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

Corinne Girarda, Michel Colombaina, Frédéric Muyardb,* Françoise Bévalota, François Tillequinb and Peter G. Watermanc

a Laboratoire de Pharmacognosie, Equipe de Chimie Thérapeutique, Faculté de Médecine et de Pharmacie, Place Saint-Jacques, 25030 Besançon Cedex, France. Fax: 33 (0)381 66 55 68. E-mail: Frederic.Muyard@univ-fcomte.fr

b Laboratoire de Pharmacognosie de l’Université René Descartes, U.M.R./C.N.R.S. N° 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l’Observatoire, 75006 Paris, France

c Centre for Phytochemistry, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia

* Author for correspondence and reprint requests

Z. Naturforsch. 57c, 39–41 (2002); received September 5/October 11, 2001

*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 Müllner, 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 CH2Cl2 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 measurement as C15H18O4. 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
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-hydroxycoumaran-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 ($\lambda_{max}$ in nm) were recorded in spectroscopic grade MeOH on a Shimadzu UV-160A spectrophotometer. IR spectra ($\nu$ 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-impulsional 2D NMR experiments ($^{13}$C–$^1$H HMOC, 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 petroleum ether (11) and extracted with CH$_2$Cl$_2$ (2 x 11) 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$_2$Cl$_2$–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, [α]$_D^0$ +12.5° (1 g/100 ml, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (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, 1143, 1116, 887, 856, 756 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz) 1.32 (3H, s, C=CH$_3$), 1.36 (3H, s, C=CH$_3$), 1.94 (1H, br, s, D$_2$O exch., OH), 2.77 (1H, dd, J = 17 Hz, J = 5.5 Hz, H-4$^\alpha$), 3.05 (1H, dd, J = 17 Hz, J = 5 Hz, H-4$^\beta$), 3.81 (1H, dd, J = 5.5 Hz, J = 5 Hz, H-5$^\alpha$), 2.72 (1H, d, J = 2 Hz, H-5$^\beta$), 7.29 (1H, dd, J = 8 Hz, J = 2 Hz, H-7$^\beta$), 7.58 (1H, d, J = 16 Hz, H-3$^\beta$); $^{13}$C NMR (CDCl$_3$, 75 MHz) δ 22.1 (C=CH$_3$), 24.9 (C=CH$_3$), 31.5 (C-4$^\alpha$), 51.5 (OCH$_3$), 69.3 (C-3$^\beta$), 77.6 (C-2$^\alpha$), 115.0 (C-2$^\beta$), 117.8 (C-8$^\alpha$), 119.3 (C-4$^\alpha$), 127.0 (C-6$^\beta$), 127.7 (C-7$^\beta$), 130.5 (C-5$^\beta$), 144.6 (C-3$^\beta$), 155.0 (C-8$^\beta$), 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.


