

NEW COUMARINS FROM *COLEONEMA ALBUM*

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(Revised received 4 November 1980)

Key Word Index—*Coleonema album*; Rutaceae; new coumarins.

Abstract—Six coumarins have been isolated from the aerial parts of *Coleonema album* and identified as ulopterol, 7-(3',3'-dimethylallyloxy)-coumarin, (R)-(+)-2',3'-epoxy-suberosin, and the novel coumarins (R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin, (R)-(+)-7-(2',3'-dihydroxy-3'-methylbutoxy)-coumarin and (R)-(+)-7-methoxy-8-(2',3'-epoxy-3'-methylbutoxy)-coumarin.

INTRODUCTION

Coleonema album Bartl. and Wendl. is a coastal shrub of Southern Africa used by local tribespeople as a 'Buchu' [1]. Previous investigation of the fruit of this species [2] afforded a single coumarin, ulopterol (5). The present study reports the isolation and identification of six coumarins, including three novel ones, from the aerial parts of the plant.

RESULTS AND DISCUSSION

The petrol extract concentrate was column chromatographed over Si gel and fractions purified by prep. TLC and crystallization. 7-(3',3'-Dimethylallyloxy)-coumarin (1) [3], (R)-(+)-2',3'-epoxysuberosin (4) [4] and ulopterol (5) [2], were isolated as minor components and identified by comparison with published data.

The major constituent (2) gave a single blue fluorescent spot on TLC and had UV, IR and ¹H NMR spectra characteristic of a 7-oxygenated coumarin [5,6]. Mass spectrometry gave the molecular formula C₁₄H₁₄O₄ with fragment ions M⁺ - C₅H₈O and base peak m/z 85 affirming a C₅H₉O moiety attached through the 7-oxygen. In the ¹H NMR spectrum (Table 1) an ABX (δ 4.30, 4.12, 3.18) and two Me groups (δ 1.39, 1.41) resolved the side chain as 2,3-epoxy-3-methylbutyl, the presence of epoxide being further substantiated by a sharp band at 830 cm⁻¹ in the IR and a fragment M⁺ - CHO in the mass spectrum. These data, together with the positive specific rotation [7], established the structure as 2. Racemic 2 was synthesized from 1 by peracid oxidation.

Another blue fluorescent constituent (3) was more polar than 2 and spectral data again indicated a 7-oxygenated coumarin. Mass spectrometry gave the molecular formula C₁₄H₁₆O₅, 18 amu greater than 2, and in the IR spectrum OH absorption at 3350 cm⁻¹ replaced the epoxide band seen in 2. The ¹H NMR (Table 1) exhibited two D₂O-exchangeable protons. That one of these was further coupled to H-2', the m(br) at δ 3.87, was shown by either addition of D₂O or by decoupling irradiation at δ 3.42 whereupon H-2' appeared as a clear dd, part of an ABX system. These data gave the structure as 3, the glycol of 2, which was confirmed by hydration of 2 with aqueous oxalic acid under reflux.

The yellow fluorescent compound (6), C₁₅H₁₆O₅, had UV, IR and ¹H NMR spectra characteristic of a 7,8-dioxygenated coumarin. The ¹H NMR (Table 1), and mass spectrum with M⁺ - CH₃, M⁺ - CHO, M⁺ - C₅H₈O and C₅H₉O peaks, confirmed the 7,8-substituents as methoxy and 2',3'-epoxy-3'-methylbutoxy and indicated structures 6 or 7 for the yellow oil. Differentiation between these two possible isomers was achieved on the basis of chemical shifts of the methoxy and 1' - CH₂ in the ¹³C NMR spectrum. It is well known [8,9] that sterically hindered (out-of-plane) methoxy groups attached to an aromatic nucleus resonate ca 5 ppm downfield from non-hindered (in-plane) methoxy groups; viz. 60–61.5 ppm vs 55.5–56.5 ppm. In the ¹³C NMR of the yellow oil the signal at 56.1 ppm indicated methoxy attachment in position 7 and this, with a further signal at 72.8 ppm for an out-of-plane 1' - CH₂ in position 8 (2

Table 1. ¹H NMR (60 MHz, CDCl₃, TMS) data of 2, 3 and 6

H	2	3	6
3	6.31 d	6.27 d	6.28 d
4	7.73 d (br)	7.68 d (br)	7.68 d
5	7.46 d	7.42 d	7.23 d
6	6.98 dd	6.92 dd	6.92 d
8	6.91 d (br)	6.82 d (br)	—
1'a	4.12 dd	4.09 dd	4.20 dd
1'b	4.30 dd	4.27 dd	4.32 dd
2'	3.18 dd	3.87 m (br)*	3.26 t
3'-Me	{ 1.39 s 1.41 s	{ 1.30 s 1.34 s	{ 1.25 s 1.33 s
3'-OH	—	2.81 s	—
2'-OH	—	3.42 s (br)	—
OMe	—	—	3.99 s

J (Hz): 3,4 = 9.6; 6,8 = 2.4; 4,8 = <1; 2,5,6 = 9; 1'a,2' = 5.5; 1'b,2' = 4.5; 1',1' = 11; 3,5,6 = 9; 1'a,2' = 6.4; 1'b,2' = 3.5; 1',1' = 9; 6,5,6 = 8.5; 1',2' = 6; 1',1' = 10.5.

