Prenylated *p*-Coumarates from the Twigs of *Phebalium rude* subsp. *amblycarpum* (Rutaceae)

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Phebalium rude ssp. *amblycarpum*, Rutaceae, Phebarudol

Phebarudol, a novel prenylated *p*-coumarate, was isolated from the twigs of *Phebalium rude* Bartl. subsp. *amblycarpum* (F. Muell.) P. G. Wilson (Rutaceae) together with the two already known related compounds, werneria chromene and methyl demethoxywutaiensate. The structure of phebarudol was established by spectroscopic methods.

Introduction

The genus *Phebalium* Vent. (Rutaceae, tribe Boronieae) includes some 45 species of shrubs and undershrubs, distributed in the south-west and south-east regions of Australia and in the northern island of New Zealand (Bentham and Müller, 1863; Engler, 1896; Wilson, 1970). In his revision, Wilson divided the genus into four sections, *Phebalium*, *Eriostemoides*, *Gonioclados*, and *Leionema* (Wilson, 1970). Section *Gonioclados* only includes two species, *Phebalium anceps* DC. and *Phebalium rude* Bartl., both characterized by an inflorescence of axillary cymes, imbricate petals, and a calyx with free sepals. In an earlier paper, we reported the isolation of several simple coumarins, furocoumarins, and dihydrofurocoumarins from the twigs of *Phebalium anceps* DC. (Bévalot *et al.*, 1988). In a continuation of our studies on Australian Rutaceous plants (Nouga Bissoue *et al.*, 1996, 1997), we report here the isolation and structure determination of a novel isoprenylcinnamate, phebarudol, together with the identification of the already known werneria chromene and methyl demethoxywutaiensate from the twigs of *Phebalium rude* Bartl. subsp. *amblycarpum* (F. Muell.) P. G. Wilson (= *Phebalium amblycarpum* (F. Muell.) Benth.).

Results and Discussion

Three secondary metabolites were isolated from the CH_2Cl_2 extract of *Phebalium rude* Bartl. subsp. *amblycarpum* (F. Muell.) P. G. Wilson twigs. Two were identified as the prenylated *p*-coumarates werneria chromene (**1**), previously isolated from *Werneria stuebelii* Hieron (Asteraceae) (Bohlmann *et al.*, 1984) and methyl demethoxywutaiensate (**2**), described from *Zanthoxylum wutaiense* Chen (Rutaceae) (Ishii *et al.*, 1982). The third compound was the novel isoprenylcinnamate phebarudol (**3**), isolated as the major secondary metabolite from the twigs. (Fig. 1)

Phebarudol (**3**) was obtained as a yellowish amorphous product. The molecular formula was determined by accurate mass measurment as $C_{15}H_{18}O_4$. The UV spectrum recorded in MeOH was suggestive of a 4-oxygenated cinnamic acid derivative (Ishii *et al.*, 1982). The IR spectrum showed characteristic bands at 3458 and 1699 cm^{-1} accounting for an alcoholic hydroxy function and for a carbomethoxy group, respectively. In the aromatic and olefinic region, the ^1H NMR spectrum displayed a pair of doublets (J = 16 Hz) at 6.26 and 7.58 ppm typical for a *trans* cinnamoyl ester, whereas a system of three signals at 6.80 (d, J = 8 Hz), 7.22 (d, J = 2 Hz), 7.29 (dd, J = 8 Hz, J = 2 Hz) ppm was consistent with the presence of a

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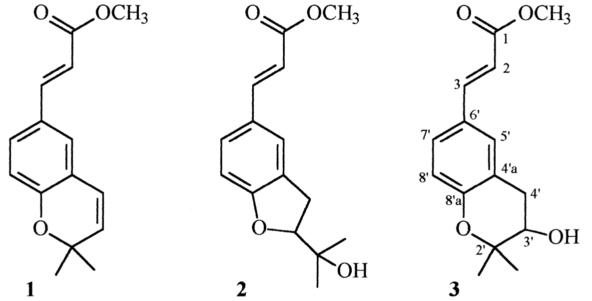

1 **2** **3**

Fig. 1. Prenylated *p*-coumarates from the twigs of *Phebalium rude* subsp. *amblycarpum.*

