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


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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-en-1-yl = (+)-6-methoxyphenhellosin (1), 1-acetoxyethyl-2-methylprop-2-en-1-yl = (+)-6-methoxysomurralongin acetate (2), 1,2-epoxy-3-hydroxy-4-(3-methylbutanoyloxy)-3-methylbutanyl = 1,2-epoxy-6-methoxycasgravol-4-(3-methyl)-butanoate (3), 1,2-hydroxy-3-methyl-2-oxobutanyl = (+)-6-methoxymurranganon (4), 3-methyl-2-oxetathydrofuran-4-yl = 6-methoxy-3,4′-dihydroisomicroaminin (5) and (E)-3-hydroxy-3-methylbut-1-en-yl = 6-methoxymurranol (6).

The substitution pattern of the coumarin nucleus of all six compounds was established through their 1H 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 I 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 13C 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 1H 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 C16H16O5. The remaining resonances in the 1H 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 phenhellosin [5, 6] and allow the isolate to be identified as (+)-6-methoxyphe-
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 $C_{31}H_{26}O_6$. 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-methoxy-murranganon (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 C₆H₂O₆. 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-CH₂-CH(R)-CH(CH₃)- 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 couma-rin 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 those 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 7'32668 and 7'32679. 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 (Rf 0.48) 1 (136 mg) was obtained pure. Band B (Rf 0.36) was further purified by PTLC over Si gel eluting with CHCl₃ to give 2 (4 mg). The following two bands (C and D, Rf 0.31, 0.29) gave esters of 7 and have been reported on elsewhere [4]. Band E (Rf 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 (Rf 0.23) required further PTLC over Si gel, eluting twice with the toluene:EtOAc (3:1) system, to give 5 (7 mg). Band F (Rf 0.21) gave 6 (10 mg).

(+)-6-Methoxyphalebolosin (1)

Needles from light petroleum (b.p. 60–80 °C), m.p. 118 °C. [α]D +22° (c = 0.13 in CHCl₃). UV λ max, nm: MeOH 250 sh, 255 sh, 287, 339. IR γ max, cm⁻¹: KBr 3085, 1730, 1480, 1400, 1350, 1140. ¹H NMR (360 MHz, CDCl₃; δ 7.59 (1 H, d, J = 9.5 Hz, H-4), 6.88 (1 H, s, H-5), 6.31 (1 H, d, J = 9.5 Hz, H-3), 5.25, 5.05 (2 × 1 H, 2 × br. s, = CH₂), 4.05 (1 H, d, J = 2.3 Hz, H-1'), 3.94 (3 H, s, 6 or 7-OMe), 3.92 (1 H, d, J = 2.3 Hz, H-2'), 3.88 (3 H, s, 6 or 7-OMe), 1.82 (3 H, br. s, 3'-Me). MS (rel. int.): m/z 288 (89) (caled for C₁₀H₁₂O₅ 288.0998, found 288.1000), 260 (7), 219 (44), 206 (26), 69 (100).

(+)-6-Methoxysomurralongin acetate (2)

Oil. [α]D +8° (c = 0.27, CHCl₃). UV λ max, nm: MeOH 250 sh, 225, 338. IR γ max, cm⁻¹: CHCl₃ 2910, 1730, 1600, 1140. ¹H NMR (250 MHz, CDCl₃; δ 7.61 (1 H, d, J = 9.5 Hz, H-4), 6.85 (1 H, s, H-5), 6.33 (1 H, d, J = 9.5 Hz, H-3), 4.97, 4.93 (2 × 1 H, 2 × br. s, = CH₂), 4.85 (1 H, dd, J = 10.8, 7.5 Hz, O-CH₂), 4.63 (1 H, dd, J = 10.8, 7.5 Hz, O-CH₂), 4.40 (1 H, tt, J = 7.5 Hz, H-1'), 3.91 (3 H, s, 6 or 7-OMe), 3.89 (3 H, s, 6 or 7-OMe), 1.96 (3 H, s, COMe), 1.71 (3 H, br. s, 2'-Me). MS (rel. int.): m/z 332 (81) (caled for C₁₄H₂₀O₅ 332.1260, found 332.1282), 272 (53), 260 (18), 242 (17), 235 (100).

