them have been isolated before: lucidin dimethyl ether **3** from *Lindera lucida* (Lauraceae) [2]; nobiletin **4** from the Chinese drug chen-pi which was made from the peel of a variety of mandarin (*Citrus nobilis* Lour.) [3], the structure being supported later by syntheses [6, 7], and 5,6,7,3′,4′-penta-*O*-methylflavone (**5**) from orange peel [4]. In addition, two new fully methylated flavones were isolated: eupalestin and 5′-methoxynobiletin.

The UV spectrum of the more polar yellow substance **2**, $C_{22}H_{24}O_9$, showed a peak at 325 nm, indicating that it is a flavone (IR bands at 1650 and 1600, 1510 cm^{-1}) rather than a flavonol [8]. The ^1H NMR spectrum (in C_6D_6) indicated the presence of seven methoxy groups and two singlets at δ 6.66 (1H) and 7.03 (2H), which could be attributed to a C-3, C-6 or a C-8 proton and two flavone-nucleus protons of the symmetrical 3′,4′,5′-trisubstituted B-ring (see Table 1). The MS showed the base peak at *m/e* 417 (M^+ – Me) characteristic of 6-methoxyflavones [9]; this peak could be attributed to structure **6**. In addition, that the NMR spectrum of **2** is different from that of 3,5,6,7,3′,4′,5′-heptamethoxyflavone [10] leads to the proposed structure.

The alkaline degradation confirmed this assumption. The acidic product was identified by its mp, IR, NMR and MS as 3,4,5-trimethoxybenzoic acid, eudesmic acid [11]. The neutral product was identified by its NMR, IR

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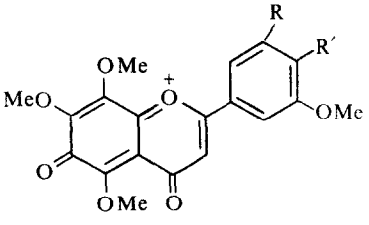
† The same compound has been independently identified as a new flavone in *Ageratum conyzoides* (see following paper).

Table 1. ^{1}H NMR data of the flavones of *Eupatorium coelestinum*

Proton	2		1	3	4	
	$CDCl_3$	C_6D_6	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_6D_6
3-H	6.62 *s*	6.66 *s*	6.55 *s*	6.55 *s*	6.60 *s*	6.65 *s*
2′-H	7.16 *s*	7.03 *s*	7.09 *d* (1.5)	7.34 *d* (1.5)	7.40 *d* (1.5)	7.21 *d* (1.5)
5′-H	—	—	—	6.90 *d* (8.5)	6.97 *d* (8.5)	6.46 *d* (8.5)
6′-H	7.16 *s*	7.03 *s*	7.15 *d* (1.5)	7.48 *dd* (8.5, 1.5)	7.55 *dd* (8.5, 1.5)	7.36 *dd* (8.5, 1.5)
OMe	3.92 *s*	3.49 *s*	3.94 *s*	3.94 *s*	3.95 *s*	3.34 *s*
	3.95 *s*	(6H)	(6H)	(6H)	(12H)	3.41 *s*
	(12H)	3.71 *s*	3.97 *s*	4.00 *s*	4.01 *s*	3.70 *s*
	4.02 *s*	3.75 *s*	4.00 *s*	4.09 *s*	4.09 *s*	3.75 *s*
	4.09 *s*	3.78 *s*	4.08 *s*			3.79 *s*
		3.81 *s*				4.03 *s*
		3.95 *s*				
O \ CH_2 / O	—	—	6.06 *s*	6.05 *s*	—	—

Spectra were run at 100 MHz and TMS was used as internal standard. Chemical shifts are in ppm relative to TMS. Signals are designated as follows: *s*, singlet; *d*, doublet. Figures in parentheses are coupling constants in hertz.



6 R = R′ = OMe
7 R, R′ = O—CH_2—O

and high resolution MS as 2-hydroxy-3,4,5,6-tetramethoxyacetophenone. The structure of this new flavone is therefore 5,6,7,8,3′,4′,5′-heptamethoxyflavone.