1,3,4-trisubstituted aromatic ring. At higher field, typical signals at 3.81 (1H, dd, J = 5.5 Hz, J = 5 Hz), 3.05 (1H, dd, J = 17 Hz, J = 5 Hz), 2.77 (1H, dd, J = 17 Hz, J = 5.5 Hz), 1.36 (3H, s), and 1.32 (3H, s) accounted for a 2,2-dimethyl-3-hydroxy-3,4-dihydro-2H-pyran subunit (Ahond *et al.*, 1979, Mitaku *et al.*, 1988). This latter statement was in full agreement with the series of signals observed at 22.1, 24.9, 31.5, 69.3, and 77.6 ppm in the ^{13}C NMR spectrum. Therefore, the structure of phebarudol can be depicted as methyl (*E*)-(2,2-dimethyl-3-hydroxychroman-6-yl)-acrylate (**3**). The absolute configuration of the chiral center at C-2′ could not be determined, due to the small amount of product isolated

From a chemotaxonomic point of view, it is interesting to note that the three prenylated *p*-coumarates from *Phebalium rude* Bartl. subsp. *amblycarpum* (F. Muell.) P. G. Wilson have the same biogenetic cinnamyl precursors as prenylated coumarins previously isolated from other species of *Phebalium.*

Experimental

General experimental procedures

Mass spectra were recorded with a Nermag R-10-10H spectrometer. UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Shimadzu UV-160A spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets on a Perkin-Elmer 257 instrument. ^{1}H-NMR (δ [ppm], J [Hz]) and ^{13}C-NMR spectra were recorded at 300 MHz and 75 MHz respectively, using a Bruker Advance-300 spectrometer. Multi-impusionnal 2D NMR experiments (^{13}C–^{1}H HMQC, and ^{13}C-^{1}H HMBC) were performed using standard Bruker microprograms, in order to assign unambiguously all carbon resonances. Column chromatographies were carried out with silica gel 20–45 μm.

Plant material

Twigs of *Phebalium rude* Bartl. subsp. *amblycarpum* (F. Muell.) P. G. Wilson were 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, pulverized twigs of *Phebalium rude* subsp. *amblycarpum* (350 g) were defatted with petrolum ether (1 l) and extracted with CH_2Cl_2 (2 × 1 l) in a Soxhlet apparatus. The solvent was removed under reduced pressure to give a crude extract (4.5 g), which was subjected to column chromatography on silica gel, using a CH_2Cl_2–EtOAc gradient of increasing polarity to yield 125 fractions. Further column chromatographies on silica gel 20–45 μm, performed on fractions 52 to 90, gave successively **1** (15 mg), **2** (18 mg), and phebarudol (**3**) (72 mg).

Spectroscopic data

Phebarudol (**1**), Amorphous yellowish solid, $[\alpha]_D$ +12.5° (1 g/100 ml, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (4.23), 234 (4.25), 296 (sh.) (4.63), 315 (4.88) nm; IR (KBr) ν_{max} 3458, 2978, 2935, 1699, 1633, 1606, 1577, 1265, 1143, 1116, 887, 856, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32 (3H, s, C–CH_3), 1.36 (3H, s, C–CH_3), 1.94 (1H, br. s, D_2O exch., OH), 2.77 (1H, dd, J = 17 Hz, J = 5.5 Hz, H-$4'_a$), 3.05 (1H, dd, J = 17 Hz, J = 5 Hz, H-$4'_b$), 3.81 (1H, dd, J = 5.5 Hz, J = 5 Hz, H-3′), 3.77 (3H, s, $COOCH_3$), 6.26 (1H, d, J = 16 Hz, H-2), 6.80 (1H, d, J = 8 Hz, H-8′), 7.22 (1H, d, J = 2 Hz, H-5′), 7.29 (1H, dd, J = 8 Hz, J = 2 Hz, H-7′), 7.58 (1H, d, J = 16 Hz, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.1 (C–CH_3), 24.9 (C–CH_3), 31.5 (C-4′), 51.5 (OCH_3), 69.3 (C-3′), 77.6 (C-2′), 115.0 (C-2), 117.8 (C-8′), 119.3 (C-4′a), 127.0 (C-6′), 127.7 (C-7′), 130.5 (C-5′), 144.6 (C-3), 155.0 (C-$8'_a$), 167.8 (C-1); HR-MS found: 262.1208 (calcd for $C_{15}H_{18}O_4$, 262.1205); EI-MS *m/z* 262 (M$^+$), 244, 229, 204, 191, 172, 160, 131, 115, 77.

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