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NEW COUMARINS FROM *COLEONEMA ALBUM*

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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′-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 ^{1}H NMR spectra characteristic of a 7-oxygenated coumarin [5,6]. Mass spectrometry gave the molecular formula $C_{14}H_{14}O_4$ with fragment ions $M^+ - C_5H_8O$ and base peak *m/z* 85 affirming a C_5H_9O moiety attached through the 7-oxygen. In the ^{1}H 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 $C_{14}H_{16}O_5$, 18 amu greater than **2**, and in the IR spectrum OH absorption at 3350 cm^{-1} replaced the epoxide band seen in **2**. The ^{1}H NMR (Table 1) exhibited two D_2O-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 D_2O 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**), $C_{15}H_{16}O_5$, had UV, IR and ^{1}H NMR spectra characteristic of a 7,8-dioxygenated coumarin. The ^{1}H NMR (Table 1), and mass spectrum with $M^+ - CH_3$, $M^+ - CHO$, $M^+ - C_5H_8O$ and C_5H_9O 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′ – CH_2 in the ^{13}C 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 ^{13}C 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′ – CH_2 in position 8 (**2**

Table 1. ^{1}H NMR (60 MHz, $CDCl_3$, 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*	1.30 *s*	1.25 *s*
	1.41 *s*	1.34 *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 D_2O or irradiation (90 MHz spectrum) of *s* (*br*) at δ 3.42.

with 1′ – CH_2 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 monoxygenase 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 monoxygenase 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 Diosmae 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; ^{1}H NMR: 60 MHz; ^{13}C NMR: 20.1 and 25.15 MHz: TMS int. standard; MS: 70 eV direct insertion probe; optical rotation: $CHCl_3$. 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, $[\alpha]_D^{21}$ +34.3° ($CHCl_3$; *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, ^{1}H 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; $C_{14}H_{14}O_4$ requires 246.0892. $[\alpha]_D^{21}$ +9° ($CHCl_3$; *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<). ^{1}H NMR, Table 1.

^{13}C NMR ($CDCl_3$TMS, 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^+ – C_5H_8O, 20), 134 (20), 133 (5), 105 (7), 85 (C_5H_9O, 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: $C_{14}H_{16}O_5$ requires 264.0998. $[\alpha]_D^{21}$ +31.1° ($CHCl_3$; *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. ^{1}H NMR, Table 1. MS, *m/z* (rel. int.): 264 (M^+, 23), 246 (M^+ – 18, 2), 162 (M^+ – $C_5H_{10}O_2$, 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 (Et_2O) afforded a yellow oil (28 mg). (Found M^+ 276.0996; $C_{15}H_{16}O_5$ requires 276.0998 $[\alpha]_D^{21}$ +4.6° ($CHCl_3$; *c* 0.24). UV λ_{max} nm: 205, 246sh, 312. IR $\nu_{max}^{liq.film}$ cm^{-1}: 2850, 1720 (>C=O) 1604, 1500, 1440, 1285, 1120, 1085, 825 (>C—C<). ^{1}H NMR, Table 1. ^{13}C NMR (C_6D_6, 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 ^{13}C NMR spectrum was recorded in C_6D_6 (owing to instability of the epoxide on prolonged dissolution in $CDCl_3$ and the possible interference from the $CDCl_3$ resonance in the region of the C-1′ signal) which led to loss of some signals under those of C_6D_6. MS, *m/z* (rel. int.): 276 (M^+, 37), 261 (M^+ – 15, 0.1), 247 (M^+ – CHO, 0.6), 205 (10), 192 (M^+ – C_5H_8O, 100), 164 (7), 163 (22), 85 (C_5H_9O, 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 Na_2CO_3 and extracted with $CHCl_3$. 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**).

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