Novel C-Geranyl 7-Hydroxycoumarins from the Aerial Parts of *Eriostemon tomentellus*

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From the aerial parts of *Eriostemon tomentellus* five coumarins have been isolated. These have been characterized as ostruthin (6-geranyl-7-hydroxycoumarin (**1**)) and the novel compounds, 8-geranyl-7-hydroxycoumarin (**2**), (E)-6-(7-hydroxy-3,7-dimethylocta-2,5-dienyl)-7-hydroxycoumarin (**3**), (E)-6-(7-hydroperoxy-3,7-dimethylocta-2,5-dienyl)-7-hydroxycoumarin (**4**), and 6-(6-hydroxy-3,7-dimethylocta-2,7-dienyl)-7-hydroxycoumarin (**5**). In addition tembamide (2-{4-methoxyphenyl}-2-hydroxy-N-benzoyl-ethylamine (**6**)) was isolated and the ¹³C NMR spectrum reported for the first time.

Introduction

Eriostemon tomentellus Diels is a minutely tomentose undershrub found widely in the south-western part of Western Australia [1]. Previous investigations have revealed the presence of the furoquinoline alkaloids skimmianine and maculosidine, the dihydrocinnamic acid derivative eriostemoic acid, and the coumarin ostruthin (**1**) [2]. We have recently been able to obtain a further sample and in this paper we wish to report the isolation and identification of a number of novel coumarins.

Results and Discussion

Sequential Soxhlet extraction of the ground aerial parts with petroleum ether (b.p. 60–80 °C), ethyl acetate and methanol followed by column chromatography of each extract over silica gel and then, in some cases, preparative TLC gave: (a) from the petroleum ether extract eriostemoic acid, ostruthin (**1**), maculosidine and one novel coumarin (b) from the ethyl acetate extract tembamide (**6**) and three further novel coumarins, and (c) from the methanol extract the flavanone glycoside hesperidin.

The novel coumarin from the petroleum ether extract showed M⁺ 298, resolving by high resolution electron impact MS for C₁₉H₂₂O₃. The ¹H

NMR spectrum (Table I) revealed signals for AB-coupling between H-3 and H-4 and between H-5 and H-6. Other resonances showed couplings identical to those observed for the geranyl side-chain in **1** (Table I). The presence of the geranyl side-chain was further confirmed by the presence of a fragment m/z 123 [C₉H₁₅]⁺ for fission of the side-chain between C-1' and C-2'. On this basis the compound must be the isomer of ostruthin with structure **2**.

The most polar of the coumarins from the ethyl acetate extract gave a highest fragment in the electron impact MS at m/z 296 [C₁₉H₂₀O₃]⁺ but fast atom bombardment MS revealed M⁺ + 1 at m/z 315 indicating an empirical formula C₁₉H₂₂O₄, one oxygen more than **1** and **2**. The ¹H NMR spectrum (Table I) revealed signals for H-3, H-4, H-5 and H-8 of the coumarin nucleus, requiring a 6-geranyl-7-hydroxycoumarin structure. A feature of the ¹H NMR spectrum was the presence of three olefinic protons attributable to the C₁₀ side chain; one of these was typical for H-2' while the others showed coupling to each other of 15.4 Hz, indicative of a *trans* relationship with one showing further coupling to a methylene group. Another feature of the ¹H NMR spectrum was the shielding of two methyl resonances to δ 1.34, typical for geminal methyls attached to a carbon also carrying an oxygen substituent.

Placement of the methylene at C-4' rather than C-6' was established by the observation of a nuclear Overhauser interaction between H-2' and the

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Table I. ¹H NMR spectra for coumarins **1–5**.

