3-Substituted Coumarins from the Twigs of *Rhadinothamnus rudis* ssp. *amblycarpus*

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The twigs of *Rhadinothamnus rudis* ssp. *amblycarpus* (*Phebalium rude* ssp. *amblycarpum*) yielded three new 3-substituted coumarins: rudicoumarin A-C (**1 – 3**). Their structures were established on the basis of their NMR and mass spectral data. In addition, ten other known compounds were also isolated, including phebaclavin I, schinicoumarin, seselin, and seven linear furocoumarins.

Key words: Rhadinothamnus rudis ssp. *amblycarpus*, *Phebalium rude* ssp. *amblycarpum*, Rutaceae, Rudicoumarin A-C, Schinicoumarin

Introduction

The genus *Rhadinothamnus* P. G. Wilson [1] was first established to accommodate the species *Nematolepis euphemiae* (syn. *Phebalium euphemiae*) that appeared to be anomalous in both of the genera into which it had been placed. Wilson (1971) noted the close relationship between *Rhadinothamnus* and species of *Phebalium* section *Gonioclados* [2], but the species in this section was not concurrently transferred in the new genus. In his recent revision of *Phebalium* [3], Wilson has included in *Rhadinothamnus* the two species of *Phebalium* belonging to the section *Gonioclados*: *Rhadinothamnus* (*Phebalium*) *anceps* (DC) P. G. Wilson and *Rhadinothamnus rudis* (*Phebalium rude*) (Bartl.) P. G. Wilson with the three subspecies *rudis*, *amblycarpus* (F. Meull) P. G. Wilson and *linearis* (C. A. Gardner) P. G. Wilson.

Previous chemical studies of the genus led to the isolation of several linear furocoumarins, dihydrofurocoumarins and 7-geranyloxycoumarins from *R. anceps* D. C. [4] and two diterpenes, (-)-kaurenoic acid and its 15β-hydroxylated derivative, from *R. rudis* ssp. *rudis* [5]. We have already reported the isolation of three prenylated *p*-coumarates from the twigs of *R. rudis* ssp. *amblycarpus* [6]. Further investigations of this latter material has now led us to report here the isolation and structure determination of three new 3-substituted coumarins, together with the identification of ten other known compounds, including phebaclavin I, schinicoumarin, seselin and seven known linear furocoumarins.

Results and Discussion

Fractionation of the CH_2Cl_2 extract of the twigs of *R. rudis* ssp. *amblycarpus* resulted in the isolation of thirteen secondary metabolites. Seven were identified as the linear furocoumarins psoralen [7], xanthotoxol [8], xanthotoxin [7], imperatorin [7], heraclenol [9], isogosferol [10] and marmesin [11]. Other known compounds identified were phebaclavin I [12], seselin [13] and schinicoumarin [14]. Previous ^{13}C NMR assignments for schinicoumarin [14] were revised as noted in the Experimental Section.

In addition three new coumarins, rudicoumarin A-C (**1 – 3**) were obtained. Rudicoumarin A (**1**) was isolated as a white amorphous solid and the molecular formula of $C_{11}H_{10}O_5$ was established by accurate mass measurement. UV absorption at 320, 263 and 235 indicated the structure of a 7,8-dioxygenated coumarin [15]. A bathochromic shift in alkaline medium indicated the presence of a free phenolic group. The IR spectrum exhibited a lactonic carbonyl absorption at 1717 cm^{-1}.

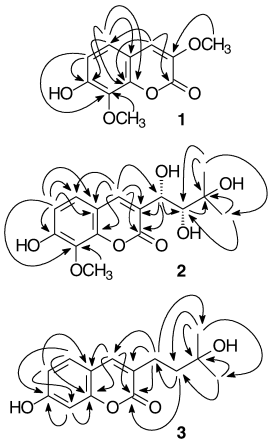


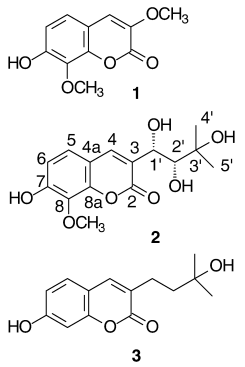
Fig. 1. H→C HMBC correlations observed for **1 – 3**.