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

Oil. [α]D +14° (c = 0.32, CHCl₃). UV λ max, nm: MeOH 248 sh, 286, 338 nm. IR γ max, cm⁻¹: CHCl₃...
3460, 1730, 1600, 1280, 1150 cm\(^{-1}\). \(^1\)H NMR (360 MHz, CDCl\(_3\)): \(\delta 7.61 (1\ H, d, J = 9.5\ Hz, H-4), 6.91 (1\ H, s, H-5), 6.35 (1\ H, d, J = 9.5\ Hz, H-3), 4.30 (1\ H, d, J = 2.3\ Hz, H-1'), 4.31 (1\ H, d, J = 11.8\ Hz, H-4'), 4.26 (1\ H, d, J = 11.8\ Hz, H-4), 3.97 (3\ H, s, 6 or 7-OMe), 3.91 (3\ H, s, 6 or 7-OMe), 3.64 (1\ H, d, J = 2.3\ Hz, H-2'), 2.10 (1\ H, m, H-3'), 1.43 (3\ H, s, 3'-Me), 0.93 (3\ H, d, J = 6.8\ Hz, 3'-Me), 0.92 (3\ H, d, J = 6.8\ Hz, 3'-Me). \(^1^3\)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 x 3'-Me), 22.7 (3'-Me), 56.1 (6-OMe), 61.8 (7-OMe). MS (rel. int.): \(m/z\) 406 (5) (calcd for C\(_{16}H_{18}O_8\) 406.1628, found 406.1700), 248 (20), 235 (100), 220 (10), 85 (38), 57 (27).

\((+)-6\)-Methoxymurraonan (4)

Oil. [\(\alpha\)]\(_D\) +65° (c = 0.22, CHCl\(_3\)). UV \(\lambda_{\text{max}}\) nm: MeOH 250sh, 260sh, 288, 340. IR \(\nu_{\text{max}}\) cm\(^{-1}\): CHCl\(_3\) 3500, 2900, 2130, 1730, 1655, 1280, 1140. \(^1\)H NMR (250 MHz, CDCl\(_3\)): \(\delta 7.65 (1\ H, d, J = 9.5\ Hz, H-4), 6.96 (1\ H, s, H-5), 6.37 (1\ H, d, J = 9.5\ Hz, H-3), 5.83 (1\ H, d, J = 4.5\ Hz, H-1'), 4.37 (1\ H, d, J = 4.5\ Hz, 1'-OH), 3.92 (3\ H, s, 6 or 7-OMe), 3.87 (3\ H, s, 6 or 7-OMe), 2.82 (1\ H, heptuplet, J = 6.7\ Hz, H-3'), 1.13 (3\ H, d, J = 6.7\ Hz, 3'-Me), 1.04 (3\ H, 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\(_2\)H\(_2\)N was treated with excess (±)-\((\beta\)-phenylethylamine (0.1 ml) 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 x 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 \(\lambda_{\text{max}}\) nm: MeOH 248 sh, 256sh, 288, 338. IR \(\nu_{\text{max}}\) cm\(^{-1}\): CHCl\(_3\) 2940, 1765, 1730, 1605, 1400, 1280, 1140. \(^1\)H NMR (250 MHz, CDCl\(_3\)): \(\delta 7.64 (1\ H, d, J = 9.5\ Hz, H-4), 6.91 (1\ H, s, H-5), 6.36 (1\ H, d, J = 9.5\ Hz, H-3), 4.56 (1\ H, t, J = 8.3\ Hz, H-5'), 4.51 (1\ H, dd, J = 8.3, 8.3 Hz, H-5'), 4.16 (1\ H, ddd, J = 10.2, 9.5, 8.3 Hz, H-4'), 3.97 (3\ H, s, 6 or 7-OMe), 3.92 (3\ H, s, 6 or 7-OMe), 3.26 (1\ H, dq, 7 = 10.2, 7.3 Hz, H-3'), 1.28 (3\ H, d, J = 7.3\ Hz, 3'-Me). MS (rel. int.): \(m/z\) 304 (100) (calcd for C\(_{16}H_{16}O_5\) 304.0937, found 304.0934), 246 (35).

6-Methoxymurraol (6)

Prisms from Me\(_2\)CO, m.p. 114 °C. UV \(\lambda_{\text{max}}\) nm: MeOH 250sh, 260sh, 300, 341. IR \(\nu_{\text{max}}\) cm\(^{-1}\): CHCl\(_3\) 3500, 2900, 1760, 1600, 1570, 1280, 1140. \(^1\)H NMR (250 MHz, CDCl\(_3\)): \(\delta 7.62 (1\ H, d, J = 9.5\ Hz, H-4), 7.04 (1\ H, d, J = 16.4\ Hz, H-1'), 6.86 (1\ H, d, J = 16.4\ Hz, H-2'), 6.81 (1\ H, s, H-5), 6.36 (1\ H, d, J = 9.5\ Hz, H-3), 3.91 (3\ H, s, 6 or 7-OMe), 3.85 (3\ H, s, 6 or 7-OMe), 1.47 (6\ H, s, 2 x 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).

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