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COUMARINS FROM TWO *COLEONEMA* SPECIES

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Key Word Index—*Coleonema aspalathoides*; *C. calycinum*; Diosmae; Rutaceae; new coumarins.

Abstract—The aerial parts of *Coleonema aspalathoides* yielded a single coumarin, 7-(3,3-dimethylallyloxy)-coumarin. *Coleonema calycinum* afforded 11 coumarins including three novel compounds identified on the basis of spectral data as 7-(2-hydroxy-3-methylbut-3-enoxy)-coumarin, 7-(*E*-3-methoxycarbonylbut-2-enoxy)-coumarin and 7-methoxy-8-(2,3-dihydroxy-3-methylbutoxy)-coumarin.

INTRODUCTION

In a recent review of the genus *Coleonema* Bartl. et Wendl., Williams [1] recognises the existence of eight species which are distributed throughout southern Africa and used locally as 'Buchu' [2]. Only one of these, *C. album* Bartl. et Wendl., has been the subject of previous chemical examination [3, 4]. In this paper we report the results of an investigation of the aerial parts of two further species *C. aspalathoides* A. Juss. and *C. calycinum* (Steud.) Williams. Eleven coumarins have been isolated, three of which appear to be novel and three others are reported for the first time from the Rutaceae [5].

RESULTS AND DISCUSSION

The petrol extract of *C. aspalathoides* was column chromatographed over silica gel and yielded a single coumarin identified as 7-(3,3-dimethylallyloxy)-coumarin (**1**) by comparison with an authentic sample and published data [4, 6]. Similar treatment of the *C. calycinum* sample followed by prep. TLC gave 11 compounds five of which were identified by comparison with authentic samples [4] as **1**, 7-(2,3-epoxy-3-methylbutoxy)-coumarin (**2**), 7-(2,3-dihydroxy-3-methylbutoxy)-coumarin (**3**), 7-methoxy-8-(2,3-epoxy-3-methylbutoxy)-coumarin (**7**) and epoxysuberosin (**10**).

Two compounds, **4** and **5**, were blue fluorescent (366 nm UV) and had spectral characteristics of 7-alkoxycoumarins. Compound **4**, $C_{14}H_{14}O_4$, had a MS fragmentation pattern similar to **2** [4] but showed IR absorption for a hydroxy group at 3420 cm^{-1}. The ^{1}H NMR spectrum (Table 1), in addition to signals typical of a 7-oxycoumarin, revealed an allylic methyl group at δ1.84, olefinic protons at δ5.08 and 5.25, a hydroxy at δ2.50 with carbinolic proton at δ4.55 and a methyleneoxy multiplet at δ3.80–4.30 characteristic of a 2-hydroxy-3-methylbut-3-enoxy side chain confirming the structure as the novel coumarin **4**.

Compound **5**, $C_{15}H_{14}O_5$, exhibited two carbonyl absorption bands, 1738 and 1715 cm^{-1}, in the IR spectrum. The MS showed peaks for loss of CH_3O and $C_6H_8O_2$ from the M$^+$ which pointed to a $C_6H_9O_2$ substituent attached to the position 7 oxygen. The ^{1}H NMR spectrum (Table 1) confirmed the presence of a carbomethoxy group (δ3.79) within the side chain. Decoupling experiments rationalized the remainder of the proton assignments of the 3-methoxycarbonylbut-2-enoxy side chain in **5** and the *trans* configuration for the double bond was indicated by the chemical shift of H-2′ at δ6.90. To our knowledge, this is the first report of **5** from a natural source although it has been synthesized by methylation of the corresponding acid isolated from *Euodia viteflora* [6].

The remaining compounds, **6**, **8**, **9** and **11**, were all yellow fluorescent (366 nm UV) 7,8-dioxycoumarins, for which two isomers were possible, depending on the placement of the aromatic methoxy and the alkoxy substituent in positions 7 and 8. In the ^{1}H NMR spectrum (Table 1) **6** showed signals for a 3,3-dimethylallyloxy group which was supported by the fragment *m/z* 69 $[C_5H_9]^+$ in the EIMS. Differentiation between the possible isomers was achieved using the NMR shift reagent $Eu(fod)_3$ [7]. Addition of $Eu(fod)_3$ caused shifts in the ^{1}H NMR spectrum which indicated complexation predominantly at the lactone carbonyl and shifts for the OMe (0.20) and OCH_2 (0.51) that supported only structure **6** in which the OCH_2R group is relatively close to the major site of complexation. This compound has previously been isolated only from *Artemisia caruifolia* Roxb. (Compositae) [8].