In the aromatic region, the ^{1}H NMR spectrum showed two mutual *ortho*-coupling signals at 7.03 and 6.91 (each 1H, d, $J = 8$ Hz) ppm, assigned to H-5 and H-6, respectively, and one proton singlet at 6.81, assigned to H-4 or H-3 of a 3,7,8- or 4,7,8-trisubstituted coumarin. Finally, two 3H singlets at 3.90 and 4.11 ppm indicated the presence of two methoxyl groups. The structure of rudicoumarin A (**1**) was elucidated unambiguously as 7-hydroxy-3,8-dimethoxycoumarin, a related compound of schinicoumarin, using HMQC and HMBC experiments (Fig. 1), the latter confirming the presence of H-4 through long range interactions with C-2, C-5 and C-10.

Rudicoumarin B (**2**) was obtained as a yellowish amorphous solid. The empirical formula was determined by accurate mass measurement as $C_{15}H_{18}O_7$. The UV spectrum recorded in MeOH was typical for a 7,8-dioxygenated coumarin [15]. A strong bathochromic shift observed in alkaline medium suggested the presence of a free phenolic group at C-7. The IR spectrum showed characteristic bands at 3395 and 1701 cm^{-1} accounting for a hydroxyl and the pyrone-carbonyl groups, respectively. In the aromatic region, the ^{1}H NMR spectrum displayed a pair of doublets ($J = 8.6$ Hz) at 6.87 and 7.25 ppm consistent with the presence of two substitutents at C-7 and C-8 on the aromatic ring, whereas a singlet at 7.97 was typical for a coumarin substituted at C-3 on the pyrone ring [16, 17]. At higher field, a 3H-singlet at 3.96 ppm indicated the presence of one aromatic methoxyl group. Additional resonances at 1.36 and 1.41 (3H, s) ppm, at 3.55 (1H, d, $J = 1.5$ Hz) and 5.14 (1H, s) gave evidence for a 3-methyl-1,2,3-trihydroxybutyl side chain [18]. Unambiguous location of the methoxyl group at C-8, of the side chain at C-3, and hence of the phenolic hydroxyl group at C-7 was carried out using COSY-LR, HMQC and HMBC experiments (Fig. 1). The relative stereochemistry of **2** was tentatively assessed by NMR considerations. It has been shown in the literature that, for coumarins sharing the same trihydroxyisoprenyl unit as the terminal moiety of their side chains [19], a $J_{1',2'}$ value of *ca.* 0 Hz was suggesting a relative configuration of the *threo*-type, *i.e.* (1'*S**, 2'*R**) for compound **2**.

Table 1. ^{13}C (75 MHz) and ^{1}H NMR (300 MHz) data of compounds **1 – 3** (CD_3OD, except for **1** in $CDCl_3$, δ in ppm, J in Hz).

	1		**2**		**3**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	157.0		162.4		162.9	
3	143.7		125.9		125.2	
4	114.2	6.81 s	141.9	7.97 s	140.2	7.71 s
4a	113.9		114.2		112.9	
5	121.8	7.03 d (8.0)	124.4	7.24 d (8.6)	129.3	7.44 d (8.4)
6	112.9	6.91 d, (8.0)	114.6	6.87 d (8.6)	113.0	6.82 dd (8.4, 2.0)
7	149.9		154.8		161.0	
8	136.7		135.4		102.7	6.74 d (2.0)
8a	144.1		148.4		155.1	
3-OCH_3	56.6	3.90 s				
8-OCH_3	62.2	4.11 s	61.4	3.96 s		
1'			69.1	5.14 s	26.0	2.57 m
2'			76.2	3.55 s	42.3	1.74 m
3'			74.7		75.4	
4'			26.9	1.34 s	26.8	1.31 s
5'			26.7	1.41 s	26.8	1.31 s



Rudicoumarin C (**3**), obtained as a yellowish amorphous powder, was assigned the molecular formula $C_{14}H_{16}O_4$ by accurate mass spectrometry. The UV spectrum, strongly modified in alkaline medium, characterized a 7-hydroxycoumarin [15]. Accordingly, the ^{1}H NMR spectrum exhibited in the aromatic region, the typical signals associated with H-5 (d, $J = 8.4$ Hz) at 7.44 ppm, H-6 (dd, $J = 2.0$ Hz, $J' = 8.4$ Hz) at 6.82, H-8 (d, $J = 2.0$ Hz) at 6.74 and H-3 (s) at 7.71. Additional resonances at 2.57 (2H, m), 1.74 (2H, m) and 1.31 (3H, s) ppm were consistent with the presence of a (3-methyl-3-hydroxybutyl) side chain. The position

of this *C*-prenyl side chain at C-3 was confirmed by HMBC correlations (Fig. 1). This molecule was previously obtained by synthesis [16] but is isolated here for the first time from a natural source.

