Eight New Prenylcoumarins from *Phebalium clavatum*

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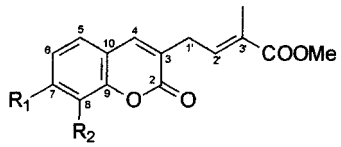
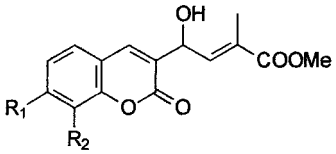
The aerial parts of *Phebalium clavatum* yielded eight new 3-prenylated coumarins, phebaclavin A−H (**1**−**8**). Their structures were established on the basis of their NMR and mass spectral data. In addition, seven known compounds were also isolated, including two 8-geranyloxy linear furocoumarins previously obtained from *Phebalium tuberculosum* ssp. *megaphyllum*, included in the same section of the genus.

The genus *Phebalium* Vent. (Rutaceae, tribe Boronieae) includes some 45 species of shrubs and undershrubs, distributed in the southwest and southeast regions of Australia and in the northern island of New Zealand.[1−3] A consistent feature of all investigated species is the presence of coumarins.[4,5] In his revision, Wilson divided the genus into four sections, *Phebalium*, *Eriostemoides*, *Gonioclados*, and *Leionema*.[3] *Phebalium clavatum* C. A. Gardn. belongs to the section *Phebalium*, which includes 19 species characterized by stem, leaves, and outside petals lepidote and by an inflorescence in terminal umbel.[3,6] Morphologically *Phebalium clavatum* is the most distinct of the West Australian species of section *Phebalium*.[3]

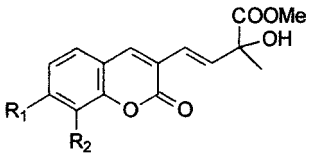
Several taxa of the section *Phebalium*, including *Phebalium tuberculosum* (F. Muell.) Benth. ssp. *megaphyllum* (Ewart) P. G. Wilson and *Phebalium filifolium* Turcz., have been previously studied chemically. Both studies led to the isolation of 7-geranyloxycoumarins, 8-prenylated coumarins, and linear furocoumarins.[7] In a continuation of our studies on Australian Rutaceous plants,[8,9] we report here the isolation and structure determination of eight new 3-prenylated coumarins from the aerial parts of *Phebalium clavatum*, together with the identification of seven known phenylpropanoids.



1 R_1 = OMe R_2 = OH
2 R_1 = OH R_2 = OMe
3 R_1 = OH R_2 = H



4 R_1 = OMe R_2 = OH
5 R_1 = OH R_2 = OMe
6 R_1 = OH R_2 = OH



7 R_1 = OMe R_2 = OH
8 R_1 = OH R_2 = OMe

Results and Discussion

Fractionation of the CH_2Cl_2 and EtOAc extracts of the aerial parts of *P. clavatum* resulted in the isolation of 15 secondary metabolites. Three were identified as the linear furocoumarins psoralen,[10] (*E*)-8-(6-hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)psoralen, and (*E*,*E*)-8-(7-hydroxy-3,7-dimethylocta-2,7-dienyloxy)psoralen, previously isolated from *Phebalium tuberculosum* (F. Muell.) Benth. ssp. *megaphyllum* (Ewart) P. G. Wilson.[7] Other known compounds included scopoletin, luvangetin, colpuchol, and methyl *p*-coumarate.[11−13] Eight 3-prenylated coumarins, named phebaclavin A−H (**1**−**8**), were new.

Phebaclavin A (**1**) was obtained as a white amorphous product. The empirical formula was determined by accurate mass measurement as $C_{16}H_{16}O_6$. The UV spectrum recorded in MeOH was typical for a 7,8-dioxygenated coumarin.[11] A bathochromic shift observed in alkaline medium suggested the presence of a free phenolic group. The IR spectrum showed characteristic bands at 3410 and 1715 cm^{-1} accounting for a hydroxy group and for the pyrone-carbonyl, respectively. In the aromatic region, the ^1H NMR spectrum (Table 1) displayed a pair of doublets (J = 8.5 Hz) at δ 6.85 and 6.97 consistent with the presence of two substitutents at 7 and 8 on the aromatic ring, whereas a singlet at δ 7.40 was typical for a coumarin substituted at C-3 on the pyrone ring.[14,15] At higher field, a 3H singlet at δ 3.98 indicated the presence of one aromatic methoxyl group. Finally, a typical set of signals, consisting of a 3H doublet at δ 1.95 (J = 1.0 Hz), a 2H doublet (J = 7.5 Hz) at 3.43, a 3H singlet at 3.77, and a 1H triplet of quartets (J = 7.5 Hz, J' = 1.0 Hz) at 6.91 accounted for a 4-(methyl 2-methyl-2-butenoate) side chain, whose *E* configuration was deduced from the chemical shift of the latter signal.[16,17] Unambiguous location of the methoxy group at C-7, of the *C*-prenyl side chain at C-3, and hence of the phenolic hydroxy group at C-8 was carried out using multi-impul-