Compound **8**, $C_{15}H_{18}O_6$, was the major constituent and in the MS underwent facile loss of $C_5H_{10}O_2$ to give the base peak *m/z* 192. The ^{1}H NMR spectrum (Table 1) confirmed the five carbon side chain as a 2,3-dihydroxy-3-methylbutoxy unit (*cf.* **3**). The presence of hydroxy groups in the side chain ruled out the use of a $Eu(fod)_3$ shift experiment to determine the substitution pattern [9]. The ^{13}C NMR spectrum, with signals for an in-plane methoxy at 56.6 ppm and an out-of-plane methylene at 76.3 ppm [4], confirmed the structure as a new coumarin **8**.

Compound **9** was 18 MU less than **8** and had a five-carbon side chain with similar ^{1}H NMR spectral characteristics to **4** (Table 1). The structure **9** was confirmed by

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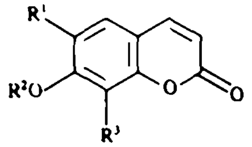

1 $R^1 = R^3 = H$, $R^2 = Pre_a$
2 $R^1 = R^3 = H$, $R^2 = Pre_b$
3 $R^1 = R^3 = H$, $R^2 = Pre_c$
4 $R^1 = R^3 = H$, $R^2 = Pre_d$
5 $R^1 = R^3 = H$, $R^2 = Pre_e$
6 $R^1 = H$, $R^2 = Me$, $R^3 = OPre_a$
7 $R^1 = H$, $R^2 = Me$, $R^3 = OPre_b$
8 $R^1 = H$, $R^2 = Me$, $R^3 = OPre_c$
9 $R^1 = H$, $R^2 = Me$, $R^3 = OPre_d$
10 $R^1 = Pre_b$, $R^2 = Me$, $R^3 = H$
11 $R^1 = H$, $R^2 = Me$, $R^3 = OH$

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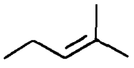

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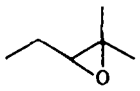

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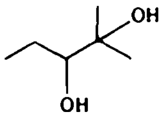

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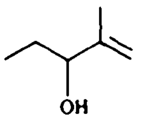

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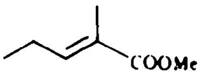

Table 1. ^1H NMR assignments for the coumarins **4**, **5**, **6**, **8** and **9**

Proton	**4**	**5**	**6**	**8**	**9**
H-3	6.28 *d* (9.6)	6.26 *d* (10.0)	6.21 *d* (9.6)	6.18 *d* (9.5)	6.27 *d* (9.7)
H-4	7.65 *dbr* (9.6)	7.64 *d* (10.0)	7.60 *d* (9.6)	7.63 *d* (9.5)	7.61 *d* (9.7)
H-5	7.20 *d* (9.0)	7.39 *d* (8.5)	7.14 *d* (8.5)	7.18 *d* (8.8)	7.20 *d* (8.5)
H-6	6.88 *dd* (2.2, 9.0)	6.86 *m*	6.84 *d* (8.5)	6.85 *d* (8.8)	6.89 *d* (8.5)
H-8	6.86 *dbr* (2.2)	6.86 *m*			
H-1′a	3.80–4.30 *m*	4.78 *dbr* (6.0)	4.67 *dbr* (7.0)	4.05 *dd* (7.8, 10.0)	3.92 *dd* (8.8, 10.4)
H-1′b				4.55 *dd* (2.6, 10.0)	4.43 *dd* (1.6, 10.4)
H-2′	4.55 *dd*	6.90 *m*	5.54 *tbr* (7.0)	3.55 *dd* (2.6, 7.8)	4.41 *dd* (1.6, 8.8)
3′-Me	1.84 *sbr*	1.95 *sbr*	1.73 *sbr* 1.77 *sbr*	1.26 *s* 1.30 *s*	1.80 *sbr*
3′-OH		–	–	3.40 *sbr*	
2′-OH	2.50 *sbr*		–	3.40 *sbr*	3.60 *sbr*
4′-CH	5.08 *m*				4.96 *m*
4′-CH	5.25 *m*				5.11 *m*
OMe		3.79 *s*	3.91 *s*	3.90 *s*	3.97 *s*

the ^{13}C NMR spectrum with methoxy at 56.7 ppm and OCH_2 at 78.4 ppm. This coumarin, trivial name ferudenol, has only previously been encountered in the umbelliferous plant *Prangos ferulacea* (L.) Lindl. [10].

Compound **11** had M^+ 192 which was also the base peak in the EIMS and a ^{1}H NMR spectrum typical of a daphnetin methyl ether. The structure was confirmed as the 7-methyl ether, **11**, by synthesis from **8** on treatment with acid. Daphnetin-7-methyl ether has previously been found in *Artemisia dracunculoides* Pursh. [11].