The finding of these coumarins in *R. rudis* confirms that this taxon has the typical coumarin-producing capacity found in many species of the Boroniae and, in particular, with the furocoumarins of the allied *R. anceps*.

Experimental Section

General experimental procedure

$[\alpha]_D$ were recorded on a Perkin-Elmer 241 polarimeter. MS were registered on a Micromass Q–T of instrument, on a Nermag R10- 10C spectrometer and a HP-5973 Mass Selective Detector. 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 MHz 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. 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 near Ravensthorpe in September 1991. A voucher sample has been deposited at the Western Australia Herbarium, Perth under the accession number PERTH 01163795.

Extraction and isolation

Dried powdered twigs (350 g) of *R. rudis* ssp. *amblycarpus* were first defatted by extraction with petroleum ether (b. p. 40 – 60 °C), then extracted sequentially with CH_2Cl_2, EtOAc and MeOH. The CH_2Cl_2 extract (4 g) of the twigs was subjected to column chromatography using silica 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. Each fraction was monitored by TLC; those containing comparable mixtures were combined and purified by repeated preparative TLC (CH_2Cl_2-MeOH (9:1)). Fractions eluted with CH_2Cl_2 gave psoralen (2 mg), seselin (7 mg) and imperatorin (2 mg). Fractions eluted with CH_2Cl_2-AcOEt (9:1) gave, xanthotoxol (3 mg), isogosferol (3 mg), xanthotoxine (22 mg), schinicoumarin (24 mg), phebaclavin I (5 mg) and compound **1** (11 mg). Fractions eluted with CH_2Cl_2-AcOEt (5:5) gave marmesin (14 mg) and compound **2** (23 mg). Fractions eluted with AcOEt gave heraclenol (6 mg) and compound **3** (14 mg).

Spectroscopic data

Schinicoumarin. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 157.8$ (C_2), 153.5 (C_7), 143.0 (C_3), 143.9 (C_{8a}), 136.2 (C_8), 121.3 (C_5), 113.5 (C_4), 114.6 (C_{4a}), 109.6 (C_6), 61.9 (8-OMe), 56.8 (7-OMe), 56.6 (3-OMe).

Rudicoumarin A (**1**). White amorphous solid. – IR (KBr disk): $\nu = 1717$ (C=O) cm^{-1}. – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 320 (3.11), 263 (2.83), 235 *sh* (3.01), 209 (3.37) nm; UV (MeOH + NaOH): λ_{max} ($\lg \varepsilon_{max}$) = 371 (3.05), 276 *sh* (2.90), 247 *sh* (3.01), 235 *sh* (3.01), 216 (3.28) nm. – for ^{1}H NMR and ^{13}C NMR see Table 1. – MS (EI, 70 eV): *m/z* (%) = 222 (18) $[M]^+$, 207 (100), 192 (62). – $C_{11}H_{10}O_5$: calcd. 222.0528; found 222.0536.

Rudicoumarin B (**2**). Yellow amorphous solid. – $[\alpha]_D + 4°$ [*c* 0.0047, MeOH]. – IR (KBr disk): $\nu = 1701$ (C=O) cm^{-1}. – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 324 (3.29), 258 (2.82), 239 *sh* (2.87), 211 (3.38) nm; UV (MeOH + NaOH): λ_{max} ($\lg \varepsilon_{max}$) = 378 (3.34), 275 (2.84), 241 *sh* (3.01), 217 (3.32) nm. – for ^{1}H NMR and ^{13}C NMR see Table 1. – MS (EI, 70 eV): *m/z* (%) = 310 (15) $[M]^+$, 295 (60), 251 (27), 236 (100). – $C_{15}H_{18}O_7$: calcd. 310.1052; found 310.1061.

Rudicoumarin C (**3**). Yellow amorphous solid. – IR (KBr disk) $\nu = 1693$ (C=O) cm^{-1}. – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 324 (3.07), 303 *sh* (2.91), 253 *sh* (2.64), 225 *sh* (3.01), 207 (3.26) nm; UV (MeOH + NaOH): λ_{max}($\lg \varepsilon_{max}$) = 367 (3.12), 241 *sh* (2.95), 231 (3.02) nm. – for ^{1}H NMR and ^{13}C NMR see Table 1. – MS (EI, 70 eV): *m/z* (%) = 248 (6) $[M]^+$, 189 (100). – $C_{14}H_{16}O_4$: calcd. 248.1048; found 248.1035.

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