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Table 1. ^{1}H NMR Data of Compounds **1**−**8** ($CDCl_3$, except for **6** in CD_3OD, δ ppm, J in Hz)

proton(s)	1	2	3	4	5	6	7	8
H-4	7.40 s	7.41 s	7.45 s	7.66 s	7.65 s	7.63 s	7.61 s	7.60 s
H-5	6.97 d (8.5)	7.08 d (8.5)	7.31 d (8.5)	7.04 d (8.5)	7.15 d (8.5)	7.32 d (8.5)	7.02 d (8.5)	7.12 d (8.5)
H-6	6.85 d (8.5)	6.91 d (8.5)	6.84 dd (8.5, 2.0)	6.88 d (8.5)	6.93 d (8.5)	6.85 d (8.5)	6.87 d (8.5)	6.90 d (8.5)
H-8			6.96 d (2.0)					
6-OMe	3.98 s			3.99 s			3.99 s	
8-OMe		4.12 s			4.12 s			4.12 s
H-1′	3.43 d (7.5)	3.43 d (7.5)	3.44 d (7.5)	5.55 br d (8.5)	5.54 br d (8.5)	5.54 br d (8.5)	6.73 d (16.0)	6.73 d (16.0)
H-2′	6.91 tq (7.5, 1.0)	6.90 tq (7.5, 1.0)	6.91 tq (7.5, 1.0)	6.84 dq (8.5, 1.0)	6.84 dq (8.5, 1.0)	6.80 dq (8.5, 1.0)	6.97 d (16.0)	6.95 d (16.0)
3′-Me	1.95 d (1.0)	1.96 d (1.0)	1.97 d (1.0)	1.98 d (1.0)	2.00 d (1.0)	1.98 s	1.60 s	1.60 s
COOMe	3.77 s	3.78 s	3.80 s	3.76 s	3.77 s	3.77 s	3.83 s	3.84 s

Table 2. ^{13}C NMR Data (δ) of Compounds **1**−**8** ($CDCl_3$, except for **6** in CD_3OD)

carbon	1	2	3	4	5	6	7	8
C-2	161.0	161.0	162.5	160.7	160.5	162.6	159.3	159.3
C-3	123.2	122.5	122.1	125.1	124.3	125.3	120.7	119.9
C-4	139.4	139.9	139.9	139.5	139.7	141.9	139.9	140.2
C-5	118.1	122.7	128.7	119.1	123.7	114.8	118.8	123.6
C-6	107.8	112.1	113.6	108.0	112.5	103.2	107.9	112.3
C-7	149.0	151.3	159.5	149.6	152.0	156.4	149.4	151.7
C-8	132.7	133.4	102.9	132.7	133.5	130.3	132.5	133.2
C-9	141.2	146.2	154.6	141.2	146.2	142.0	140.9	145.9
C-10	113.9	113.6	112.7	113.5	113.2	113.2	114.0	113.7
7-OMe	56.5			56.5			56.5	
8-OMe		61.7			61.8			61.7
C-1′	29.3	29.3	29.3	67.1	67.2	66.3	123.7	123.9
C-2′	136.9	136.9	137.4	138.9	138.8	141.0	135.2	135.1
C-3′	130.4	130.4	130.3	130.6	130.7	130.8	74.7	74.7
C═O	168.2	168.3	168.6	168.1	168.1	170.0	175.9	175.9
Me	12.5	12.6	12.6	13.2	13.2	13.2	26.4	26.4
CO-O*Me*	51.9	51.9	52.1	52.1	52.1	52.5	53.3	53.3

sional COSY-LR, HMQC, and HMBC experiments. Indeed, the COSY-LR spectrum, optimized for ^5J correlations, showed a strong cross-peak between the aromatic methoxy signal at δ 3.98 and the H-6 doublet at 6.85, whereas the typical three-bond HMBC connectivities were observed between (i) CH_2-1′ at δ 3.43 and C-2 at δ 161.0 and C-4 at δ 139.4, (ii) O*C*H_3-7 at δ 3.98 and C-7 at δ 149.0. These data defined the structure of phebaclavin A as **1**.