* m(br) → dd, J = 3.5 and 6.4, after D₂O or irradiation (90 MHz spectrum) of s (br) at δ 3.42.

with 1' - CH₂ at 67.8 ppm, in-plane), confirmed the structure as **6**.

All of the optically active coumarins isolated in this study had the *R* configuration. It has been suggested [7] that epoxidation of *O*- and *C*-prenylated coumarins, in Rutaceae and Umbelliferae, is controlled by separate monooxygenase enzymes because of the isolation of both prenylated types having different configurations from the same plant species. The isolation of *O*- and *C*-prenylepoxides having the same absolute configuration from *C. album* in the present study could imply their formation by a single monooxygenase enzyme. However, the presence of two distinct enzymes producing epoxides of the same configuration cannot be ruled out.

The majority of natural coumarins have 7-oxygenation and are derived from *p*-coumaric acid [10] and more highly oxygenated coumarins arise by subsequent hydroxylation of the coumarin nucleus. The ability to perform stepwise hydroxylation could have taxonomic value and among taxa of the tribe Diosmeae so far investigated *C. album* produces 7-oxy and 7,8-dioxy coumarins while *Agathosma puberula* Fourc. [11] and *Phyllosma capensis* Bolus [12] produce 6,7,8-trioxy coumarins. These differences indicate the potential of coumarins as taxonomic markers.

EXPERIMENTAL

Mps are uncorr.; IR:KBr; UV: EtOH; ¹H NMR: 60 MHz; ¹³C NMR: 20.1 and 25.15 MHz: TMS int. standard; MS: 70 eV direct insertion probe; optical rotation: CHCl₃. The plant material (aerial parts) was collected at Voelklip, Cape Province, South Africa (Voucher: Williams 2047, National Botanic Garden, Kirstenbosch, Cape Town).

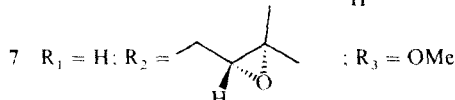
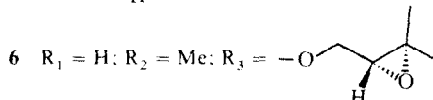
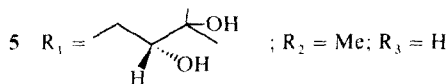
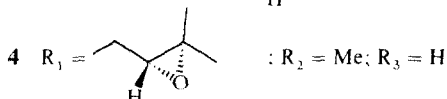
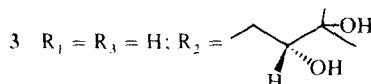
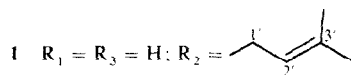
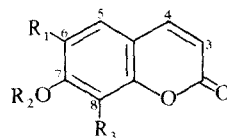
Extraction and isolation. The air-dried, powdered aerial parts (800 g), consisting mainly of leaf and stem, were Soxhlet extracted with petrol (bp 40–60°) and the extract concd *in vacuo*. Column chromatography of the residue over Si gel with petrol and petrol–EtOAc mixtures of increasing polarity, followed by prep. TLC of fractions, yielded 7-(3',3'-dimethylallyloxy)-coumarin (**1**, 60 mg, *R_f* 0.68) [3], (*R*)-(+)-2',3'-epoxysuberosin (**4**, 54 mg, *R_f* 0.54, [α]_D²¹ + 34.3° (CHCl₃; *c* 0.07) [4], ulopterol (**5**, 15 mg, *R_f* 0.13) [2] and 3 novel coumarins.

Identification of components. The known coumarins **1**, **4** and **5** were identified by comparison of their mp, IR, UV, ¹H NMR and MS with published data. *R_f* values relate to TLC over Si gel with EtOAc–petrol (2:1).

(*R*)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin (**2**). Recrystallization of the residue from the fraction *R_f* 0.60 yielded colourless crystals (3.1 g) mp 113–114° (petrol–EtOAc). (Found *M*⁺ 246.0887; C₁₄H₁₄O₄ requires 246.0892. [α]_D²¹ + 9° (CHCl₃; *c* 0.3). UV λ_{\max} nm: 299 sh, 320. IR ν_{\max} cm⁻¹: 1720 (>C=O), 1615, 1298, 1130, 1120, 1020, 830 (>C–C<). ¹H NMR, Table 1.

