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ISOLATION AND SYNTHESIS OF TWO NEW FLAVONES FROM *CONOCLINIUM COELESTINUM**†

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Key Word Index—*Conoclinium coelestinum*; Compositae; Eupatorieae; flavones; 3′,4′-methylenedioxy-5,6,7,8,5′-pentamethoxyflavone; 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone.

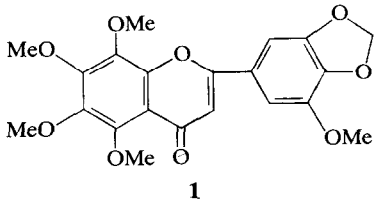
Abstract—Two new flavones, 3′,4′-methylenedioxy-5,6,7,8,5′-pentamethoxyflavone and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone, have been isolated from *Conoclinium coelestinum*. Final structure proof was accomplished by synthesis.

INTRODUCTION

We report the isolation and synthesis of two new flavones, 3′,4′-methylenedioxy-5,6,7,8,5′-pentamethoxyflavone (**1**) and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**2a**), from the non-polar extract of the herbaceous parts of *Conoclinium coelestinum* (L.) DC. [1], tribe Eupatorieae, Gyptis group [2]. Methylenedioxyflavones are quite rare and have not, as far as we are aware, been isolated previously from the Compositae [3]. An earlier report from our laboratory [4] dealt with flavonol 3-glycosides from the polar extract; since then the occurrence of sesamin in the roots and aerial parts of *C. coelestinum* has been described [5].

RESULTS AND DISCUSSION

Flavone **1**, mp 190–190.5°, $C_{21}H_{20}O_9$ (high resolution MS), had four methoxyls and one methylenedioxy group (NMR spectrum). The presence of a narrowly split low field AB quartet ($J_{AB} = 1.5$ Hz) centered at



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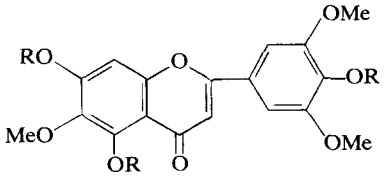

2a R = H
2b R = Ac
2c R = Me
2d R = CH_2Ph

7.11 ppm and a singlet at 6.55 ppm indicated that positions 3,2′ and 6′ of the flavone system were unoccupied; this was confirmed and the locus of the methylenedioxy group at C-3′ and C-4′ established by the mass spectral fragmentation pattern, which also showed that ring A was fully substituted (Scheme 1) [3].

Compound **2a**, mp 223–224°, $C_{18}H_{16}O_8$ (high resolution MS), had three methoxyl groups and three hydroxyl groups (formation of a triacetate **2b** and trimethyl ether **2c**), one of which was on C-5 (NMR signal at 13.08 ppm which disappeared on exchange, bathochromic shift of 31 nm in band I on addition of $AlCl_3$). The presence of a second hydroxyl group at C-7 was indicated by the appearance of a band at 327 nm on adding NaOMe; the same reagent produced a large bathochromic shift (71 nm) of band I, which revealed that the third hydroxyl was located either on C-3 or C-4′. The former possibility was excluded by the NMR spectra of **2a** and derivatives **2b** and **2c**, which exhibited a two-proton singlet at 7.33 ppm (7.06 in **2b**, 7.07 in **2c**) thus showing that H-2′ and H-6′ were equivalent and not coupled to a proton on C-4′ and establishing the substitution pattern of ring B. Attachment of the third methoxyl group to C-6 (or possibly C-8) rather than C-3 was suggested by the

