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STRUCTURE OF GRANDINOL: A NOVEL ROOT INHIBITOR FROM *EUCALYPTUS GRANDIS*

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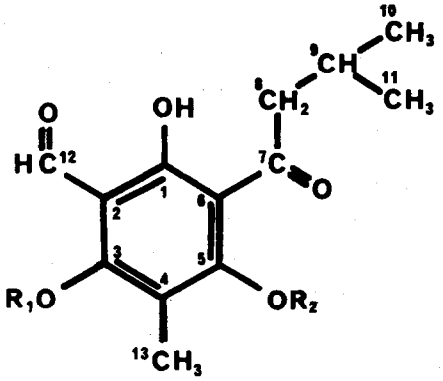
The existence of rooting inhibitors in adult tissues of *Eucalyptus grandis*, shown in physiological studies[1], was confirmed by the isolation of the three root inhibitors, G1, G2 and G3. Their structures were determined by X-ray crystallographic analysis[2] and G1, G2 and G3 were synthesized[3]. Recently, we succeeded in detecting a new root inhibitor from adult leaves of *E. grandis* and in this paper we wish to report the isolation and structural elucidation of the active principle, grandinol (*1*).



(*1*) $R_1 = R_2 = H$
(*2*) $R_1 = CH_3$, $R_2 = H$
(*3*) $R_1 = H$, $R_2 = CH_3$

Fractionation of the methanol extract was guided by a combination of cress seed germination test and mung bean rooting test. An ethyl acetate-water partition followed by column chromatography on silica gel (*n*-hexane-ethyl acetate) gave a new active fraction. Preparative TLC on silica gel of this fraction afforded grandinol (*1*, 0.0015%) : m.p. 130-132°C; λ_{max}(EtOH) 278nm (ε=27700), 345nm (ε=3800); ν_{max}^{nujol} 3340, 1650, 1630 and 1600 cm^{-1}. The high resolution mass spectrum showed M^+=252.0997 ($C_{13}H_{16}O_5$), with the expected fragmentation pattern of the isobutyryl sidechain (M-CH_3, M-C_3H_6, M-C_3H_7, M-C_4H_9), with the base peak (M-C_4H_9) showing subsequent loss of CO. ^{1}H-n.m.r. ($CDCl_3$) : δ2.05 (*s*, 13-CH_3); 1.00 (*d*, J=7, 10/11-CH_3); 2.24 (*m*, J=7 and 8, 9-CH); 3.00 (*d*, J=8, 8-CH_2); 10.2 (*s*, 12-CH).

Treatment of *1* with diazomethane gave two monomethyl ethers [*2* : $C_{14}H_{18}O_5$; m.p. 89-89.5°C; λ_{max}(EtOH) 278nm (ε=39900) and 346nm (ε=4800), and *3*: $C_{14}H_{18}O_5$; m.p. 78-78.5°C; λ_{max}(EtOH) 270nm (ε=39900)]. In the n.m.r. spectra of *2* and *3* ($CDCl_3$), the methoxyl signals appear at δ3.80 and δ3.79 respectively. The mass spectra of *2* and *3* show the same molecular ion peak (m/e 266) and the main peaks were observed at similar *m/e* values. The ^{13}C-n.m.r. spectrum of *2*($CDCl_3$) indicated fourteen carbons: δ8.0 (*q*,13-C); 22.8 (*q*, 10-C and 11-C); 24.9 (*d*, 9-C); 53.1 (*t*, 8-C); 62.9 (*q*, 14-C); 106.4, 107.2 and 111.1 (*s*, 2-C, 4-C and 6-C, not assigned); 166.3, 167.3 and 172.8 (*s*, 1-C, 3-C and 5-C, not assigned); 192.6 (*d*, 12-C); 207.0 (*s*, 7-C).

The structure of *2* was determined by direct single-crystal X-ray analysis (no satisfactory crystal of *1* could be obtained). Recrystallization from *n*-hexane-ethyl acetate gave well formed crystals: orthorhombic, space group *Pbcn*, a=20.609, b=8.957, c=14.697Å, with a unit cell containing eight molecules. Intensity data were collected on a Picker automatic diffractometer (Mo-Kα radiation); a total of 1685 independent reflections with non-zero intensities [$I \geq 3\delta(I)$] was obtained. Corrections were made in the usual way for Lorentz and polarization factors, but not for absorption. The structure was solved by application of the program MULTAN[4] and refined by a block-diagonal least squares method. From bond lengths and temperature factors, five of nineteen atoms appeared to be oxygen. After refinement using anisotropic temperature factors for carbon and oxygen, all the hydrogen atoms were located on a difference map; the R factor for 'observed reflections' is 0.059 at the present stage.

Grandinol (*1*) is presumed to be biogenetically derived from phloroglucinol, as are G1, G2 and G3, and shows inhibitory effect on cress seeds at 10^{-5} M and on mung bean cuttings at 10^{-4} M concentration. Further studies on the synthesis and biological activity of *1* and other derivatives are in progress, and we hope to report on this point later.

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