¹³C NMR (CDCl₃TMS, 25.15 MHz): 19.0 (*q*, *cis*-Me), 24.6 (*q*, *trans*-Me), 58.1 (*s*, C-3'), 60.8 (*d*, C-2'), 67.8 (*t*, C-1'), 101.7 (*d*, C-8), 112.7 (*d*, C-3), 112.9 (*s*, C-10), 113.3 (*d*, C-6), 128.9 (*d*, C-5), 143.2 (*d*, C-4), 155.7 (*s*, C-9), 160.8* (*s*, C-2), 161.7* (*s*, C-7). MS, *m/z* (rel. int.): 246 (*M*⁺, 35), 217 (*M*⁺ – CHO, 0.1), 162 (*M*⁺ – C₅H₈O, 20), 134 (20), 133 (5), 105 (7), 85 (C₅H₉O, 100).

(*R*)-(+)-7-(2',3'-dihydroxy-3'-methylbutoxy)-coumarin (**3**). Prep. TLC of the fraction *R_f* 0.23 over Si gel (EtOAc–petrol, 2:1) and crystallization from petrol–EtOAc gave needles (95 mg) mp



94–95°. Found *M*⁺ 264.1004; C₁₄H₁₆O₅ requires 264.0998. [α]_D²¹ + 31.1° (CHCl₃; *c* 0.15). UV λ_{\max} nm: 298sh, 320. IR ν_{\max} cm⁻¹: 3350 (OH), 1710 (>C=O), 1618, 1280, 1230, 1125, 1020, 820. ¹H NMR, Table 1. MS, *m/z* (rel. int.): 264 (*M*⁺, 23), 246 (*M*⁺ – 18, 2), 162 (*M*⁺ – C₅H₁₀O₂, 100), 134 (39).

(*R*)-(+)-7-methoxy-8-(2',3'-epoxy-3'-methylbutoxy)-coumarin (**6**). Prep. TLC of the fraction *R_f* 0.45 over Si gel (Et₂O) afforded a yellow oil (28 mg). (Found *M*⁺ 276.0996; C₁₅H₁₆O₅ requires 276.0998 [α]_D²¹ + 4.6° (CHCl₃; *c* 0.24). UV λ_{\max} nm: 205, 246sh, 312. IR $\nu_{\max}^{\text{liq, film}}$ cm⁻¹: 2850, 1720 (>C=O), 1604, 1500, 1440, 1285, 1120, 1085, 825 (>C–C<). ¹H NMR, Table 1. ¹³C NMR

(C₆D₆, TMS, 20.1 MHz): 18.8 (*cis*-Me), 24.6 (*trans*-Me), 56.1 (7-OMe), 72.8 (C-11), 108.8 (C-6), 114.2 (C-10, C-3), 123.9 (C-5), 159.7 (C-2), 166.1 (unassigned). The ¹³C NMR spectrum was recorded in C₆D₆ (owing to instability of the epoxide on prolonged dissolution in CDCl₃ and the possible interference from the CDCl₃ resonance in the region of the C-1' signal) which led to loss of some signals under those of C₆D₆. MS, *m/z* (rel. int.): 276 (*M*⁺, 37), 261 (*M*⁺ – 15, 0.1), 247 (*M*⁺ – CHO, 0.6), 205 (10), 192 (*M*⁺ – C₅H₈O, 100), 164 (7), 163 (22), 85 (C₅H₉O, 100), 59 (68).

Preparation of (±)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin (2). To a cooled (0°) EtOAc soln of **1** was added *m*-chloroperbenzoic acid and the progress of the reaction monitored by TLC. Work-up yielded racemic **2**.

Preparation of 7-(2',3'-dihydroxy-3'-methylbutoxy)-coumarin (3). The epoxide **2** was heated under reflux with 1% aq. oxalic acid for 1.5 hr, the cooled mixture neutralized (pH 7) with Na₂CO₃ and extracted with CHCl₃. The solid remaining on

*Assignments may be reversed.

concn of the dried (Na_2SO_4) CHCl_3 recrystallised from petrol-EtOAc and was identical with the natural product (3).

Acknowledgements—Dr. P. G. Waterman, University of Strathclyde for MS, ^1H NMR (90 MHz) and ^{13}C NMR (25.15 MHz); Dr. I. Williams for supply and authentication of plant material; Dr. D. Cardin, Trinity College Dublin for ^{13}C NMR (20.1 MHz).

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