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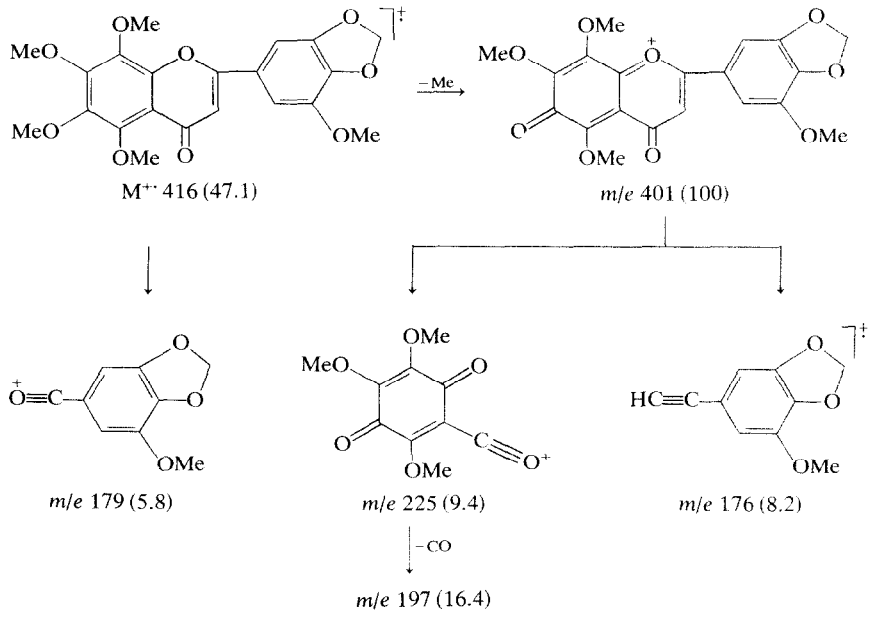
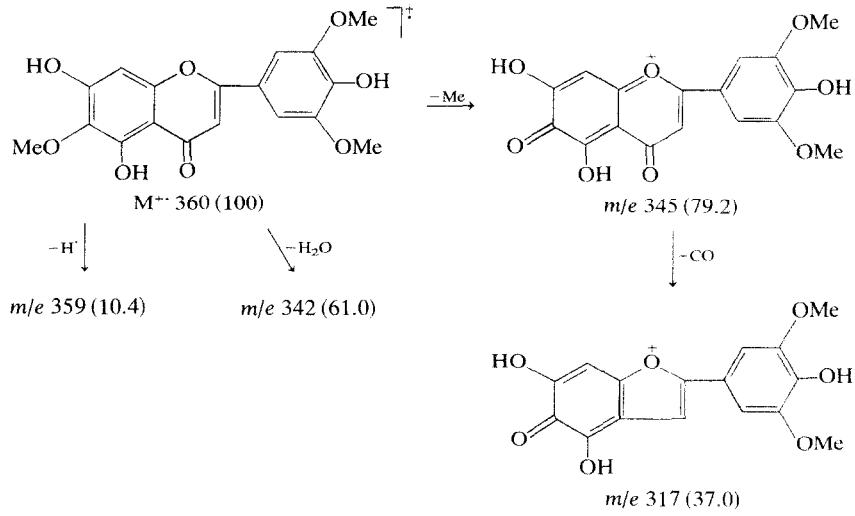


Scheme 1. Selected fragments from MS of **1**.



Scheme 2. Selected fragments from MS of **2a**.

NMR spectrum which had one-proton singlets at 6.98 and 6.67 ppm, only the former of which experienced a significant downfield shift on acetylation. A decision in favour of C-6 was reached by considering the UV spectrum in the presence of $AlCl_3$, which showed a bathochromic shift in band I of only 22 nm and from the mass spectrum (Scheme 2). The relative intensities of the M^+, M^+-15 and M^+-18 peaks were characteristic of 5,7-dihydroxy-6-methoxy- rather than 5,7-dihydroxy-8-methoxyflavones [6].

The ^{13}C NMR spectrum of **2a** (Table 1) was in accord with the proposed formula. Since **1** was insoluble in DMSO-d_6, the ^{13}C NMR spectrum was run in $CDCl_3$ and is also given in Table 1. However, the absence of literature data on 5,6,7,8-tetrahydroxylated flavonoids and the possibility of solvent shifts interfered with unambiguaus assignment of many frequencies.

