The Structures of Podolactones A and B, Inhibitors of Expansion and Division of Plant Cells

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Summary Podolactones A and B, which strongly inhibit expansion and mitosis of plant cells, are shown to be norditerpene dilactones with structures (I) and (II).

A PARTIALLY purified extract of the bark of *Podocarpus* species (*cf. P. neriifolius*) from Northern Queensland was found to inhibit strongly cell expansion in the pea-segment system described by Adamson *et al.*[1] Further fractionation afforded two compounds, podolactones A and B, with activities equal to or greater than that of abscisic acid in the above system. We now suggest structures (I) and (II) respectively for these lactones.

Molecular formulae were established by microanalysis: podolactone A, (m.p. 291—293°, decomp.), $C_{19}H_{22}O_8$ and

podolactone B, (m.p. 272—275°, decomp.) $C_{19}H_{22}O_9$. Both compounds have u.v. absorption (ethanol) at 218 nm (ϵ 12,500) and i.r. bands (KBr) near 1715 and 1640 cm^{-1}, suggesting the presence of an $\alpha\beta$-unsaturated δ-lactone, while bands near 1775 and 3500 cm^{-1} indicate γ-lactone and hydroxy-groups.

These data are closely similar to those for inumakilactone (III) [λ_{max} 220 nm (ϵ 11,000), ν_{max} 3460, 1760, 1705, and 1640 cm^{-1}], a bisnorditerpenoid dilactone isolated[2] recently from *Podocarpus macrophyllus* D. Don. The n.m.r. data (Table) further confirm the similarity, and indicate that

Chemical shifts (δ) *and coupling constants* (*Hz*) *of proton resonances*[a]

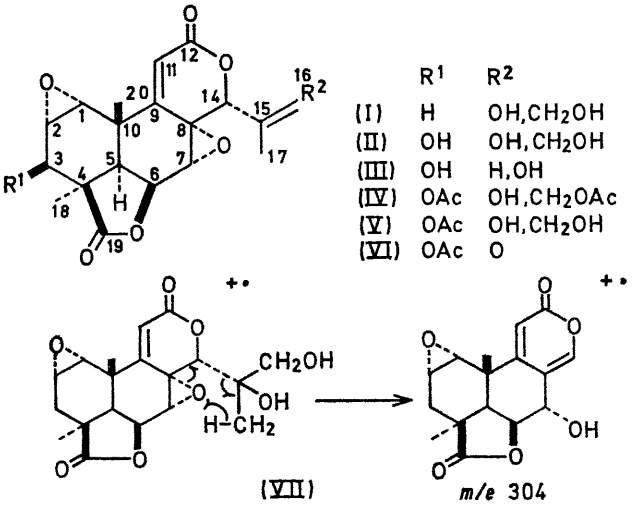
	Podolactone A	Podolcatone B	Inumakilacetone[2]
1-H	3·23(4·5)	3·59(4)	3·62(4)
2-H	3·36(4·5,1,2)	3·46(4,6)	3·51(4,6)
3α-H	2·17(2,15)	4·59(6)	4·65(6)
3β-H	1·70(1,15)	—	—
5-H	1·77(5)	2·06(5)	2·13(5·5)
6-H	5·07(5, <1)	4·99(5, <1)	5·08[b]
7-H	5·18(<1)	5·23(<1)	5·08[b]
11-H	6·11	6·69	6·73
14-H	4·97	5·05	4·72
16-H	4·25(11·5)	4·17(12)	—
	4·48(11·5)	4·45(12)	—
17-H_3	1·80	1·78	1·56(16-H_3)
18-H_3	1·45	1·53	1·53
20-H_3	1·41	1·34	1·40

[a] In [$^2H_5$]pyridine.
[b] Chemical shifts and coupling constants unclear due to overlapping. The diacetate shows $J_{5,6}$ 5·0, $J_{6,9}$ 1·5 Hz.

podolactone B and inumakilactone differ only in the nature of their side-chains. The side-chain structure of podolactone B follows from the presence of a pair of doublets (broadened by coupling with hydroxyl) centred at δ 4·17 and δ 4·45 (J 12 Hz), attributed to the methylene protons of a primary alcohol, and a three-proton methyl singlet at δ1·78. A sharp one-proton singlet at δ5·05 is assigned to the 14-hydrogen.

