Further Novel 6,7-Dimethoxy-8-Prenylated Coumarins from the Aerial Parts of *Phebalium elatius* ssp. *beckleri*

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Z. Naturforsch. **45c**, 927–930 (1990); received June 11, 1990

Rutaceae, *Phebalium*, 8-Prenyl-6,7-methoxycoumarins

From the aerial parts of *Phebalium elatius* ssp. *beckleri* six novel coumarins have been isolated. These compounds are all based on a 6,7-dimethoxycoumarin nucleus with a modified C-8 isoprenyl substituent. The prenyl side chains were identified, primarily on the basis of NMR studies, as 1,2-epoxy-3-methylbut-3-enyl = (+)-6-methoxyphebalosin (**1**), 1-acetoxymethyl-2-methylprop-2-enyl = (+)-6-methoxyisomurralonginol acetate (**2**), 1,2-epoxy-3-hydroxy-4-(3-methylbutanoyloxy)-3-methylbutanyl = 1′,2′-epoxy-6-methoxycasegravol-4′-(3-methyl)-butanoate (**3**), 1-*(S)*-hydroxy-3-methyl-2-oxobutanyl = (+)-6-methoxymurranganon (**4**), 3-methyl-2-oxotetrahydrofuran-4-yl = 6-methoxy-3′,4′-dihydroisomicrominutin (**5**) and *(E)*-3-hydroxy-3-methylbut-1-enyl = 6-methoxymurraol (**6**).

Introduction

Of the 44 species of *Phebalium* Vent. recognized by Wilson [1] phytochemical data is available on only 10 [2–4]. A consistent feature of all investigated species is the presence of coumarins. In a continuation of our investigation of the secondary metabolites from *Phebalium* species [2–4] we have undertaken the analysis of *Phebalium elatius* (F. Muell.) Benth. ssp. *beckleri* (F. Muell.) Wilson [1]. In a previous paper [4] we reported the isolation of four new coumarins, all of which were different 1′ esters of 6-methoxy-3′,4′-dehydromurranganon (**7**). We now wish to report the results of a further investigation which has led to the isolation of six more novel coumarins.

Results and Discussion

TLC analysis of a petroleum ether (b.p. 60–80 °C) extract of the aerial parts revealed the presence of seven blue fluorescent bands (bands A–G). The four previously reported compounds [4] were obtained from bands C and D; the six further compounds that are the subject of this paper were derived from the remaining bands by chromatographic separations described in the Experimental.

The substitution pattern of the coumarin nucleus of all six compounds was established through their ^{1}H NMR spectra. In each case resonances for H-3 and H-4 were observed as doublets (J 9.5 Hz) centred at δ 6.31–6.37 and δ 7.59–7.64, respectively. Other signals common to all compounds were a single aromatic proton (δ 6.81–6.96) and two methoxyls. Assignment of the aromatic proton to H-5 was confirmed for **1** by an NOE experiment which showed a 10% enhancement of H-5 on irradiation of H-4. The relatively shielded position of H-4 in all of the compounds indicated that C-5 was not oxygenated. In **3** a ^{13}C NMR study revealed signals for two oxygen bearing aromatic carbons at 147.7 and 149.5 p.p.m., each comparatively shielded because of one *ortho* carbon also being oxygenated. These must be placed at C-6 and C-7 which must, therefore, carry the two methoxyl substituents. In view of the close similarity in ^{1}H NMR data for these six compounds and those isolated previously [4] it follows that all of the coumarins obtained in this study are 6,7-dimethoxycoumarins carrying a substituent at C-8.

Band A gave a single compound, M^+ 288, resolving by high resolution electron impact MS for $C_{16}H_{16}O_5$. The remaining resonances in the ^{1}H NMR spectrum were indicative of an isopropenyl group and two oxymethine protons which, as the substituent contained only one oxygen, must be due to an epoxide. These data were in agreement with that reported for phebalosin [5, 6] and allow the isolate to be identified as (+)-6-methoxyphe-

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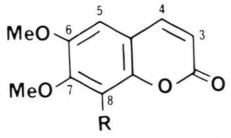