The UV spectrum of the less polar compound **1**, $C_{21}H_{20}O_9$, showed a peak at 333 nm, characteristic of a flavone. Two other peaks at 246 and 272 nm could be due to an unsymmetrical 3′,4′,5′-trisubstituted B-ring. The two doublets at δ 7.15 and 7.09 (J = 1.5 Hz) in ^{1}H NMR (in $CDCl_3$) could be attributed to the 2′-H and 6′-H protons of the unsymmetrical 3′,4′,5′-trisubstituted B-ring. The ^{1}H NMR spectrum indicated the presence of five methoxy groups and exhibited two further singlets at δ 6.55 (1H) and 6.06 (2H) due to the C-3 and 3′,4′-methylenedioxy protons (Table 1). The MS showed the base peak at *m/e* 401 (M^+ − Me, structure **7**) characteristic of 6-methoxyflavones. Alkaline degradation afforded the same acetophenone as given by **2**, 3-methoxy-4,5-methylenedioxybenzoic acid, or myristicic acid, identified by mp, NMR, IR and MS [12, 13]. The structure of the flavone is therefore 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxyflavone.

EXPERIMENTAL

UV were run in MeOH; IR in CCl_4 or $CHCl_3$; ^{1}H NMR spectra at 100 MHz; MS at 70 eV, direct probe; mps were determined in capillaries and uncorr.

Eupatorium coelestinum. The air-dried plant material collected on 11 October, 1975 in Louisiana: Baton Rouge: River Road (Urbatch No. 2225. Voucher deposited at Louisiana State University Herbarium at Baton Rouge), was extracted with cold $CHCl_3$ and worked up in the usual manner. The crude syrup (*ca* 4 g) was chromatographed over Si gel, using petrol–Et_2O and Et_2O–MeOH mixtures as eluants. The aerial part (390 g) of *E. coelestinum* L afforded 400 mg of coumarin. The Et_2O–MeOH fractions gave a mixture of several flavonoids which could be separated with difficulty after repeated recrystallizations and TLC yielding 50 mg **1**; 500 mg **3**; 150 mg **2**; 110 mg **4** and 40 mg **5**.

5′-Methoxynobiletin. Light yellow crystals (Et_2O), mp 102°. UV λ_{max}^{MeOH} nm: 275 and 325. IR ($CHCl_3$) cm^{-1}: 1650 (crossed conjugated ketone); 1600, 1510 (aromatics). MS m/e (rel. int.): 432.142 (M^+, 30.6) (calc. for $C_{22}H_{24}O_9$, 432.142); 417 (M − Me, 100); 403 (M − CO − H, 2.5); 401 (M − OMe, 4.8); 389 (417 − CO, 3.8); 374 (389 − Me, 6.0); 387 (417 − CH_2O, 7.6); 359 (387 − CO, 6.4); 356 (387 − OMe, 6.3).

Alkaline degradation of **2**. A soln of **2** (40 mg) in 50% KOH (3 ml) and EtOH (3 ml) was refluxed for 24 hr. The reaction mixture was cooled and acidified with 20% H_2SO_4 then extracted 4× with Et_2O. The ethereal extract was washed with 5% $NaHCO_3$, then H_2O, dried ($MgSO_4$) and evapd to dryness to yield the bright yellow oily 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (20 mg) [6, 14]. UV λ_{max}^{MeOH} nm: 280 and 342; ^{1}H NMR ($CDCl_3$): δ 2.67 (*s*, ArCOC$\underline{H}_3$), 3.80, 3.85, 3.95, 4.07 (4*s*, 4 OMe) and 13.10 (*s*, OH); IR ($CHCl_3$) cm^{-1}: 3540 (OH), 1630 (ketone) and 1590 (aromatics); MS *m/e* (rel. int.): 256.095 (M^+, 100) (calc. for $C_{12}H_{16}O_6$, 256.095); 241 (M − Me, 57.9); 213 (241 − CO, 47.9); 195 (213 − H_2O, 43.6). The H_2O phase

was acidified with 50% H_2SO_4 and extracted with Et_2O (4 ×); the ethereal soln was dried and evapd to afford a colourless solid. Recrystallization from MeOH gave 3,4,5-trimethoxybenzoic acid as colourless crystals; mp 170° (lit. 169° [11]); UV λ_{max}^{MeOH} nm: 255; ^{1}H NMR ($CDCl_3$): δ 3.92 (*s(br)*, 3OMe) and 7.34 (*s*, 2- and 6-H); IR ($CHCl_3$) cm^{-1}: 3200–3540 and 1690 (ArCOOH), 1590 (aromatics); MS *m/e* (rel. int.): 212.069 (M^+, 100) (calc. for $C_{10}H_{12}O_5$, 212.069); 197 (M − Me, 34.4).

