

3,4,8-TRIMETHOXY-2-QUINOLONE: A NEW ALKALOID  
FROM *ERIOSTEMON GARDNERI*

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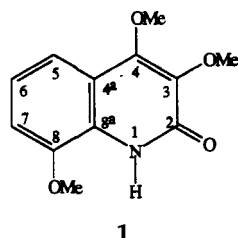
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ABSTRACT.—A new alkaloid, 3,4,8-trimethoxy-2-quinolone [**1**], and six known compounds including two pyranocoumarins, avicennol and *cis*-avicennol, two dihydrocinnamic acid derivatives, eriostemoic acid and eriostoic acid, and two flavonoid glycosides, hesperidin and diosmin, have been isolated from the aerial parts of *Eriostemon gardneri*.

*Eriostemon gardneri* P.G. Wilson (Rutaceae), an undershrub found near the south coast from Cheyne Bay east to beyond Esperance, and north towards Lake King in Western Australia, is one of eighteen species placed in *Eriostemon* sect. *Nigrostipulae* (1–3). As part of our phytochemical survey on the genus *Eriostemon* (4–6), we wish to report on the major secondary metabolites found in a sample of the aerial parts of *Eriostemon gardneri*.

Seven compounds were isolated and characterized as 3,4,8-trimethoxy-2-quinolone [**1**], avicennol (7,8), *cis*-avicennol (7,9), eriostemoic acid (10), eriostoic acid (10), hesperidin (11,12) and diosmin (11,12). All except **1** were identified by direct comparison of physical and spectroscopic properties with those of authentic samples.

The new alkaloid **1**, C<sub>12</sub>H<sub>13</sub>NO<sub>4</sub>, was observed on tlc as a quenching spot under uv light (254 nm) and visualized as an orange colored spot by spraying with Dragendorff's reagent. The uv absorption revealed maxima at 316, 282, 274, and 246 nm, suggestive of a 2-quinolinone system and the ir absorptions at 1660–1620 cm<sup>-1</sup> were characteristic for the carbonyl group in the 2-quinolinone system without *N*-methylation. The <sup>1</sup>H-nmr spectrum showed an ABC system assigned for the protons at C-5, C-6, and C-7, and a deshielded broad one-proton singlet for the presence of N-H.



Unambiguous assignment of all the protons and carbons was achieved by an HMBC nmr experiment. The H-5 proton showed <sup>3</sup>J couplings to the oxygen-bearing quaternary carbon at C-4, a quaternary carbon at C-8a, and the methine C-7. The methoxyl protons (δ 4.23) also showed a correlation with C-4. In addition, H-6 showed <sup>2</sup>J couplings with two methine carbons at C-7 and C-5 and <sup>3</sup>J coupling with quaternary carbons at C-8 and C-4a. Likewise, H-7 coupled (<sup>2</sup>J) to the methine carbon at C-6 and showed <sup>3</sup>J couplings with C-8a and C-5. The 8-methoxyl (δ 3.96) coupled to the oxygen-bearing quaternary carbon C-8. The remaining methoxyl (δ 3.97) showed a strong correlation to another oxygen-bearing quaternary carbon which must be at C-3. In an nOe difference experiment, irradiation of the C-8 methoxy (δ 3.96) resulted in the enhancement of the signal for H-7. On this basis, the structure of the compound was assigned as **1**. This alkaloid differs radically from other known trimethoxy-2-quinolones due to the un-

usual occurrence of oxygenation at C-3, a position that is normally prenylated in alkaloids of this type found in the Rutaceae (13).

The presence of avicennol-type angular pyranocoumarins supports the placement of this species in *Eriostemon* sect. *Nigrostipulae* (1). In contrast, 2-quinolone alkaloids have only been reported from *E. australias* (14).

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Ir and uv spectra were recorded on Mattson Genesis Series Ft-ir and Perkin-Elmer 552 spectrophotometers, respectively. The ms was recorded on an AEI MS 902 spectrometer.  $^1\text{H}$ -,  $^{13}\text{C}$ -, HMBC, and nOe difference nmr spectra were recorded on a Bruker AMX-400 instrument. Chemical shifts were reported in ppm relative to solvent ( $\text{CDCl}_3/\text{DMSO}$ ). The following types of Si gel were used: Si gel (Merck 7734.1000) for cc and Si gel 60-PF<sub>254</sub> for tlc.

**PLANT MATERIAL.**—Aerial parts of *Eriostemon gardneri* P.G. Wilson were collected from near Pingrup, about 350 km southeast of Perth, Australia. A voucher specimen (PERTH 01155628) representing this collection is housed in the Western Australian Herbarium.

**EXTRACTION AND ISOLATION.**—Powdered aerial parts of *E. gardneri* (25 g) were extracted in a Soxhlet with, successively, petroleum ether (40–60°),  $\text{CHCl}_3$ , and MeOH. The concentrated petroleum ether extract (0.7 g) was subjected to cc, and the fractions eluted with hexane-EtOAc (9:1 to 4:1) were subjected to prep. tlc (hexane-EtOAc, 4:1) to obtain two bands, which were then further purified by prep. tlc ( $\text{CHCl}_3$ -EtOAc, 4:1) to give avicennol (1.4 mg) from the top band and *cis*-avicennol (1.5 mg), eriostemoic acid (2.6 mg), eriostoic acid (2.0 mg), and compound **1** (1.8 mg) from the lower band.