To verify the proposed structures, **1** and **2a** were synthesized as follows. For the synthesis of **1**, 2-hydroxy-3,4,5,6-tetramethoxyacetophenone [7] was acylated with myristicoyl chloride [8] to give 2,3,4,5,-tetramethoxy-6-(3,4-methylenedioxy-5-methoxy)-benzoxyloxyacetophenone (**3**). Baker–Venkataraman rearrangement of the latter furnished 1-(2-hydroxy-3,4,5,6-tetramethoxy)-phenyl-3-(3,4-methylenedioxy-5-methoxy)-phenylpropanedione, which afforded on ring closure with acetic acid–sodium acetate, 3',4'-methylenedioxy-5,6,7,8,5'-pentamethoxyflavone (**1**).

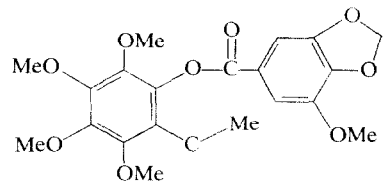


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Table 1. ^{13}C NMR spectra of flavones **1** and **2a***

Carbon atom	**1**†(in $CDCl_3$)	**2a**‡(in DMSO-d_6)
C-2	160.63	163.65
C-3	100.38*d*	102.60*d*
C-4	177.15	182.01
C-5	151.50	152.53†
C-6	138.34‡	131.32
C-7	147.62‡	152.37‡
C-8	138.06‡	94.41*d*
C-9	148.38§	157.30
C-10	114.87	103.97
C-1′	125.90	120.43
C-2′	107.28*d*†	104.22*d*
C-3′	144.17§	148.10
C-4′	149.60§	139.76
C-5′	143.88§	148.10
C-6′	106.70*d*†	104.22*d*
OCH_3	56.74*q*‖	59.93*q*¶
	62.23*q*, 61.95*q*	56.28*q*, 56.28*q*‖
	61.77*q*, 61.62*q*¶	
$O-CH_2-O$	102.32*t*	

*Run at 67.09 MHz on a Bruker HX-270 instrument with TMS as internal standard.

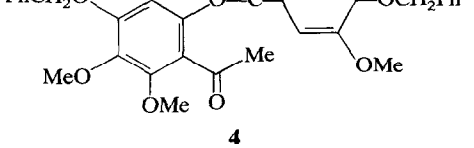
†‡§Assignments may be interchanged.

‖Ring B methoxyls.

¶Ring A methoxyls.

The synthetic material was identical in all respects (mpp, TLC, IR) with material isolated from the plant. This appears to be the first synthesis of a flavone with 3′,4′-methylenedioxy-5′-methoxy substitution.

The synthesis of **2a** was accomplished in analogous fashion beginning with 2,5-dihydroxy-4-benzyloxy-6-methoxyacetophenone [9] which was selectively methylated to give 2-hydroxy-4-benzyloxy-5,6-dimethoxyacetophenone. Acylation of this substance with 4-benzylsyringoyl chloride gave 2,3-dimethoxy-4-benzyloxy-6-(3,5-dimethoxy-4-benzyloxy)-benzoyloxyacetophenone (**4**). Baker–Venkataraman rearrangement of **4** gave 1-(2-hydroxy-4-benzyloxy-5,6-dimethoxy)-phenyl-3-(3,5-dimethoxy-4-benzyloxy)-phenylpropanedione, which on ring closure first afforded 7,4′-dibenzyloxy-5,6,3′,5′-tetramethoxyflavone (**2d**) and after treatment with boiling HCl–acetic acid, 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone. The latter was identical in all respects (mpp, TLC, IR) with **2a** from the plant.