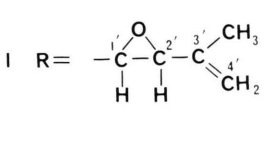

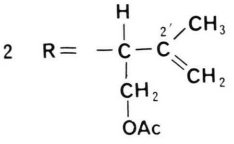

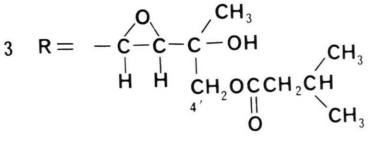

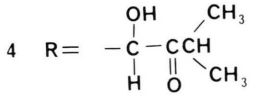

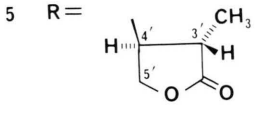

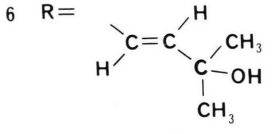

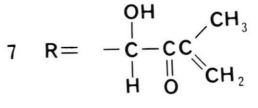

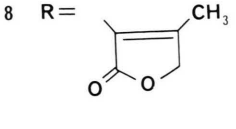

balosin (**1**). Band B, after further purification by PTLC, yielded a compound which was characterized as (+)-6-methoxyisomurralonginol acetate (**2**) by comparison of ^{1}H NMR data with that recorded for isomurralonginol acetate isolated from *Murraya exotica* [5].

Band E yielded two compounds after further PTLC. The more abundant of these analyzed, by MS, for $C_{21}H_{26}O_8$. The ^{1}H and ^{13}C NMR spectra showed resonances for the normal 6,7-dimethoxycoumarin nucleus together with two oxymethine signals for an epoxide (as in (**1**)). Other resonances suggested an isolated methyl (δ 1.43), deshielded by attachment to a quaternary carbinol (69.4 p.p.m.), and an hydroxymethyl (δ 4.31, 4.26, *J* 11.8 Hz; 67.9 p.p.m.). The relatively deshielded chemical shift values for the hydroxymethyl protons indicated esterification and the identification of the esterifying group as 3-methylbutanoic acid followed from significant ions at *m/z* 85 and 57 in the MS and from the ^{1}H NMR spectrum. On this evidence the coumarin was identified as **3** and named as 6-methoxy-1′,2′-epoxycasegravol-4′-(3-methyl)butanoate. The minor compound obtained from this band proved to be 6-methoxymurranganon (**4**) which had previously been isolated from this species only in an impure form [4]. The stereochemistry of the C-1′ hydroxyl of **4** was investigated by the application of Horeau's method [7], which suggests that at C-1′ **4** is *(S)*.

Band F, after PTLC, gave a further coumarin which analyzed by MS for $C_{16}H_{16}O_6$. After sub-

traction of the dimethoxycoumarin nucleus the C-8 substituent is $C_5H_7O_2$. The seven protons were all clearly visible in the ^1H NMR spectrum and through chemical shift values and a series of decoupling experiments could be placed in an $-O-CH_2-CH(R)-CH(CH_3)-$ system. In order to accommodate the remaining carbon and oxygen and to explain the coupling constants obtained this must form a tetrahydrofuran-2-one system in which R is the point of attachment to the coumarin nucleus. This leads to assignment of structure **5.** Relative stereochemistry of H-3′ and H-4′ were determined as *trans* by an NOE experiment in which the 3′-methyl resonance (δ 1.28) was irradiated resulting in a 6% enhancement of H-4′. This compound is obviously formed by cyclization of an isomurralonginol type (*e.g.* **2**) side chain. The closest known compound is microminutin (**8**) [8]. The new compound has been assigned the trivial name of 6-methoxy-3′,4′-dihydroisomicrominutin.

The final coumarin, obtained pure from band G, gave spectral data in agreement with that recorded previously for murraol [5] and was thus characterized as 6-methoxymurraol (**6**).

Together with the four previously reported 6-methoxy-3′,4′-dehydromurranganon derivatives [4] a total of ten novel 6,7-dimethoxy-8-prenylated coumarins have now been isolated from *P. elatius* ssp. *beckleri*. To date this species is unique among *Phebalium* species in yielding 6-oxygenated coumarins [1, 2]. The 8-prenyl substituents produced continue to show a remarkable convergence with those of species of *Murraya* and the allied genera *Micromelum* and *Merrillia* [9]. While *Phebalium* and *Murraya* are both members of the Rutaceae they are not considered to be closely allied.

Experimental

Plant material

Selected from wild-collected plants cultivated at the Australian National Botanic Gardens under accession numbers 732668 and 732679. Vouchers of these accessions are lodged in the ANBG Herbarium.

Isolation of compounds

Ground plant material (320 g) was extracted by Soxhlet with, successively, light petroleum (b.p. 60–80 °C), EtOAc and MeOH. The concentrated petrol extract was separated on Si gel PTLC, developing with petrol:EtOAc (1:1) to give seven bands (A–G). From band A (R_f 0.48) **1** (136 mg) was obtained pure. Band B (R_f 0.36) was further purified by PTLC over Si gel eluting with $CHCl_3$ to give **2** (4 mg). The following two bands (C and D, R_f 0.31, 0.29) gave esters of **7** and have been reported on elsewhere [4]. Band E (R_f 0.26) was a mixture which was separated by PTLC over silica gel eluting with toluene:EtOAc (3:1) to give **4** (4 mg) followed by **3** (20 mg). Band F (R_f 0.23) required further PTLC over Si gel, eluting twice with the toluene:EtOAc (3:1) system, to give **5** (7 mg). Band F (R_f 0.21) gave **6** (10 mg).