The structure (II) was confirmed by acetylation of podolactone B to the 3,16-diacetate (IV), selective hydrolysis to the 3-acetate (V), and oxidation of (V) with sodium metaperiodate in aqueous ethanol, which afforded a methyl ketone, m.p. 210—213° (decomp.), λ_{max} 219 nm (ϵ 10,500), ν_{max} 1778, 1740, 1725, and 1645 cm^{-1}. The properties of the latter agreed with those reported[2] for the compound (VI) obtained from inumakilactone 3-acetate by oxidation (m.p. 215—218° decomp., ν_{max} 1780, 1742, 1722 and 1642 cm^{-1}). The n.m.r. data for the methyl ketone in deuteriochloroform and deuteriopyridine were in complete agreement with the values given[2] for (VI).

Podolactone A has one oxygen less than podolactone B and on acetylation forms a monoacetate (m.p. 271—275°, ν_{max} 3500, 1780, 1730, 1715, 1640, and 1260 cm^{-1}, three-proton singlet at δ 1·93). From n.m.r. spectra podolactone A has no hydroxy-group at C-3. In the spectrum of A the C-3 protons appear as a pair of doublets (J 15 Hz) with each proton weakly coupled to 2-H. The small coupling constants $J_{2,3\alpha}$ and $J_{2,3\beta}$ obtained for A are in accord with values calculated from measurements made with Dreiding models, and using the modified Karplus constants suggested[3] for use with the protons adjacent to an epoxide group. The spectra of podolactones A and B are otherwise closely similar and podolactone A can be assigned structure (I).



The mass spectra of the podolactones do not show molecular ion peaks. The base peak in the spectrum of podolactone A at m/e 304 ($M-74$) is ascribed to the product of a McLafferty rearrangement[4] involving the 7,8-epoxide, with loss of the side-chain (VII). The spectrum of podolactone B shows a corresponding peak at m/e 320.

The podolactones strongly inhibited growth of hook and apical segments of pea stems at a concentration of $2·5 \times 10^{-5}$M. After 24 hr. growth the increase in weight of hook segments as a percentage of control [$=100 \pm 11\%$ (mean deviation)] in the presence of podolactones A and B was 25 and 29%, respectively. No such activity has been reported for inumakilactone, but nagilactone C,[5] a lactone of related structure isolated[6] from *P. elatus* R.Br. showed inhibitory activity at $1·0 \times 10^{-5}$M (increase in weight 56%). In the presence of (±)-abscisic acid (2×10^{-5}M) the increase was 53%. Podolactone A also acted as an inhibitor of cell divison in the Jerusalem artichoke system.[1,7] Freshly cut slices treated with auxin, cytokinin, and calcium chloride showed a mitotic frequency of 5·7% after 37 hr. and 7·0% after 50 hr. Addition of podolactone A ($2·5 \times 10^{-5}$M) prevented mitosis until 50 hr., when the mitotic frequency was only 2·4%.

(*Received, December 1st*, 1969; *Com.* 1825).

[1] D. Adamson, V. H. K. Low, and H. Adamson, in "Biochemistry and Physiology of Plant Growth Substances," ed. F. Wightman and G. Setterfield, Runge Press, Ottawa, 1968, p. 505.
[2] S. Ito, M. Kodama, M. Sunagawa, T. Takahashi, H. Imamura, and O. Honda, *Tetrahedron Letters*, 1968, 2065.
[3] K. Tori, T. Komeno, and T. Nakagawa, *J. Org. Chem.*, 1964, **29**, 1136.
[4] H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, 1967, p. 454.
[5] Y. Hayashi, S. Takahashi, H. Ono, and T. Sakan, *Tetrahedron Letters*, 1968, 2071.
[6] Unpublished data.
[7] D. Adamson, *Canad. J. Botany*, 1962, **40**, 719.