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EXPERIMENTAL

Isolation of flavones. Herbaceous parts of *Conoclinium coelestinum* (L.) DC. (for provenance see [4]) wt 1.9 kg, were extracted with $CHCl_3$ and worked up in the usual fashion [10]. The crude gum, wt 22.15 g, was preadsorbed on 45 g of silicic acid (Mallinckrodt 100 mesh) and chromatographed over 580 g of the same material. 250 ml fractions being eluted as follows. Fractions 1–4 (C_6H_6), 5–12 (C_6H_6–$CHCl_3$, 9:1), 13–20 (C_6H_6–$CHCl_3$, 1:1), 21–35 ($CHCl_3$), 36–42 ($CHCl_3$–MeOH, 97:3) and 43–48 ($CHCl_3$—MeOH, 9:1). The material from fractions 26–30 (wt 9.28 g) which showed identical spots on TLC was combined and rechromatographed to yield **1** (1.21 g) which was recryst. from $CHCl_3$–MeOH. Fractions 33 and 34 of the original chromatogram were combined (wt 0.97 g). Trituration with C_6H_6 afforded a yellow solid (**2a**), which was recryst. from MeOH, yield 0.13 g. Examination of the other fractions under UV light indicated the presence of complicated mixtures of flavonoids which resisted separation into homogeneous crystalline materials.

3′,4′-Methylenedioxy-5,6,7,8,5′-pentamethoxyflavone. Compound **1**, pale yellow, mp 190–190.5°, had IR bands (KBr) at 1630,1580, 1560, 1510, 1350, 1170, 1120 and 1050 cm^{-1}; NMR signals (270 MHz, $CDCl_3$) at 7.14*d* and 7.09*d* ($J = 1.5$ Hz, AB system of H-2′ and H-6′), 6.08 (2H, methylenedioxy), 4.09, 4.00, 3.97 and 3.94 ppm (methoxyls); UV (λ_{max}, nm) (MeOH) 252 (sh), 335; (MeOH—$AlCl_3$) unchanged; (MeOH—$AlCl_3$—HCl) unchanged; (MeOH—NaOAc) unchanged; (MeOH—NaOAc—H_3BO_3) unchanged; (MeOH—NaOMe) 276 (sh), 334. (Analysis: Calc. for $C_{21}H_{20}O_9$: MW, 416.1106. Found: MW(MS), 416.1122 (26.7%)). Other significant peaks in the high resolution MS were at *m/e* (composition, %) 401 ($M^+ - Me$, 100), 371 ($C_{19}H_{15}O_8$, 11.4), 358 ($C_{18}H_{14}O_8$, 13.4), 225 ($C_{10}H_9O_6$, 1.3), 197 (C_9H_5O, 13.0) and 176 ($C_{10}H_8O_3$, 5.4). In the low resolution MS significant peaks appeared at *m/e* (%): 416 (47.1), 401 (100), 386 1(11.1), 371 (25.8), 358 (15.3), 340 (8.2), 225 (9.4), 197 (16.4), 182 (14.1), 179 (5.8), 176 (8.2), 131 (4.7) and 83 (10.6).

5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone. Compound **2a**, bright yellow needles, mp 223,224°, had IR bands (KBr) at 3500, 3430, 1655, 1610, 1520, 1470, 1360, 1170, 1125, 1010, and 820 cm^{-1}; NMR signals (270 MHz, DMSO-d_6) at 13.08 *br* (5-OH, exchanges with D_2O), 7.33 (2H, H-2′ and H-6′), 6.98 (H-8), 6.67 (H-3), 3.89 (6H, 3′- and 5′-OMe), 3.76 (6-OMe); UV (λ_{max}, nm) (MeOH) 240 (sh), 276, 351; (MeOH—$AlCl_3$) 281,307 (sh), 382, 389 (sh); (MeOH—$AlCl_3$—HCl) 252 (sh), 284, 302 (sh), 373; (MeOH—NaOAc) 277, 318, 381 (sh), 405; (MeOH—NaOAc—H_3BO_3) 277, 353, 381 (sh); (MeOH—NaOMe) 264, 277 (sh), 327 (sh), 382 (sh), 422. (Analysis: Calc. for $C_{18}H_{16}O_8$: MW, 360.0844. Found: MW(MS), 360.0793 (100%)). Other significant peaks in the high resolution MS were at *m/e* (composition, %): 359 ($M^+ - H_2O$, 55.4), 331 ($C_{17}H_{15}O_7$, 7.8), 330 ($C_{17}H_{14}O_7$, 8.9), 317 ($C_{16}H_{13}O_7$, 42.5) and 314 ($C_{17}H_{14}O_6$, 13.5). In the low resolution MS significant peaks appeared at *m/e* (%): 360 (100) 359 (10.4), 345 (79.2), 342 (61.0), 330 (14.2), 317 (37.0), 181 (3.8), 179 (13.0), 178 (5.2), 171 (7.8), 167 (9.0), 164 (7.8), 167 (9.0), 164 (7.8), 153 (4.5), 139 (9.7), 114 (3.8) and 69 (28.5). Acetylation of 60 mg of **2a** (Ac_2O—dry Py) at room temp. for 24 hr followed by the usual work-up and purification of the crude solid, wt 22 mg, by PLC ($CHCl_3$—MeOH, 9:1) gave the triacetate **2b**, which had IR bands ($CHCl_3$) at 1770, 1640, 1460, 1370, 1180 and 1140 cm^{-1}; NMR signals (270 MHz, $CDCl_3$) at 7.06 (2H, H-2′ and H-6′), 7.33 (H-8), 6.58 (H-3),