	1	2	3	4	5
H-3	6.24 (1 H, d, <i>J</i> = 9.5 Hz)	6.25 (1 H, d, <i>J</i> = 9.5 Hz)	6.21 (1 H, d, <i>J</i> = 9.4 Hz)	6.24 (1 H, d, <i>J</i> = 9.5 Hz)	6.26 (1 H, d, <i>J</i> = 9.3 Hz)
H-4	7.65 (1 H, d, <i>J</i> = 9.5 Hz)	7.64 (1 H, d, <i>J</i> = 9.5 Hz)	7.62 (1 H, d, <i>J</i> = 9.4 Hz)	7.65 (1 H, d, <i>J</i> = 9.5 Hz)	7.67 (1 H, d, <i>J</i> = 9.3 Hz)
H-5	7.20 s	7.25 (1 H, d, <i>J</i> = 8.4 Hz)	7.17 s	7.19 s	7.38 s
H-6		6.81 (1 H, d, <i>J</i> = 8.4 Hz)			
H-8	7.01 s		6.95 s	7.00 s	6.97 s
H-1'	3.40 (2 H, d, <i>J</i> = 7.2 Hz)	3.66 (2 H, d, <i>J</i> = 6.9 Hz)	3.37 (2 H, d, <i>J</i> = 7.4 Hz)	3.39 (2 H, d, <i>J</i> = 7.2 Hz)	3.65 (2 H, d, <i>J</i> = 7.4 Hz)
H-2'	5.33 (1 H, tq, <i>J</i> = 7.2, 1.2 Hz)	5.29 (1 H, tq, <i>J</i> = 6.9, 1.1 Hz)	5.34 (1 H, br.t, <i>J</i> = 7.4 Hz)	5.38 (1 H, br.t, <i>J</i> = 7.2 Hz)	5.72 (1 H, tq, <i>J</i> = 7.4, 1.3 Hz)
CH ₃ -3'	1.74 (3 H, d, <i>J</i> = 1.2 Hz)	1.86 (3 H, d, <i>J</i> = 1.1 Hz)	1.71 (3 H, br.s)	1.74 (3 H, br.s)	1.82 (3 H, d, <i>J</i> = 1.3 Hz)
H-4'	2.10 (2 H, m)	2.10 (2 H, m)	2.76 (2 H, br.d, <i>J</i> = 5.6 Hz)	2.80 (2 H, br.d, <i>J</i> = 6.6 Hz)	2.38 (2 H, m)
H-5'	2.10 (2 H, m)	2.10 (2 H, m)	5.65 (1 H, dt, <i>J</i> = 15.4, 5.6 Hz)	5.72 (2 H, dt, <i>J</i> = 15.7, 6.6 Hz)	1.95 (2 H, m)
H-6'	5.09 (1 H, br.t, <i>J</i> = 6.8 Hz)	5.05 (1 H, br.t, <i>J</i> = 6.7 Hz)	5.67 (1 H, d, <i>J</i> = 15.4 Hz)	5.61 (1 H, d, <i>J</i> = 15.7 Hz)	4.37 (1 H, t, <i>J</i> = 6.7 Hz)
CH ₃ -7'	1.60 (3 H, br.s)	1.60 (3 H, br.s)	1.34 (3 H, s)	1.35 (3 H, s)	1.86 (3 H, br.s)
H-8'	1.69 (3 H, br.s)	1.68 (3 H, br.s)	1.34 (3 H, s)	1.35 (3 H, s)	4.93 (1 H, m) 5.22 (1 H, m)
OOH-7'				6.90 (1 H, br.s)	

1 was run at 250 MHz in CDCl₃, **2–4** at 400 MHz in CDCl₃ and **5** at 400 MHz in C₅D₅N.

H-4' methylene protons. The nOe also confirmed a *cis* relationship between H-1' and the 3'-methyl allowing assignment of structure **3**. Finally a heteronuclear multiple bond coupling (HMBC) study [3] was used to assign the ¹³C NMR chemical shifts (Table II).

The least polar coumarin from the ethyl acetate extract also failed to show an M⁺ in the electron impact MS but fast atom bombardment MS showed a pseudomolecular ion [M⁺ + 1] at *m/z* 331, indicative of an empirical formula C₁₉H₂₂O₅. Significant fragments in the electron impact MS could be allocated to the loss of OOH[−] and H₂O₂, suggesting the presence of a hydroperoxy group. The ¹H NMR spectrum (Table I) and ¹³C NMR spectrum (Table II) were closely comparable to those of **3** for the coumarin nucleus and for C-1' to C-4' of the side chain but the carbon spectrum showed strong deshielding for C-5' and C-7' and shielding for C-6', C-8' and the C-7' methyl. These

Table II. ¹³C NMR spectra for **3–5**.

Carbon	3	4	5
2	162.2 s	162.4 s	162.2 s
3	112.2 d	112.2 d	112.3 d
4	144.1 d	144.2 d	144.1 d
5	128.3 d	128.2 d	128.4 d
6	125.6 s	125.6 s	125.6 s
7	158.5 s	158.4 s	158.5 s
8	103.1 d	103.1 d	103.1 d
9	154.1 s	154.1 s	154.1 s
10	112.2 s	112.2 s	112.2 s
1'	28.4 t	28.4 t	28.6 t
2'	122.0 d	122.2 d	121.7 d
3'	137.0 s	136.7 s	137.9 s
3'-CH ₃	16.3 q	16.3 q	16.2 q
4'	42.3 t	42.5 t	*32.9 t
5'	124.8 d	129.4 d	*35.8 t
6'	139.6 d	135.1 d	75.9 d
7'	71.1 s	82.2 s	147.1 s
7'-CH ₃	29.8 q	24.2 q	17.7 q
8'	29.8 q	24.2 q	111.3 t

* Assignments interchangeable; spectra run at 100.6 MHz in CDCl₃.

changes are in agreement with those observed in conversion of an isopropanol terminal group to the corresponding peroxide and permit assignment of structure **4** [4]. Again an HMBC study allowed unambiguous assignment of all carbon resonances.