This is the first report of **4**, **5** and **8** from a natural source. The range of coumarin types encountered in *C. calycinum* and *C. album* [4] are similar but 7-alkoxycoumarins predominate in the latter while 7,8-dialkoxycoumarins abound in the former. The abundance of coumarins in both of these species contrasts sharply with the single compound found in *C. aspalathoides*. The latter sample was collected in January, after the flowering/fruiting season, whereas both of the other species were collected within that season. This may explain the paucity of coumarins in *C. aspalathoides* as it is well known that coumarin production is generally highest around the time of flowering [12]. Other genera of the tribe Diosmae (subfamily Rutoideae) have recently been shown to produce simple coumarins [13]. The genera *Agathosma* Willd. and *Empleurum* Soland *ex* Ait. synthesize 6,7-dioxycoumarins while *Agathosma* and *Diosma* L. produce 7,8-dioxy- and 6,7,8-trioxycoumarins. The latter genera have so far been found to prenylate only the 7-oxygen in contrast to *Coleonema* which ornaments both 7- and 8-oxygens with a variety of oxidized prenyl groupings commonly encountered in the Rutoideae [14].

EXPERIMENTAL

Plant material. Aerial parts of *C. aspalathoides* and *C. calycinum* were collected in the Cape Province, S.A. and vouchers (Williams 2023 and 2350, respectively) are deposited at the Compton Herbarium, Kirstenbosch, S.A.

Extraction and isolation. Ground plant material was extracted with petroleum ether (bp 60–80°) to exhaustion (*C. aspalathoides*—350 g; *C. calycinum* 87 g). The conc. extracts were subjected to CC over silica gel eluting with petrol (bp 60–80°) and then petrol containing increasing amounts of EtOAc. Fractions containing similar mixtures of components were further purified by prep. TLC on silica gel using EtOAc petrol mixtures as developing solvent (R_f values quoted relate to EtOAc petrol 2:1).

Examination of C. aspalathoides. A single blue fluorescent compound, R_f 0.68, was isolated as an oil (250 mg) and identified as 7-(3,3-dimethylallyloxy)-coumarin, **1**, by comparison with an authentic sample [4].

Examination of C. calycinum. A total of 11 compounds were visualized on TLC in UV light (254 and 366 nm). Five were identified as 7-(3,3-dimethylallyloxy)-coumarin, **1** (84 mg), 7-(2,3-epoxy-3-methylbutoxy)-coumarin, **2** (33 mg), 7-(2,3-dihydroxy-3-methylbutoxy)-coumarin, **3** (85 mg), 7-methoxy-8-(2,3-epoxy-3-methylbutoxy)-coumarin, **7** (15 mg) and epoxysuberosin, **10** (trace) by comparison of TLC, $[\alpha]_D$, UV, IR, ^{1}H NMR and MS with authentic samples [4]. The six other compounds were characterized as **4** (33 mg, R_f 0.36), **5** (56 mg, R_f 0.52), **6** (45 mg, R_f 0.64), **8** (202 mg, R_f 0.12), **9** (50 mg, R_f 0.31) and **11** (29 mg, R_f 0.23).

7-(*2-Hydroxy-3-methylbut-3-enoxy)-coumarin* (**4**). Isolated as an oil. Found: M^+ 246.0875; $C_{14}H_{14}O_4$ requires 246.0892. UV λ_{max} nm: 223, 296, 323. IR ν_{max} (liq. film) cm^{-1}: 3420, 2930, 1725, 1710, 1610, 1555, 1495, 1405, 1350, 1130, 910, 835. ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1. MS m/z (rel. int.): 246 $[M]^+$ (34), 175 (33), 162 (100), 147 (61), 89 (19), 71 (21), 63 (10), 57 (12).

7-(*3-Methoxycarbonylbut-2-enoxy)-coumarin* (**5**). Colourless needles from EtOAc–petrol, mp 170.5–172° (lit. [6] 170–172°). Found: M^+ 274.1; $C_{15}H_{14}O_6$ requires 274.1. UV λ_{max} nm: (EtOH) 208, 255, 292, 317. IR ν_{max} cm^{-1}: 3040, 1738, 1715, 1620, 1510, 1418, 1355, 1130, 1020, 985, 899, 845. ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1. MS m/z (rel. int.): 274 $[M]^+$ (14), 243 (12), 242 (12), 162 (100), 134 (32), 113 (57), 81 (14), 53 (11).

7-*Methoxy-8-(3,3-dimethylallyloxy)-coumarin* (**6**). Yellow oil. Found: M^+ 260.1, $C_{15}H_{16}O_4$, not observed. UV λ_{max} nm: 202, 321. IR ν_{max} cm^{-1}: 2920, 1730, 1610, 1440, 1290, 1120, 1090, 825. ^{1}H NMR (60 MHz, $CDCl_3$): see Table 1. ^{1}H NMR: $Eu(fod)_3$-induced shifts (relative to H-3 = 1.00) [7]; H-4 (0.32), H-5 (0.24), H-6 (0.29), OMe (0.20), OCH_2 (0.51), 2′-CH (0.43), 3′-Me (0.19), 3′-Me (0.25). MS m/z (rel. int.): 260 $[M]^+$ (not seen), 192 (72), 149 (54), 125 (17), 111 (29), 97 (45), 69 (55), 57 (100).