Eupalestin. Recrystallization from Et_2O gave fine light yellow crystals, mp 165°, which raised to 185° after recrystallization from MeOH. UV λ_{max}^{MeOH} nm: 246, 272 (sh) and 333. IR ($CHCl_3$) cm^{-1}: 1640 (crossed conjugated ketone); 1590 and 1515 (aromatics). MS *m/e* (rel. int.): 416.111 (M^+, 27.7%) (calc. for $C_{21}H_{20}O_9$, 416.111); 401 (M − Me, 100); 387 (M − CO − H, 25.7); 371 (401 − CH_2O, 17.0); 340 (371 − $^{\cdot}$OMe, 7.1); 358 (401 − CO − Me, 14.6).

Alkaline degradation of **1**. A solution of **1** (35 mg) in 50% KOH (3 ml) and EtOH (3 ml) was refluxed for 24 hr. The reaction mixture was cooled and neutralized with 20% H_2SO_4, then extracted 4 × with Et_2O. The ethereal extract was washed with 5% $NaHCO_3$, then water, dried and evapd to dryness to afford the same acetophenone as above (21 mg). The H_2O phase was acidified with 50% H_2SO_4 and extracted with Et_2O (4 ×); the ethereal soln was dried and evapd to yield colorless solid. Recrystallization from MeOH gave 3-methoxy-4,5-methylenedioxybenzoic acid as colorless crystals (10 mg), mp 208° (lit. 206–7° [12], 209–210° [13]); UV λ_{max}^{MeOH} nm: 270; ^{1}H NMR ($CDCl_3$): δ 3.93 (*s*, OMe), 6.05 (*s*, OCH_2O), 7.24 and 7.38 (*d*, *J* = 1.5 Hz, 2- and 6-H); IR ($CHCl_3$) cm^{-1}: 3200–3540 and 1690 (ArCOOH), 1600 (aromatics); MS *m/e* (rel. int.): 196.037 (M^+, 100) (calc. for $C_9H_8O_5$, 196.037); 181 (M − Me, 5.5).

Lucidin dimethyl ether (**3**). Colorless crystals, mp 160° (MeOH) (lit. 163–168° [2]); UV λ_{max}^{MeOH} nm: 247, 270 and 332; IR ($CHCl_3$) cm^{-1}: 1650 (ketone) and 1600, 1500 (aromatics); MS *m/e* (rel. int.): 386.100 (M^+, 31.6) (calc. for $C_{20}H_{18}O_8$, 386.100); 371 (M − Me, 100); 355 (M − OMe, 5.2); 341 (371 − CH_2O, 18.1); 343 (371 − CO, 11.6); 328 (343 − Me, 19.4).

Nobiletin (**4**). Light yellow crystals, mp 130° (Et_2O) (lit. 129–130° [3]); UV λ_{max}^{MeOH} nm: 247, 270 and 332; IR ($CHCl_3$) cm^{-1}: 1645 (ketone) and 1615, 1525 (aromatics); MS *m/e* (rel. int.): 402.131 (M^+, 18.8) (calc. for $C_{21}H_{22}O_8$, 402.131); 387 (M − Me, 100); 371 (M − OMe, 9.1); 359 (387 − CO, 4.9); 357 (387 − CH_2O, 10.4); 344 (359 − Me, 11.7).

5,6,7,3′,4′-*penta*-O-*methylflavone* (**5**). Colourless crystals, mp 173° (MeOH) (lit. 172–3° [15] and 177–8° [16]). UV λ_{max}^{MeOH} nm: 240, 265 (sh) and 328; IR ($CHCl_3$) cm^{-1}: 1645 (ketone) and 1610, 1530 (aromatics); MS *m/e* (rel. int.): 372 (M^+, 25.0); 357 (M − Me, 100); 341 (M − OMe, 21.5); 326 (357 − OMe, 7.8).

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