The molecular formula of phebaclavin B (**2**), $C_{16}H_{16}O_6$, was deduced to be the same as that of **1** from the HRMS data. The UV spectrum was also typical for a 7,8-dioxygenated coumarin, but the bathochromic shift observed upon alkali addition was dramatically increased when compared to that obtained for **1** under similar conditions, suggesting the presence of a free phenolic group at C-7. Both ^{1}H and ^{13}C NMR data (Tables 1 and 2) were nearly identical with those of **1**. Nevertheless, significant differences were observed in the chemical shifts of the methoxyl group signals (δ_H = 4.12 ppm, δ_C = 61.7 ppm), suggesting location of the Ar-OCH_3 group at C-8. In good agreement with this statement, a typical three-bond HMBC cross-peak was observed between the O*C*H_3 signal at 4.12 ppm and that of C-8 at 133.4 ppm, whereas no ^5J correlation concerning the methoxyl group could be observed on the COSY-LR spectrum. Therefore, the structure of phebaclavin B was established as **2**.

The empirical formula of phebaclavin C (**3**) was determined by accurate mass spectrometry as $C_{15}H_{14}O_5$. The UV spectrum, strongly modified in alkaline medium, characterized a 7-hydroxycoumarin.[11] Accordingly, the ^{1}H NMR spectrum (Table 1) exhibited in the aromatic region the typical signals associated with H-5 (d, J = 8.5 Hz) at δ 7.31, H-6 (dd, J = 8.5 Hz, J' = 2.0 Hz) at δ 6.84, and H-8 (d, J = 2.0 Hz) at δ 6.96. The other features of the ^{1}H and ^{13}C NMR spectra were closely related to those of **1** and **2**, revealing the presence of the same prenyl side chain at C-3. Consequently, the structure of phebaclavin C was concluded to be **3**.

Phebaclavin D (**4**) was assigned the molecular formula $C_{16}H_{16}O_7$ by accurate mass measurement. The UV spectrum, nearly identical with that of **1**, was typical for a 7,8-dioxygenated coumarin.[11] The ^{1}H NMR spectrum (Table 1) showed typical signals associated with H-3, H-5, and H-6 comparable to those observed for compounds **1** and **2**. A 3H singlet at δ 3.99 was suggestive of a methoxyl group at C-7. Additional resonances at δ 6.84 (dq, J = 8.5 Hz, J' = 1.0 Hz), 5.55 (br d, J = 8.5 Hz), 3.76 (3H, s), and 1.98 (3H, d, J = 1.0 Hz) gave evidence for a 4-(methyl 4-hydroxy-2-methyl-2-butenoate) side chain of *E* configuration.[18,19] Positions of the *C*-prenyl chain at C-3, of the methoxyl group at C-7, and of the free phenolic group at C-8 were finally confirmed by typical COSY-LR and HMBC correlations, similar to those observed for phebaclavin A. Therefore, the structure of phebaclavin D was established as **4**. The absolute configuration of the chiral center at C-1′ could not be determined, due to the small amount of product isolated.

The empirical formula of phebaclavin E (**5**), $C_{16}H_{16}O_7$, determined by accurate mass spectrometry, was the same as that of phebaclavin D (**4**). The UV, IR, and ^{1}H and ^{13}C NMR data (Tables 1 and 2) were essentially similar to those of **4**. Nevertheless, a characteristic difference was observed in the UV, which displayed a strong bathochromic shift in alkaline medium, indicating a free phenolic group at C-7. Evidence for the presence of a methoxyl group at C-8 was obtained by the observation of the corresponding typical signals at δ_H = 4.12 and δ_C = 61.8 in NMR spectroscopy. Hence, the structure of phebaclavin E was depicted as **5**.

The empirical formula of phebaclavin F (**6**) was determined as $C_{15}H_{14}O_7$ by HRMS. The UV spectrum, dramatically modified in alkaline medium, was typical for a 7,8-dihydroxycoumarin. In agreement with this statement, the ^{1}H NMR spectrum (Table 1) displayed in the aromatic region two *ortho*-coupled doublets assigned to H-5 and H-6 and the singlet due to H-4, associated with a lack of aromatic methoxyl resonance. Additional signals similar to those encountered in the spectra of **4** and **5** accounted for a 4-(methyl 4-hydroxy-2-methyl-2-butenoate) substituent at C-3. Consequently, the structure of phebaclavin F was established as **6**.