3.91 (3′- and 5′-OMe), 3.88 (6′-OMe), 2.55, 2.40 and 2.37 ppm (three acetates). Methylation of 20 mg of **2a** in 15 ml dry Me_2CO with 0.2 ml of dimethyl sulfate and 1 g of dry K_2CO_3 (reflux, N_2 atm), removal of solvent at red. pres. dilution with H_2O, extraction with $CHCl_3$, and evapn yielded solid **2c** which was purified by PLC ($CHCl_3$—MeOH, 9:1) and recryst. from MeOH, yield 14 mg, mp 114–116°; IR bands at 1640,1600, 1460, 1420, 1360, 1250, 1140 and 1130 cm^{-1}; NMR signals (270 MHz, $CDCl_3$) at 7.07 (2H, H-2′ and H-6′), 6.80 (H-8), 6.60 (H-3), 3.99 (6H), 3.95 (6H) and 3.93 (6H), significant peaks in the low resolution MS at *m/e* (%) 402 (M^+, 73.9), 387 (100), 371 (26.0), 357 (48.9), 341 (17.3), 326 (28.2), 195 (7.6), 192 (2.1), 186 (6.5), 172 (14.1), 167 (17.3), 157 (13.0), 69 (6.5).

2,3,4,5-*Tetramethoxy*-6-(3,4-*methylenedioxy*-5-*methoxy*)-*benzoyloxyacetophenone* (**3**). A mixture of 500 mg 2-hydroxy-3,4,5,6-tetramethoxyacetophenone [7], 400 mg of freshly prepared myristicoyl chloride [8], 10 ml dry Me_2CO and 1 g fused K_2CO_3 was refluxed for 2 hr with exclusion of moisture. After filtration and evapn of solvent, the residue was recryst. from EtOH, yield 770 mg (91%), mp 109–110°; UV (MeOH) λ_{max} 277 (4.128); TLC (Si gel, toluene—Me_2CO, 9:1) R_f 0.62; NMR signals (60 MHz, $CDCl_3$) at 7.45*d* and 7.35*d* ($J = 1.5$ Hz, H-2′ and H-6′), 6.11 (O—CH_2—O), 4.00, 3.97, 3.95, 3.90, 3.83 (-OMe) and 2.49 ppm (Ac). (Analysis: Calc. for $C_{21}H_{22}O_{10}$: C, 58.06, H, 5.11. Found: C, 58.14; H, 5.08%).

Synthesis of **1**. A mixture of 150 mg **3**, 40 mg of powdered KOH and 1.5 ml Py was heated at 60° with stirring for 30 min and cooled, acidified cautiously with HCl to pH 5 and diluted with H_2O. The dione product was extracted with $CHCl_3$, the organic solvent evapd and the residue refluxed with 1 ml of HOAc and 400 mg of dry NaOAc for 2 hr. Dilution of the cooled mixture with H_2O pptd the flavone which was filtered, washed and recryst. from EtOH—C_6H_{14} and then from Me_2CO. The light yellow crystals, yield 90 mg (62%), mp 191–192° (no depression on admixture of isolated material) UV, NMR and IR identical, TLC (Si gel, toluene—MeOH, 9:1) R_f 0.43, identical with isolated material. (Analysis: Calc. for $C_{21}H_{20}O_9$: C, 60.58, H, 4.84. Found: C, 60.42; H, 4.93%).