On standing, the MeOH extract gave a yellowish-white precipitate which was filtered out and washed with absolute EtOH to obtain a 1:2 mixture of hesperidin and diosmin.

**3,4,8-Trimethoxy-2-quinolone [1] (1.8 mg).**—Amorphous; uv  $\lambda$  max (log  $\epsilon$ ) (EtOH) 316 (3.70), 282 (3.78), 274 (3.86), and 246 (3.90) nm; ir  $\nu$  max 3250, 3010, 1622  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr  $\delta$  9.03 (1H, br s, H-1), 7.45 (1H, dd,  $J=8.0$  and 1.0 Hz, H-5), 7.14 (1H, dd,  $J=8.0$  and 8.0 Hz, H-6), 6.93 (1H, dd,  $J=8.0$  and 1.0 Hz, H-7), 3.97 (3H, s, OMe-3), 4.23 (3H, s, OMe-4), and 3.96 (3H, s, OMe-8);  $^{13}\text{C}$  nmr  $\delta$  160.2 (C-2), 136.0 (C-3), 154.2 (C-4), 118.8 (C-4a), 115.2 (C-5), 122.3 (C-6), 109.4 (C-

7), 145.5 (C-8), 125.1 (C-8a), 61.1 (OMe-3)\*, 60.8 (OMe-4)\*, and 56.3 (OMe-8) (\*assignments are interchangeable); eims  $m/z$   $[\text{M}]^+$  235.0840 (calcd 235.0845 for  $\text{C}_{12}\text{H}_{13}\text{NO}_4$ ), 234  $[\text{M}-1]^+$  (15), 220  $[\text{M}-15]^+$  (100), 205  $[\text{M}-30]^+$  (9), 204  $[\text{M}-31]^+$  (10), 192  $[\text{M}-43]^+$  (13), 191  $[\text{M}-44]^+$  (7), 189  $[\text{M}-46]^+$  (10), 177  $[\text{M}-58]^+$  (7), 175  $[\text{M}-60]^+$  (10), 149  $[\text{M}-86]^+$  (25), 121  $[\text{M}-114]^+$  (8), and 75  $[\text{M}-160]^+$  (20).

**Avicennol (1.4 mg).**—Yellow plates from *n*-hexane-EtOAc (19:1); mp, uv, ir,  $^1\text{H}$ -nmr, and eims data in agreement with literature (8);  $^{13}\text{C}$ -nmr data in agreement with literature (7).

**cis-Avicennol (1.5 mg).**—Amorphous; mp, uv, ir,  $^1\text{H}$ -nmr, and eims data in agreement with literature (9);  $^{13}\text{C}$ -nmr data in agreement with literature (7).

**Eriostemoic acid (2.6 mg).**—Colorless gum; uv, ir,  $^1\text{H}$ -nmr, and eims data in agreement with literature (10).

**Eriostoic acid (2.0 mg).**—Needles from *n*-hexane/EtOAc; Uv, ir,  $^1\text{H}$ -nmr, and eims data in agreement with literature (10).

**Mixture of hesperidin and diosmin (25 mg).**—Amorphous; uv, ir,  $^1\text{H}$ -nmr, and  $^{13}\text{C}$ -nmr data in agreement with literature (11,12).

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## LITERATURE CITED

1. P.G. Wilson, *Nuytsia*, **1**, 1 (1970).
2. P.G. Wilson, *Nuytsia*, **1**, 197 (1971).
3. P.G. Wilson, *Nuytsia*, **4**, 47 (1982).
4. S.D. Sarker, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Biochem. Syst. Ecol.*, **22**, 641 (1994).
5. M.A. Rashid, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Phytochemistry*, **30**, 4033 (1991).
6. S.D. Sarker, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Biochem. Syst. Ecol.*, **22**, 863 (1994).
7. S.D. Sarker, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *J. Nat. Prod.*, **57**, 324 (1994).
8. A.I. Gray, R.D. Waigh, and P.G. Waterman, *J. Chem. Soc., Perkin Trans. I*, 488 (1975).
9. A.I. Gray, R.D. Waigh, and P.G. Waterman, *Phytochemistry*, **16**, 1017 (1977).
10. A.M. Duffield and P.R. Jefferies, *Aust. J. Chem.*, **16**, 123 (1963).

11. M.A. Rashid, "Phytochemical and Chemosystematic Studies in Eriostemoninae (Rutaceae)," Ph.D. Thesis, University of Strathclyde, Glasgow, UK, 1991.
12. H. Wagner, V.M. Chari, and J. Sonnenbichler, *Tetrahedron Lett.*, 1799 (1976).
13. I. Mester, in: "Chemistry and Chemotaxonomy of the Rutales." Ed. by P.G. Waterman and M.F. Grondon, Academic Press, London, 1983, pp. 31-96.
14. E.V.L. Da Cunha, J.A. Armstrong, A.I. Gray, D.C.R. Hockless, P.G. Waterman, and A.H. White, *Aust. J. Chem.*, **46**, 1507 (1993).

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