Identical molecular formulas, $C_{16}H_{16}O_7$, were established for both phebaclavins G (**7**) and H (**8**) by accurate mass measurement of the molecular ions. The ^{1}H and ^{13}C NMR data of the two compounds (Tables 1 and 2) displayed striking similarities. The salient features of the ^{1}H NMR spectra were (i) two aromatic doublets and a singlet associated with H-5, H-6, and H-4 of a 3-substituted

coumarin, (ii) the resonance of an aromatic methoxyl group, and (iii) a typical series of signals accounting for a 4-(*E*)-(methyl 2-hydroxy-2-methyl-3-butenoate) side chain, including two olefinic 1H doublets (J = 16.0 Hz), a carbomethoxyl 3H singlet, and a 3H singlet at δ 1.60. Differences only concerned the greater bathochromic shift observed for phebaclavin H in UV spectroscopy upon alkali addition and the ^{1}H and ^{13}C chemical shifts of the aromatic methoxyl resonances, which appeared at δ_H = 3.99 and δ_C = 56.5 for phebaclavin G and δ_H = 4.12 and δ_C = 61.7 for phebaclavin H. These data led us to establish the structures of phebaclavins G and H as **7** and **8**, respectively.

All the secondary metabolites isolated from the aerial parts of *P. clavatum* in this study belong to the phenylpropanoid series. Most of them are coumarins, showing once again the chemical homogeneity of the genus *Phebalium*.[4,5] Interestingly, the two linear furocoumarins bearing a geranyloxy-derived substituent at C-8 isolated here were previously only obtained from *Phebalium tuberculosum* ssp. *megaphyllum*, which is included in the same section of the genus.[7] Another interesting relationship is with *Phebalium canaliculatum*, which is also characterized by 7,8-dioxygenated coumarins.[4] All the novel compounds are members of the uncommon 3-*C*-prenylcoumarin series. Their biosynthetic homogeneity should be emphasized, since the prenyl side chains at C-3 of phebaclavins D−H can be considered as arising from the oxidation of that present at the same position in phebaclavins A, B, and C.

Experimental Section

General Experimental Procedures. UV spectra were recorded in MeOH on a Shimadzu UV 160A UV spectrometer and IR spectra in KBr on a Shimadzu FTIR-8201PC IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ or CD_3OD on a Bruker Avance 300 (300 and 75 MHz respectively) NMR spectrometer. ^{1}H−^{1}H COSY, COSY-LR, ^{13}C−^{1}H HMQC, and HMBC experiments were performed using the standard Bruker microprograms. MS were recorded using a Nermag R-10-10-H instrument in the El (70 eV) mode. Extractions were carried out using a Soxhlet apparatus (24 h and 3 L for each solvent used).

Plant Material. The plant material used in this study was collected in bushland near Coolgardie in September 1991. A voucher sample has been deposited at the Western Australia Herbarium, Perth, under the accession number PERTH 01194313.

Extraction and Isolation. Dried powdered twigs (262 g) and leaves (227 g) of *P. clavatum* were treated separately and were defatted by extraction with petroleum ether (bp 40−60 °C), then extracted sequentially with CH_2Cl_2, EtOAc, and MeOH. The AcOEt extract (6 g) of the twigs was subjected to column chromatography using Si gel 60 (Merck; 0.063−0.200 mm) packed in CH_2Cl_2. Elution was performed with CH_2Cl_2 containing increasing amounts of AcOEt, then AcOEt containing increasing amounts of MeOH. Each fraction was monitored by TLC; those containing comparable mixtures were combined and purified by repeated preparative TLC (CH_2Cl_2−MeOH (19:1)). Fractions eluted with CH_2Cl_2−AcOEt (17:3) gave colpuchol (4 mg), luvangetin (10 mg), and compound **2** (87 mg). Fractions eluted with CH_2Cl_2−AcOEt (4:1) gave compounds **3** (19 mg) and **1** (65 mg). Fractions eluted with CH_2Cl_2−AcOEt (3:1) gave compounds **8** (66 mg), **5** (9 mg), **6** (8 mg), and **7** (40 mg). Fractions eluted with CH_2Cl_2−AcOEt (2:1) gave **4** (80 mg). The CH_2Cl_2 extract (1.3 g) of the leaves was subjected to column chromatography using Si gel 60 (Merck; 0.063−0.200 mm) packed in CH_2Cl_2. Elution was performed with CH_2Cl_2 containing increasing amounts of AcOEt. Fractions eluted with CH_2Cl_2−AcOEt (4:1) gave psoralen (6 mg), and a mixture of (*E*,*E*)-8-(7-hydroxy-3,7-dimethylocta-2,5-dienyloxy)psoralen (4 mg) and (*E*)-8-(6-hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)-psoralen (3 mg) separated by preparative TLC (CH_2Cl_2−MeOH (49:1)). Finally, fractions eluted with CH_2Cl_2−AcOEt (3:1) led to the isolation of scopoletin (4 mg) and methyl *p*-hydroxycoumarate (6 mg).