2,3-*Dimethoxy*-4-*benzyloxy*-6-(3,5-*dimethoxy*-4-*benzyl-oxy*)-*benzoyloxyacetophenone* (**4**). Reaction of 150 mg of 2-hydroxy-4-benzyloxy-5,6-dimethoxyacetophenone [9] with 200 mg of freshly prepared 4-benzylsyringoylchloride and 400 mg fused K_2CO_3 in the manner described for **3** and recryst. from EtOH gave 140 mg (52%) of **4**, colourless needles, mp 91–92°, UV (MeOH) λ_{max} 270 nm, TLC (Si gel, toluene-Me_2CO 19:1) R_f 0.53; IR bands (KBr) 2950, 1730, 1680, 1590 cm^{-1}; NMR signals (20 MHz, $CDCl_3$) at 7.5–7.3 *m* (2 phenyls, H-2′, H-6′), 6.68 (H-5), 5.12 (-OCH_2-), 3.98, 3.87, 3.85 (4 OMe) and 2.51 ppm (Ac). (Analysis: Calc. for $C_{33}H_{32}O_9$: C, 69.22; H, 5.63. Found: C, 69.32; H, 5.62%).

7,4′-*Dibenzyloxy*-5,6,3′,5′-*tetramethoxyflavone* (**2d**). A mixture of 150 mg of **4**, 1.5 ml of Py and 30 mg of powdered KOH was heated with stirring at 60° for 30 min. The dione was isolated and cyclized as described in the synthesis of **1**. The flavone was pptd from the cooled reaction mixture by trituration with H_2O, filtered, washed and purified by PLC (Si gel, toluene—MeOH, 9:1) and subsequent recryst. from $CHCl_3$—EtOH, yield of white prisms 80 mg (55%), mp 146–147°, UV (MeOH) λ_{max} 240 sh, 267 (4.163), 320 nm (4.453), TLC (Si gel, toluene—MeOH 9:1) R_f 0.47; NMR signals (60 MHz, $CDCl_3$) at 7.6–7.3 m (two phenyls), 7.06 (H-2′, H-6′), 6.88 (H-8), 660 (H-3), 5.25, 5.12 (2-OCH_2-), and 4.02, 3.93, 3.91 ppm (4-OMe). (Analysis: Calc. for $C_{33}H_{30}O_8$: C, 71.47, H, 5.45. Found: C, 71.18; H, 5.40%).

Synthesis of **2a**. A mixture of 80 mg of **2d**, 2 ml conc HCl and 2 ml HOAc was refluxed for 3 hr, poured into ice H_2O and refrigerated overnight. The ppt. (**2a**) was filtered, washed and recryst. from Me_2CO—$CHCl_3$, yield (yellow needles) 32 mg, wt 32 mg (61%), mp 218–219° (no depression of material from plant) UV, NMR and IR identical; TLC (Si gel, toluene—MeOH, 8:2) R_f 0.32, identical with isolated material. (Analysis: Calc. for $C_{18}H_{16}O_8$: C, 60.00; H, 4.48. Found: C, 59.74; H, 4.54%). The triacetate was recryst. from EtOH—Py, mp 197–199°, UV (MeOH) λ_{max} 265 sh, 312 nm; IR and NMR identical with triacetate from isolated material; TLC (Si gel, toluene—EtOAc, 5:4) R_f 0.44. (Analysis: Calc. for $C_{24}H_{22}O_{11}$: C, 59.26; H, 4.56. Found: C, 59.30; H, 4.64%).

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NOTE ADDED IN PROOF

After the acceptance of our manuscript, Le Van, N. and Phan, T. V. C. [(1979) *Phytochemistry*, **18,** 1859] reported the isolation of **1**, mp 185°, and 5′-methoxynobiletin from *C. conoclinium* using the old binomial *Eupatorium conoclinium* L.