Two New Phloroglucinol Derivatives with Phosphodiesterase Inhibitory Activity from the Leaves of *Eucalyptus robusta*

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Two simple, new phloroglucinol derivatives have been isolated from the leaves of *Eucalyptus robusta* Smith, used in the Chinese traditional medicine “Da Ye An.” These phenolic compounds were purified using a phosphodiesterase inhibition bioassay to guide the isolation. Both compounds had moderate activity in this bioassay.

The leaves of *Eucalyptus robusta* Smith (Myrtaceae) are used in China to prepare the anti-malarial traditional medicine “Da Ye An” [1]. Previous work on the extract of the leaves has yielded the bisphloroglucinol robustol [2] as well as the robustolides A and B [3]. The known phenols eguglobal Ia, and Ia2, originally reported from *E. globulus* [4], were also isolated [3]. In a broad screening for biological activity, the crude ethanol extract of the leaves showed phosphodiesterase inhibitory activity. Using this bioassay to guide the isolation, two simple, though to the best of our knowledge previously unreported, phloroglucinols, 1 and 2, were isolated from the crude ethanol extract.

The high resolution mass spectrum of 1 revealed the molecular formula C_{14}H_{20}O_{4} (m/z 238.1204, M+, caled for C_{14}H_{20}O_{4} 238.1205). A hexasubstituted aromatic compound with the 1,3,5-trioxygenated ring pattern was immediately apparent from the $^{13}$C NMR spectrum (Table I). The $^1$H NMR spectrum readily accounted for five of the six substituents: one methoxyl group at $\delta$ 3.68, two aromatic methyl singlets at $\delta$ 2.09 and 2.10, and two phenolic hydroxyl groups, $\delta$ 3.42 and 13.22, which exchanged with deuterium upon addition of D$_2$O. The $^{13}$C NMR spectrum indicated that the only oxygenated sp$^3$ carbon was the methoxyl methyl carbon. The shift of the low field phenolic OH group was independent of concentration clearly indicating intramolecular hydrogen bonding. The remaining substituent was determined to be an acyl group by the low field signal at 210.6 in the $^{13}$C NMR spectrum of a ketone homologue of 1. The sole difference was in the alkyl substituent; the methoxyl group was replaced by an acetyl group. Inspection of the ‘H and $^{13}$C NMR data (Table I) indicated that 2 was an homologue of 1. The sole difference was in the aliphatic chain of the ketone which contained an additional carbon. An APT experiment indicated the four carbons of the chain to be two methyls, a methylene and a methine. The COSY spectrum of 2 easily mapped out the spin system of a 2-methylbutanoyl group as the acyl substituent. The substitution pattern of the aromatic ring was established by nOe studies analogous to those for 1, and selective INEPT experiments enabled assignment of all aromatic carbons. The absolute stereochemistry of the stereocenter at C-8 was not defined.

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Simple phloroglucinol derivatives are frequently encountered substituents of *Eucalyptus* species [6–8]. The frequent co-occurrence of the isobutanoyl group with higher homologues as the acyl group in phloroglucinol derivatives has been previously noted [9–11]. In the PDE bioassay, the ED$_{50}$ of 1 was 100 µg/ml, while that of 2 was 125 µg/ml. In addition to 1 and 2, the well known *Eucalyptus* flavonoid eucalyptin [12, 13] and the 7-O-D-glucoside of 5,7-dihydroxy-2-methylchromone [14–16], which has been found in several species, were also isolated.

**Experimental**

**General procedures**

Plant collection and ethanol extraction are previously described [3]. A voucher specimen is on deposit in the Chemistry Department at Boston University. The NMR spectra were recorded on a Varian XL-400 (93.93 kG, 400 MHz for $^1$H, 100 MHz for $^{13}$C) in CDCl$_3$. Residual CHCl$_3$ ($< 5$ 7.24 ppm) and $^{13}$CDCl$_3$ (J 77.0 ppm) were used as internal references for $^1$H and $^{13}$C, respectively. Assignment of “OH” protons were confirmed by D$_2$O exchange. Mass spectra (medium and high resolution) were recorded on a Finnigan MAT-90 in the EI mode (70 eV); IR spectra were recorded on a Perkin-Elmer 1800 FTIR spectrometer.

**Isolation of 1 and 2**

The crude ethanol extract was partitioned between equal volumes (11) of water and ethyl acetate, and the ethyl acetate fraction (700 mg) subsequently partitioned between equal volumes (250 ml) of petroleum ether (30–60 °C) and 5% aqueous methanol. The water fraction was likewise partitioned between equal volumes of n-butanol and water. The phosphodiesterase inhibition activity [17] was located in the aqueous methanol fraction (0.5 g) which was further fractionated by flash chromatography on silica gel using methylene chloride as eluant. Phenols 1 and 2 were purified from the active fraction by reverse phase HPLC (Microsorb-C$_{18}$ Rainin, 5 µm, 4.6 X 250 mm; flow rate 1.4 ml/min; UV detection, 254 nm) using 20% aqueous methanol as eluant.

**Phenol 1**

Colorless film: UV $\lambda_{max}$ (MeOH) 206 (ε 28000), 280 (32000), 312 (11000); IR (CCl$_4$) cm$^{-1}$ 3600, 2950, 1650, 1630, 1590, 1550, 1461, 1410, 1350, 1320, 1150, 1110, 1000; HRMS 238.1205 (calcd for C$_{14}$H$_{10}$O$_4$ 238.1205); EIMS $m/z$ (%) 238 (M$^+$, 18), 195 (100), 180 (5), 152 (7); $^1$H and $^{13}$C NMR (CDCl$_3$) see Table.

**Phenol 2**

Colorless film: UV $\lambda_{max}$ (MeOH) 208 (ε 21000), 280 (28000), 312 (19000); IR (CCl$_4$) cm$^{-1}$ 3600, 2950, 1650, 1630, 1590, 1550, 1460, 1410, 1350, 1320, 1150, 1110, 1000; HRMS 252.1362 (calcd for C$_{14}$H$_{10}$O$_4$ 252.1362); EIMS $m/z$ (%) 252 (M$^+$, 17), 195 (100), 180 (3), 152 (5); $^1$H and $^{13}$C NMR (CDCl$_3$) see Table.

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[17] The phosphodiesterase bioassay was performed at Schering-Plough Corp., Bloomfield NJ. Details of the procedure were not divulged.