

The Structure of Wortmannin, a Steroidal Fungal Metabolite

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WORTMANNIN was first isolated as a neutral solid, m.p. 240°, from culture filtrates of *Penicillium wortmanni* Klocker by Brian *et al.*¹ who reported that the metabolite showed highly specific anti-fungal activity and caused a characteristic spiral waving of *Botrytis allii* hyphae. On the chemical and spectroscopic evidence summarised below, we propose structure (I) for wortmannin.

With boiling 2N-hydrochloric acid, wortmannin, C₂₃H₃₄O₈,[‡] yielded acetic acid (1.0 mol.), methoxy-acetaldehyde (0.5 mol.), and two acids, C₂₁H₂₂O₇ (0.5 mol.) m.p. 236—238°, and C₁₈H₁₆O₅ (0.5 mol.) m.p. 253—256°. Structure (II; R¹=R²=H) was established for the acid, C₁₈H₁₆O₅; for brevity, evidence is discussed only for the methyl ester (II; R¹=Me, R²=H) m.p. 219—220°. In the n.m.r. spectrum, a sharp one-proton singlet (1.84 τ in CDCl₃) was assigned to an α -hydrogen in a furan ring (ν_{C-H} 3150 and 1550 cm.⁻¹); this low-field proton was shown to be deshielded by a β -methoxycarbonyl group ($\nu_{C=O}$ 1715 cm.⁻¹) by LiAlH₄ reduction to a triol, m.p. 209—210.5° in which the low-field proton had moved upfield to τ 2.59. A fully substituted aromatic ring was indicated by the infrared spectrum ($\nu_{C=C}$ 1620 and 1580 cm.⁻¹) and by the absence of aromatic proton signals in the n.m.r. spectrum; an aryl methyl group was inferred from the presence of a three-proton singlet (τ 7.3—7.5) in the n.m.r. spectrum of the methyl ester (II; R¹=Me, R²=H) and its

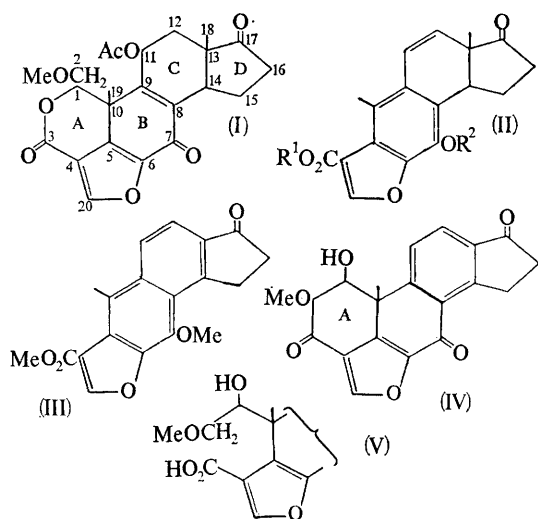
reduction products and a phenolic hydroxyl group was characterised by the formation of a methyl ether, m.p. 153—154°, and of a monoacetate (1765 cm.⁻¹) m.p. 236—237.5°. An AB-system (τ 3.26 and 3.62; J 10 c./sec.) in the n.m.r. spectrum of the methyl ester (II; R¹=Me, R²=H) was absent in the dihydro-derivative, m.p. 196—198°, and was consistent with the presence of a *cis*-olefinic double bond, with no allylic hydrogen atoms, and in a six-membered ring. The steroidal five-membered ring was diagnosed by intense peaks in the mass spectra of the methyl ether (II; R¹=R²=Me) at ($M-56$)⁺ and ($M-57$)⁺ and of its di-deuteration product at ($M-58$)⁺ and ($M-59$)⁺. The ring D structure was further supported by reduction of the saturated carbonyl group ($\nu_{C=O}$ 1730 cm.⁻¹) with NaBH₄ to an alcohol, m.p. 223—225°, whose n.m.r. spectrum in C₅D₅N showed a new one-proton triplet at τ 5.54 (J 7 c./sec.) and a tertiary methyl signal deshielded by τ 0.36 compared to the tertiary methyl signal at τ 9.15 in the original ketone (II; R¹=Me, R²=H) in the same solvent.

Orientation of these deduced structural features as in (II) was established by: (i) comparison of the u.v. spectra of the methyl ester (II; R¹=Me, R²=H) and of its dihydro-derivative which showed that the double bond was conjugated with the benzene ring; (ii) the upfield shift of the n.m.r. resonance of the aromatic methyl group by

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‡ The molecular formulae of all compounds reported were obtained by high resolution mass spectra on pure crystalline solids.

LiAlH_4 reduction of the carboxymethyl group, or by hydrogenation of the double bond, showing the close proximity of these three functions; and (iii) by the small upfield shift in the n.m.r. resonance of the tertiary methyl protons on hydrogenation of the double bond. Structure (II) for the acid, $\text{C}_{18}\text{H}_{16}\text{O}_5$, was finally confirmed by dehydrogenation of the methyl ester methyl ether (II; $\text{R}^1=\text{R}^2=\text{Me}$) with 20% palladium on charcoal to the methyl ester methyl ether (III) of the oxidation product² of the mould metabolite, viridin³ (IV).



From these results, structure (I) can readily be deduced for wortmannin, methoxyacetaldehyde and the acid (II; $\text{R}^1=\text{R}^2=\text{H}$) arising by an acid catalysed retro-aldol reaction after hydrolysis of the lactone and loss of acetic acid. Structure (I) is also supported by spectroscopic data. The i.r. spectrum shows absorption due to the five-ring ketone (1750 cm^{-1}), lactone (1732 cm^{-1}), cyclohexadienone (1684 and 1656 cm^{-1}) and furan ring (3125 and 1540 cm^{-1}). *Inter alia* the n.m.r. spectrum (τ in CDCl_3) contains: (i) four methyl singlets of aliphatic methoxyl (6.8), acetoxyl (7.9), and two tertiary methyls (8.28 and 9.02), (ii) an ABX system of the 1-, 2- and 2'-protons; (iii) an AMXY system respectively assigned to the 11-, 14-, 12- and 12'-protons; and (iv) a one-proton singlet at τ 1.78 of the 20-proton. The most intense peaks in the mass spectrum correspond to the separate or successive loss from the molecular ion of 74 (cleavage of ring A at the C(1)-C(10) and C(3)-oxygen bonds), 60 (acetic acid), 57 (ring D cleavage) and 28 [CO from C(3) or C(7)] mass units.

Wortmannin (I) is, therefore, a modified 4-methyl-steroid related to viridin (IV) and ring A of the former may be envisaged as arising from ring A of the latter by fission to (V), then lactonisation.

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