(+)-6-Methoxyphebalosin (**1**)

Needles from light petroleum (b.p. 60–80 °C), m.p. 118 °C. $[\alpha]_D$ +22° (c = 0.13 in $CHCl_3$). UV λ_{max}, nm: MeOH 250 sh, 255 sh, 287, 339. IR γ_{max}, cm^{-1}: KBr 3085, 1730, 1600, 1480, 1400, 1350, 1140. ^1H NMR (360 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 9.5 Hz, H-4), 6.88 (1H, s, H-5), 6.31 (1H, d, J = 9.5 Hz, H-3), 5.25, 5.05 (2 × 1H, 2 × br. s, $=CH_2$), 4.05 (1H, d, J = 2.3 Hz, H-1′), 3.94 (3H, s, 6 or 7-OMe), 3.92 (1H, d, J = 2.3 Hz, H-2′), 3.88 (3H, s, 6 or 7-OMe), 1.82 (3H, br. s, 3′-Me). MS (rel. int.): m/z 288 (89) (calcd for $C_{16}H_{16}O_5$ 288.0998, found 288.1002), 260 (7), 219 (44), 206 (26), 69 (100).

(+)-6-Methoxyisomurralonginol acetate (**2**)

Oil. $[\alpha]_D$ +8° (c = 0.27, $CHCl_3$). UV λ_{max}, nm: MeOH 250 sh, 285, 338. IR γ_{max}, cm^{-1}: $CHCl_3$ 2910, 1730, 1600, 1140. ^1H NMR (250 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 9.5 Hz, H-4), 6.85 (1H, s, H-5), 6.33 (1H, d, J = 9.5 Hz, H-3), 4.97, 4.93 (2 × 1H, 2 × br. s, $=CH_2$), 4.85 (1H, dd, J = 10.8, 7.5 Hz, $O-CH_2$), 4.63 (1H, dd, J = 10.8, 7.5 Hz, $O-CH_2$), 4.40 (1H, t, J = 7.5 Hz, H-1′), 3.91 (3H, s, 6 or 7-OMe), 3.89 (3H, s, 6 or 7-OMe), 1.96 (3H, s, COMe), 1.71 (3H, br. s, 2′-Me). MS (rel. int.): m/z 332 (81) (calcd for $C_{18}H_{20}O_6$ 332.1260, found 332.1282), 272 (53), 260 (18), 242 (17), 235 (100).

1′,2′-Epoxy-6-methoxycasegraveol-4′-(3-methyl)butanoate (**3**)

Oil. $[\alpha]_D$ −14° (c = 0.32, $CHCl_3$). UV λ_{max}, nm: MeOH 248 sh, 286, 338 nm. IR γ_{max}, cm^{-1}: $CHCl_3$

3460, 1730, 1600, 1280, 1150 cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 9.5 Hz, H-4), 6.91 (1H, s, H-5), 6.35 (1H, d, J = 9.5 Hz, H-3), 4.30 (1H, d, J = 2.3 Hz, H-1′), 4.31 (1H, d, J = 11.8 Hz, H-4′), 4.26 (1H, d, J = 11.8 Hz, H-4′), 3.97 (3H, s, 6 or 7-OMe), 3.91 (3H, s, 6 or 7-OMe), 3.64 (1H, d, J = 2.3 Hz, H-2′), 2.25 (2H, d, J = 6.7 Hz, H-2″), 2.10 (1H, m, H-3″), 1.43 (3H, s, 3′-Me), 0.93 (3H, d, J = 6.8 Hz, 3″-Me), 0.92 (3H, d, J = 6.8 Hz, 3″-Me). ^{13}C NMR (90.56 MHz, $CDCl_3$): p.p.m. s at 69.4 (C-3′), 114.3 (C-10), 118.1 (C-8), 147.7 (C-6), 149.5 (C-7), 152.3 (C-9), 160.0 (C-2), 172.9 (C-1″); d at 25.4 (C-3″), 48.5 (C-2′), 61.5 (C-1′), 109.7 (C-5), 115.1 (C-3), 142.9 (C-4); t at 43.0 (C-2″), 67.9 (C-4′); q at 22.3 (2 × 3″-Me), 22.7 (3′-Me), 56.1 (6-OMe), 61.8 (7-OMe). MS (rel. int.): m/z 406 (5) (calcd for $C_{21}H_{26}O_8$ 406.1628, found 406.1700), 248 (20), 235 (100), 220 (10), 85 (38), 57 (27).

