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ROOT INHIBITORS IN EUCALYPTUS GRANDIS: NATURALLY OCCURRING DERIVATIVES OF THE 2,3-DIOXABICYCLO[4.4.0]DECANE SYSTEM

W. D. Crow*, W. Nicholls+ and M. Sterns*

*Chemistry Department /+Botany Department, Australian National University, Canberra, A.C.T. 2600

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Paton, Willing, Nicholls and Pryor[1] have shown in physiological studies that adult tissue of E. grandis contains compounds which inhibit root formation in cuttings, and suitable bioassays have been developed. The present communication is concerned with the structures of three inhibitors, G1, G2 and G3 (Table I) which have been isolated from mature leaves of E. grandis.

	Formula, m.p.	$\lambda_{max.}^{EtOH}$ (nm.)	$[\alpha]_D^{20}$	ν_{OH}	ν_{CO}	ν_{CO} —C=C		(cm^{-1})
G1	$C_{15}H_{22}O_5$ 98.5	241 (7440)	±0°	3380	1715	1690	1635	mull
G2	$C_{15}H_{22}O_5$ 127-8	242 (7200)	±0°	3360	1717	1682	1630	mull
G3	$C_{14}H_{20}O_5$ 169-70 d.	240 (7300)	±0°	3500	1718	1690	1640	mull

Table I: Physical Data for Inhibitors G1, G2 and G3.

Inhibitor G1 crystallised from cyclohexane in the monoclinic system, with 4 molecules per unit cell of dimensions a=11.767, b=12.063, c=10.906 Å, β=103.2°, the space group being $P2_1/c$. Intensities of 2411 reflections (1829 statistically significant) were measured on a Picker automatic single crystal diffractometer (CuK_α radiation), and the structure was solved[2] by iterative application of Sayre's equation[3], using a modification of Long's programme[4]. Atomic parameters were refined by full matrix least-squares calculations to a reliability index of R=0.046 for the "observed" reflections. Hydrogen atoms, located from difference syntheses, were included in the final structure calculation, but not in the least squares refinement. A view of the molecular skeleton is shown in Chart I.

The structure of G1 was now used to assign structures to G2 and G3 from the appropriate spectroscopic data. We had noted during isolation that both G1 and G2, but not G3, isomerised on silica gel TLC plates. Their identical mass spectra, and the existence of the hemiacetal link, suggested that they were related as the diastereomers I and II, and should be interconvertible