Phebaclavin A (1): amorphous solid; IR (KBr disk) ν_{max} 3410, 2900, 1715, 1610, 1510 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 323 (4.71), 261 (4.51), 225 sh (4.42), 208 (5.08) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 378 (4.53), 336 (4.51), 280 (4.69), 216 (5.01) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 304 [M]$^+$ (38), 272 (27), 244 (100), 229 (17); HREIMS *m/z* 304.0958 (calcd for $C_{16}H_{16}O_6$, 304.0947).

Phebaclavin B (2): amorphous solid; IR (KBr disk) ν_{max} 3420, 2950, 1720, 1600 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 327 (4.42), 262 sh (4.21), 225 sh (4.59), 208 (4.78) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 383 (4.49), 276 (4.16), 250 sh (4.59), 214 (4.70) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 304 [M]$^+$ (47), 272 (35), 244 (100), 229 (28); HREIMS *m/z* 304.0932 (calcd for $C_{16}H_{16}O_6$, 304.0947).

Phebaclavin C (3): amorphous solid; IR (KBr disk) ν_{max} 3380, 2960, 1700, 1610, 1260 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 327 (4.60), 293 sh (4.33), 235 sh (4.39), 215 sh (4.76), 207 (4.81) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 372 (4.70), 248 sh (4.39), 212 (4.97) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 274 [M]$^+$ (5), 242 (22), 215 (40), 214 (100); HREIMS *m/z* 274.0832 (calcd for $C_{15}H_{14}O_5$, 274.0841).

Phebaclavin D (4): amorphous solid; $[\alpha]_D$ 0° [*c* 0.01, $CHCl_3$]; IR (KBr disk) ν_{max} 3410, 2915, 1720, 1625, 1510, 1105 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 324 (4.57), 262 (4.44), 225 sh (4.62), 207 (4.95) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 333 (4.46), 281 (4.61), 216 (4.86) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 320 [M]$^+$ (6), 288 (50), 259 (31), 245 (25), 219 (100); HREIMS *m/z* 320.0891 (calcd for $C_{16}H_{16}O_7$, 320.0896).

Phebaclavin E (5): amorphous solid; $[\alpha]_D$ −6° [*c* 0.0035, $CHCl_3$]; IR (KBr disk) ν_{max} 3430, 2920, 1710, 1600, 1260 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 329 (4.36), 262 sh (4.06), 224 sh (4.41), 205 (4.71) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 386 (4.44), 277 (4.07), 252 sh (4.14), 214 (4.60) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 320 [M]$^+$ (5), 288 (43), 259 (25), 245 (20), 219 (100); HREIMS *m/z* 320.0905 (calcd for $C_{16}H_{16}O_7$, 320.0896).

Phebaclavin F (6): amorphous solid; $[\alpha]_D$ +7° [*c* 0.002, MeOH]; IR (KBr disk) ν_{max} 3405, 2930, 1710, 1610, 1575, 1260 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 329 (4.21), 260 sh (4.08), 223 sh (4.44), 204 (4.77) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 378 (4.29), 254 sh (4.08), 214 (4.50) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 306 [M]$^+$ (12); HREIMS *m/z* 306.0748 (calcd for $C_{15}H_{14}O_7$, 306.0739).

Phebaclavin G (7): amorphous solid; $[\alpha]_D$ −22° [*c* 0.01, $CHCl_3$]; IR (KBr disk) ν_{max} 3390, 2900, 1725, 1625 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 347 (4.79), 265 (4.43), 248 (4.31), 220 sh (4.64), 206 (4.71) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 352 (4.52), 289 (4.58), 234 sh (4.46), 216 (4.63) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 320 [M]$^+$ (26), 303 (8), 261 (30), 245 (29), 219 (100); HREIMS *m/z* 320.0907 (calcd for $C_{16}H_{16}O_7$, 320.0896).

Phebaclavin H (8): amorphous solid; $[\alpha]_D$ −38° [*c* 0.008, $CHCl_3$]; IR (KBr disk) ν_{max} 3410, 2920, 1720, 1600 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 348 (4.63), 266 sh (4.35), 250 (4.37), 219 sh (4.69), 206 (4.76) nm; UV (MeOH + NaOH) λ_{max} (log ϵ) 400 (4.69), 279 (4.40), 264 sh (4.34), 214 (4.66) nm; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 320 [M]$^+$ (17), 303 (8), 261 (21), 245 (12), 219 (100); HREIMS *m/z* 320.0883 (calcd for $C_{16}H_{16}O_7$, 320.0896).

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