The final coumarin showed a comparable coumarin nucleus and C-1' to C-3' side chain to **3** and **4**. The electron impact MS showed M^+ 314, solving for $C_{19}H_{22}O_4$, identical to **3**. Differences from **3** observed in both 1H (Table I) and ^{13}C (Table II) NMR spectra indicated the presence of a terminal isopropenyl moiety and a secondary alcohol. Placement of the hydroxyl at C-6' was established from a NOESY study which showed a strong interaction between the δ 1.86 methyl of the isopropenyl group and the oxymethine proton at δ 4.37. On this basis the coumarin can be assigned structure **5**, the stereochemistry of the 6'-hydroxyl being unknown.

Tembamide (**6**) was also isolated from the ethyl acetate extract. While this amide has been found quite widely in the Rutaceae [5] this is the first report of its occurrence in *Eriostemon*. The opportunity was taken to record, for the first time, the ^{13}C NMR spectrum, assignments again being made unambiguously through application of the HMBC technique.

Experimental

Plant material

Eriostemon tomentellus was collected on September 5, 1989 from Ennuin Station, South Western Australia. A voucher specimen, Perth 01185853 has been deposited at the Western Australian Herbarium.

Isolation of compounds

The ground aerial parts (300 g) were extracted successively with petroleum ether (b. p. 60–80 °C), EtOAc and MeOH. The petrol extract was concentrated (15 g) and an aliquot (5 g) subjected to vacuum liquid chromatography over Si gel, eluting with petrol containing increasing amounts of EtOAc. Elution with 20% EtOAc, followed by removal of pigments (Sephadex LH 20 column eluting with $CHCl_3$) gave eriostemoic acid (274 mg). Further elution with 30% EtOAc, followed by treatment with Sephadex LH 20 and then PTLC (Si gel, solvent toluene:EtOAc 8:2), gave **2** (4 mg) and **1** (215 mg). Finally elution with EtOAc gave maculosidine (5 mg).

Concentration of the EtOAc extract gave a solid (11 g) an aliquot of which (7 g) was subjected to vacuum liquid chromatography eluting with petrol:EtOAc and then EtOAc:MeOH mixtures of increasing polarity. Elution with 50% EtOAc in petrol yielded a mixture which was separated by PTLC (Si gel, solvent $CHCl_3$:MeOH 49:1) to give **4** (16 mg) and **5** (13 mg). Concentration of the 65% EtOAc in petrol eluant gave crystals of **6** (13 mg). PTLC of the supernatant from which **6** had been crystallized (Si gel, toluene:EtOAc 8:3) yielded **3** (6 mg).

Concentration of the MeOH extract yielded small amounts of hesperidin (12 mg).

Eriostemoic acid

Identical (UV, IR, 1H NMR, MS) with authentic sample [6].

Ostruthin (1)

Plates from petrol:EtOAc, m.p. 117–119 °C (Lit. [7] 117–118 °C). UV λ_{max} [nm]: MeOH 245, 257, 334. IR ν_{max} [cm^{-1}]: KBr 3160, 1685, 1600, 1570, 1440, 1390, 1270, 1235, 1150, 1130, 980, 850, 745, 660. 1H NMR – see Table I. MS (rel. int.): m/z 298 (34) (calcd for $C_{19}H_{22}O_3$ 298.1569, found 298.1559), 229 (35), 175 (70), 123 (68), 69 (100).

8-Geranyl-7-hydroxycoumarin (2)

Gum. UV $\lambda_{\max}$ [nm]: MeOH 235, 253 sh, 280 sh, 322. IR $\nu_{\max}$ [cm⁻¹]: film 3300, 1730, 1710, 1605, 1570, 1450, 1255, 1120, 830. ¹H NMR – see Table I. MS (rel. int.): *m/z* 298 (34) (calcd for C₁₉H₂₂O₃ 298.1569, found 298.1585), 229 (59), 175 (100), 123 (98), 69 (48).