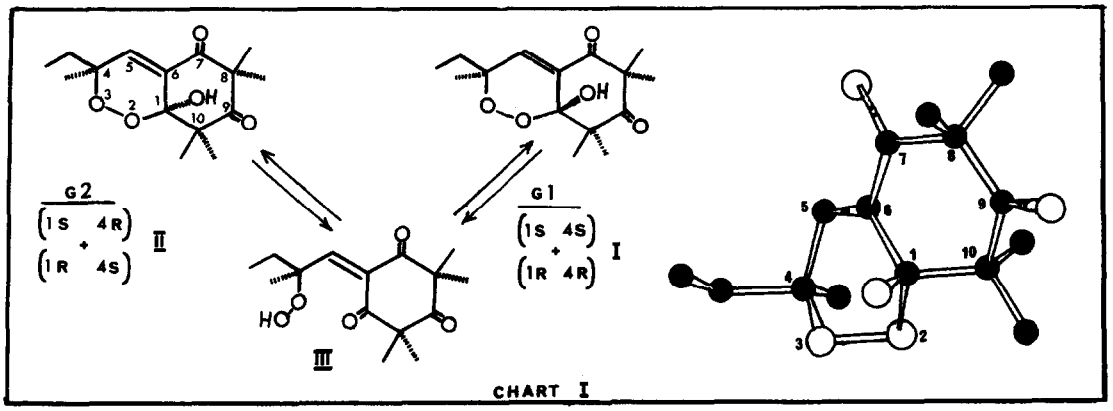


CHART I

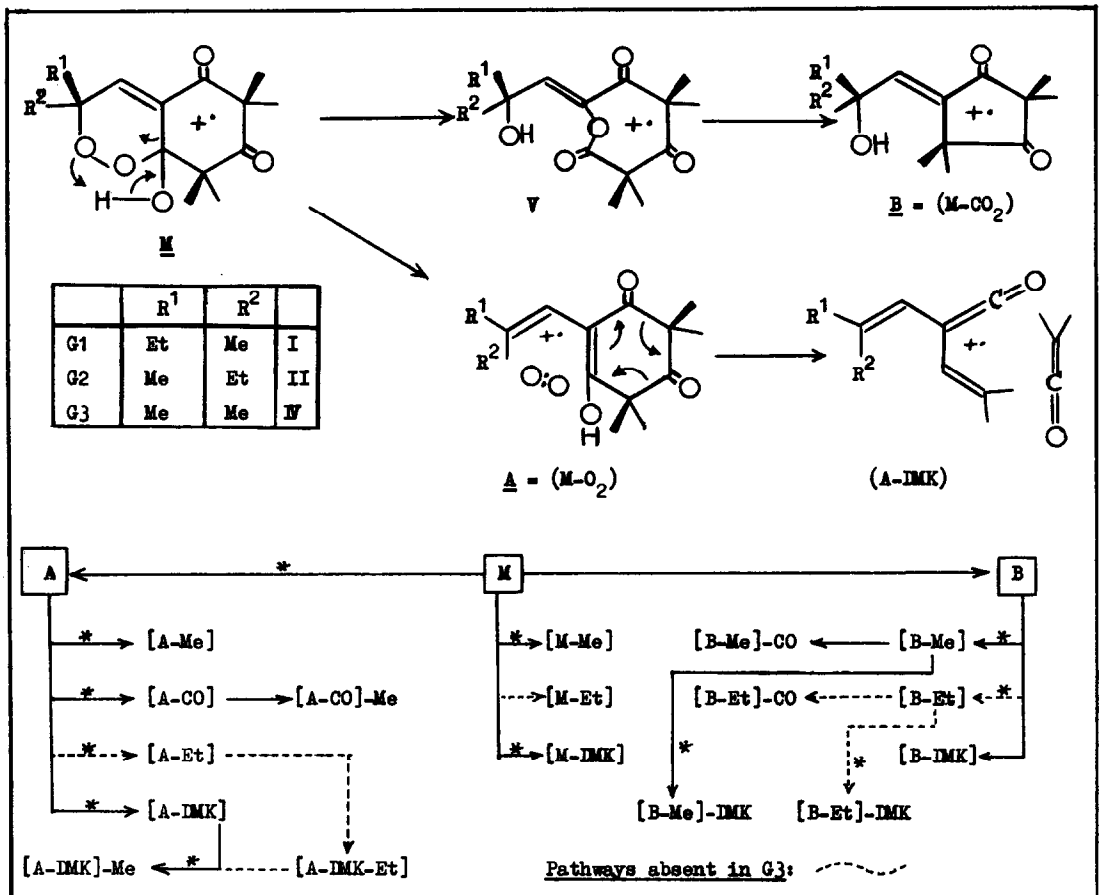
through the hydroperoxide III. Examination of the PMR spectrum in 70% CD_3OD in D_2O at pH 8-11 readily demonstrated that this was so. A 1:1 equilibrium mixture was attained over some 48 hr. at 30°, no signals other than those of G1 and G2 being observed. Rapid decomposition occurred at higher pH, as might be anticipated.

The PMR spectrum of G3 indicated the absence of the 4-Et group, and consisted solely of six overlapping methyl singlets, an olefinic singlet, and a broad peak due to OH (exchanged D_2O). The structure IV was assigned on the basis of the identical mass spectral fragmentation pathways observed for all three compounds (Chart II).

Mass Spectra of G1, G2 and G3.