7-*Methoxy-8-(2,3-dihydroxy-3-methylbutoxy)-coumarin* (**8**). Yellow oil. Found: M^+ 294.1; $C_{15}H_{18}O_6$ requires 294.1. UV λ_{max} nm: 222, 258, 320. IR ν_{max} cm^{-1}: 3320, 2980, 1730, 1650, 1500, 1285, 1090, 980, 825. ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (25.15 MHz, $CDCl_3$, ppm): 25.2 (Me), 26.0 (Me), 56.6 (OMe), 76.3 (OCH_2), 108.7 (C-3), 114.0 (C-6), 123.1 (C-5), 143.4 (C-4). MS m/z (rel. int.): 294 $[M]^+$ (5), 192 (100), 163 (20), 149 (9), 92 (6), 59 (22).

7-*Methoxy-8-(2-hydroxy-3-methylbut-3-enoxy)-coumarin* (*ferudenol*, 9). Yellow oil. Found: M^+ 276.1; $C_{15}H_{16}O_5$ requires 276.1. UV λ_{max} nm: 225, 257, 320. IR ν_{max} cm^{-1}: 3560, 3150, 1720, 1610, 1560, 1500, 1450, 1400, 1290, 1090, 835. ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (25.15 MHz, $CDCl_3$, ppm): 29.8 (Me), 56.7 (OMe), 73.9 (2′-CH), 78.4 (OCH_2), 109.6 (C-3), 112.6 (C-6), 114.0 (4′-CH_2), 123.3 (C-5), 143.5 (C-4). MS m/z (rel. int.): 276 $[M]^+$ (3), 206 (15), 192 (100), 163 (14), 149 (7), 95 (5), 83 (6), 69 (7), 55 (8).

7-*Methoxy-8-hydroxycoumarin* (*daphnetin-7-methylether*, **11**). Yellow oil. Found: M^+ 192.1; $C_{10}H_8O_4$ requires 192.1. UV λ_{max} nm: 220, 263, 295, 320. IR ν_{max} cm^{-1}: 3400, 2990, 1725, 1710, 1645, 1575, 1525, 1500, 1200, 725. ^{1}H NMR (80 MHz, $CDCl_3$): δ 3.98 (3H, *s*, OMe), 5.70 (1H, *sbr*, OH), 6.23, 7.62 (2H, ABq, $J = 9.5$ Hz, H-3, H-4), 6.85, 7.00 (2H, ABq, $J = 7.8$ Hz, H-6, H-5). MS m/z (rel. int.): 192 $[M]^+$ (100), 177 (11), 164 (10), 149 (25), 121 (11), 65 (18), 51 (5).

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REFERENCES

1. Williams, I. (1981) *J. S. Afr. Botany* **47**, 63.
2. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd. edn, p. 909.
3. Dreyer, D. L., Pickering, M. V. and Cohan, P. (1972) *Phytochemistry* **11**, 705.
4. Gray, A. I. (1981) *Phytochemistry* **20**, 1711.

5. Gray, A. I. and Waterman, P. G. (1978) *Phytochemistry* **17**, 845.
6. Lassak, E. V. and Southwell, I. A. (1972) *Austral. J. Chem.* **25**, 2491.
7. Gray, A. I., Waigh, R. D. and Waterman, P. G. (1978) *J. Chem. Soc. Perkin Trans. II*, 391.
8. Barua, N. C., Sharma, R. P., Madhusudanan, K. P., Thyagarajan, G. and Herz, W. (1980) *Phytochemistry* **19**, 2217.
9. Gray, A. I., Waigh, R. D. and Waterman, P. G. (1977) *Phytochemistry* **16**, 1017.
10. Abyshev, A. Z. (1974) *Khim. Prir. Soedin.* **10**, 568.
11. Herz, W., Bhat, S. V. and Santhanam, P. S. (1970) *Phytochemistry* **9**, 891.
12. Murray, R. D. H., Mendez, J. and Brown, S. A. (1982) *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*, Chs. 5 and 8. Wiley, Bristol.
13. Campbell, W. E., Majal, T. and Bean, P. A. (1986) *Phytochemistry* **25**, 655.
14. Gray, A. I. (1983) in *Chemistry and Chemical Taxonomy of the Rutales* (Waterman, P. G. and Grundon, M. F., eds), p. 97. Academic Press, London.