Maculosidine

Identical (mixed m.p., UV, IR, ¹H NMR, MS) with authentic sample [6].

(E)-6-(7-Hydroxy-3,7-dimethylocta-2,5-dienyl)-7-hydroxycoumarin (3)

Gum. UV $\lambda_{\max}$ [nm]: MeOH 235, 256 sh, 274 sh, 280, 320 sh, 331. IR $\nu_{\max}$ [cm⁻¹]: film 3315, 1710, 1620, 1570, 1440, 1390, 1255, 1130, 820, 750. ¹H NMR – see Table I. ¹³C NMR – see Table II. FAB-MS (rel. int.): *m/z* 315.1596 (C₁₉H₂₃O₄ – M⁺ + 1). EI-MS (rel. int.): *m/z* 296 (66), 215 (39), 175 (100), 121 (47).

(E)-6-(7-Hydroperoxy-3,7-dimethylocta-2,5-dienyl)-7-hydroxycoumarin (4)

Gum. UV $\lambda_{\max}$ [nm]: MeOH 232, 244 sh, 256 sh, 330. IR $\nu_{\max}$ [cm⁻¹]: film 3340, 1715, 1620, 1570, 1440, 1390, 1260, 1135, 810, 735. ¹H NMR – see Table I. ¹³C NMR – see Table II. FAB-MS (rel. int.): *m/z* 331.1545 (C₁₉H₂₃O₅ – M⁺ + 1). EI-MS (rel. int.): *m/z* 312 (6), 297 (2), 296 (12), 229 (5), 175 (100), 137 (11).

6-(6-Hydroxy-3,7-dimethylocta-2,7-dienyl)-7-hydroxycoumarin (5)

Gum. [α]_D +4° (*c* = 0.3, CHCl₃). UV $\lambda_{\max}$ [nm]: MeOH 244, 258, 332. IR $\nu_{\max}$ [cm⁻¹]: KBr 3385, 3120, 1690, 1620, 1445, 1390, 1255, 1165, 1055,

880, 815. ¹H NMR – see Table I. ¹³C NMR – see Table II. MS (rel. int.): *m/z* 314 (23) (calcd for C₁₉H₂₂O₄ 314.1518, found 314.1505), 296 (18), 229 (31), 175 (100), 147 (13).

Tembamide (6)

Needles from petrol:EtOAc, m.p. 146–148 °C (Lit. [8] 147–149 °C). [α]_D +13° (*c* = 0.3, CHCl₃) (Lit. [8] +23°). UV $\lambda_{\max}$ [nm]: MeOH 228, 275, 280. IR $\nu_{\max}$ [cm⁻¹]: KBr 3420, 3100, 1620, 1540, 1510, 1300, 1245, 1180, 1080, 1030, 830, 690. ¹H NMR (400 MHz, CDCl₃): δ 3.20 (1H, d, *J* = 3.5 Hz, OH), 3.51 (1H, ddd, *J* = 11.6, 8.0, 3.5 Hz, H-1), 3.81 (3H, s, OMe), 3.88 (1H, ddd, *J* = 11.6, 8.0, 4.8 Hz, H-1), 4.90 (1H, dt, *J* = 3.5, 8.0 Hz, H-2), 6.62 (1H, dd, *J* = 4.8, 3.5 Hz, N-H), 6.90 (2H, dd, *J* = 8.8, 2.2 Hz, H-3', H-5'), 7.34 (2H, dd, *J* = 8.8, 2.2 Hz, H-2', H-6'), 7.44 (2H, dt, *J* = 1.5, 7.3 Hz, H-3'', H-5''), 7.51 (1H, tt, *J* = 1.5, 7.3 Hz, H-4''), 7.76 (2H, dd, *J* = 7.3, 1.5 Hz, H-2'', H-6''). ¹³C NMR (100.6 MHz, CDCl₃): δ 47.7 (t, C-1), 55.3 (q, OMe), 73.3 (d, C-2), 114.0 (d, C-3', C-5'), 127.0 (d, C-2'', C-6''), 127.1 (d, C-2', C-6'), 128.6 (d, C-3'', C-5''), 131.7 (s, C-4''), 133.8 (s, C-1'), 134.1 (s, C-1''), 159.3 (s, C-4'), 168.5 (s, C=O). MS (rel. int.): *m/z* 271 (2) (calcd for C₁₆H₁₇NO₃ 271.1209, found 271.1219), 150 (61), 137 (63), 135 (100), 109 (27), 105 (78).

Hesperidin

Identical (co-TLC, UV, IR, ¹H NMR) with authentic sample [6].

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