(+)-6-Methoxymurranganon (**4**)

Oil. $[\alpha]_D$ +65° (c = 0.22, $CHCl_3$). UV λ_{max} nm: MeOH 250sh, 260sh, 288, 340. IR ν_{max} cm^{-1}: $CHCl_3$ 3500, 2920, 1730, 1610, 1565, 1280, 1140. ^1H NMR (250 MHz, $CDCl_3$): δ 7.65 (1H, d, J = 9.5 Hz, H-4), 6.96 (1H, s, H-5), 6.37 (1H, d, J = 9.5 Hz, H-3), 5.83 (1H, d, J = 4.5 Hz, H-1′), 4.37 (1H, d, J = 4.5 Hz, 1′-OH), 3.92 (3H, s, 6 or 7-OMe), 3.87 (3H, s, 6 or 7-OMe), 2.82 (1H, heptuplet, J = 6.7 Hz, H-3′), 1.13 (3H, d, J = 6.7 Hz, 3′-Me), 1.04 (3H, d, J = 6.7 Hz, 3′-Me). MS (rel. int.): m/z 306 (15) (calcd for $C_{16}H_{18}O_6$ 306.1103, found 306.1061), 235 (100). Compound **4** (3 mg) in 0.1 ml C_5H_5N was treated with excess (±)-α-phenylbutyric anhydride and kept in a sealed vial at 40 °C for 2 h. (+)-(*R*)-α-phenylethylamine (0.1 ml) was added and the mixture agitated for 15 min. It was then diluted with dry EtOAc (0.5 ml) and the sample analyzed by GC (BP-1 column 25 m × 0.33 mm, SGE (Australia), isocratic at 200 °C). The relative proportions of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid were calculated from the areas under the respective peaks. The peak increment for the *R*-acid was 1.4% when compared with the result for a racemic mixture of cyclohexanol.

6-Methoxy-3′,4′-dihydroisomicrominutin (**5**)

Clusters from MeOH, m.p. 110 °C. $[\alpha]_D$ −10° (c = 0.15, $CHCl_3$). UV λ_{max} nm: MeOH 248sh, 256sh, 288, 338. IR ν_{max} cm^{-1}: $CHCl_3$ 2940, 1765, 1730, 1600, 1565, 1400, 1280, 1140. ^1H NMR (250 MHz, $CDCl_3$): δ 7.64 (1H, d, J = 9.5 Hz, H-4), 6.91 (1H, s, H-5), 6.36 (1H, d, J = 9.5 Hz, H-3), 4.56 (1H, t, J = 8.3 Hz, H-5′), 4.51 (1H, dd, J = 9.5, 8.3 Hz, H-5′), 4.16 (1H, ddd, J = 10.2, 9.5, 8.3 Hz, H-4′), 3.97 (3H, s, 6 or 7-OMe), 3.92 (3H, s, 6 or 7-OMe), 3.26 (1H, dq, J = 10.2, 7.3 Hz, H-3′), 1.28 (3H, d, J = 7.3 Hz, 3′-Me). MS (rel. int.): m/z 304 (100) (calcd for $C_{16}H_{16}O_6$ 304.0947, found 304.0939), 246 (35).

6-Methoxymurraol (**6**)

Prisms from Me_2CO, m.p. 114 °C. UV λ_{max} nm: MeOH 250sh, 260sh, 300, 341. IR ν_{max} cm^{-1}: $CHCl_3$ 3500, 2960, 1720, 1600, 1570, 1280, 1140. ^1H NMR (250 MHz, $CDCl_3$): δ 7.62 (1H, d, J = 9.5 Hz, H-4), 7.04 (1H, d, J = 16.4 Hz, H-1′), 6.86 (1H, d, J = 16.4 Hz, H-2′), 6.81 (1H, s, H-5), 6.36 (1H, d, J = 9.5 Hz, H-3), 3.91 (3H, s, 6 or 7-OMe), 3.85 (3H, s, 6 or 7-OMe), 1.47 (6H, s, 2 × 3′-Me). MS (rel. int.): m/z 290 (35) (calcd for $C_{16}H_{18}O_5$ 290.1155, found 290.1154), 275 (41), 247 (100).

Acknowledgements

The authors wish to thank Dr. I. H. Sadler, Department of Chemistry, Edinburgh University, for high-field NMR studies. One of us (J. A. E.-T.) acknowledges the award of a scholarship from the Libyan Government.

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