The high resolution mass spectra of G1 and G2 could be distinguished only by small changes in peak heights, or in the ratio of isobaric ions for a given peak. The 1,2-dioxolane system largely controls the fragmentation through ions A and B; direct fragmentation of the parent M by other routes can be detected, but makes only a minor contribution. Loss of a single O can also be detected, but the retro-Diels Alder loss of O_2 to give A is an almost exclusive process. The carbocyclic ring shows its presence by the losses of dimethylketene which occur in the cascades from most of the major ions. Greater interest is attached to the generation of the ion B ($M-CO_2$) in a major fragmentation pathway. Although this could arise by stepwise loss of O, then CO, the [M-O] peak is almost negligible, and we prefer to formulate the reaction as a type of Bayer-Villiger rearrangement to give the lactone V, followed by ring contraction. Unfortunately, no metastables were observed to substantiate either pathway. Since both A and B would arise by paths which destroy the difference between G1 and G2, the similarity in mass spectra is expected.

Inhibitor G3 showed essentially the same fragmentation pathways as did G1 and G2, with the exception that pathways involving the 4-Et group were, naturally, absent. Below m/e 130 the mass



	R^1	R^2	
G1	Et	Me	I
G2	Me	Et	II
G3	Me	Me	IV

Chart II: Mass Spectral Fragmentation Pathways in Inhibitors G1, G2 and G3.
(Empirical formulae of all ions were determined by high resolution measurement. Metastables were observed in most cases for transitions marked with an asterisk.)

spectra of all three compounds were identical. One significant difference shown by G3 was a relative enhancement of the pathway through the ion A. It is probable that the retro-Diels Alder transition state is favoured in G3 by the decreased steric pressure resulting from the replacement of ethyl by methyl.

60 MHz PMR Spectra of G1, G2 and G3.

The PMR spectra of the compounds were initially of little diagnostic value, in that most of the signals consisted of methyl singlets crowded into the range τ8.5-9.0; only in the cases of G1 and G2 was any spin splitting observed. The data are set out in Table II, with appropriate notes. With only chemical shift data to go by, assignments must be treated with some reserve, but the interconversion of G1 and G2 permits the assignment of the signals for the 4-α-methyl group, and

(with less certainty) for the 4-β-methyl. All three compounds show a methyl singlet at high field which we have tentatively assigned to the axial 10-α- methyl, on the basis of long-range shielding by the anisotropic[5] (sterically fixed) 2,3-peroxide linkage.

Table II: 60 MHz PMR Signals for G1, G2 and G3 in CCl_4 (τ)

	5-H	1-OH *	4-Et **	4-α-Me	4-β-Me, 8-Me, 10-β-Me	10-α-Me
G1	2.90	6.08	8.26 q - 9.02 t	8.54	-- 8.68, 8.70, 8.72	8.98
G2	2.88	5.98	8.30 q - 9.02 t	--	8.71, 8.73, 8.75, 8.78	9.02
G3	2.81	6.20	--- ---	8.49	8.62, 8.62, 8.62, 8.66	8.93

*Broad, exchanged D_2O. **Secondary splitting due to asymmetric environment.

Derivatives of 2,3-dioxabicyclo[2.2.2]octane are known as growth inhibitors[6], and are formed together with 2,3-dioxabicyclo[4.4.0]decane derivatives in photochemical oxidation[7] of 1-vinyl-1,2-dihydrobenzenes. The lack of optical purity at C_4 in the compounds reported here possibly indicates a similar non-enzymic step in their formation in E. grandis[8]. The possibility of ring-chain tautomerism in solution is implied by the interconversion of G1 and G2, and by the absence of optical purity at C_1. Our PMR measurements in a variety of solvents (CCl_4, C_6H_6, C_5H_5N and CD_3OD), while they show substantial solvent shifts[9], give no evidence for such tautomerism, and we conclude that the hydroperoxide III exists only as a transient intermediate. Further studies on synthesis and biological activity are in progress, and we hope to report on these points later.

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