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CANADENSOLIDE - AN ANTIFUNGAL METABOLITE OF PENICILLIUM CANADENSE

N.J. McCorkindale, J.L.C. Wright, P.W. Brian, S.M. Clarke, and S.A. Hutchinson,
Joint Mycology Laboratory, Departments of Chemistry and Botany,
The University of Glasgow, Glasgow, W.2.

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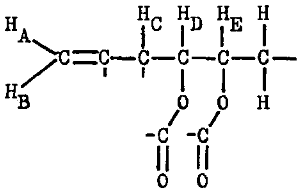
Culture filtrates of the fungus Penicillium canadense have been found to inhibit the germination of fungi, e.g. Botrytis alii. We report the isolation and structure of the metabolite responsible, canadensolide (I) and of some closely related compounds.

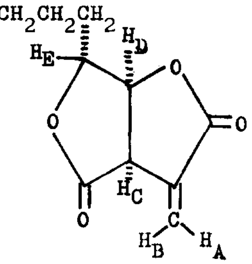
Canadensolide (I) $C_{11}H_{14}O_4$, m.p. 46-47·5°, $[\alpha]_D$ -141°, shows absorption in the IR at 1780 cm.$^{-1}$ (ε 1320), this position and intensity indicating the presence of two γ-lactone functions. A band also appears at 1667 cm.$^{-1}$ (ε 73) due to a terminal methylene group exocyclic to a five membered ring,[1] the presence of this grouping being confirmed by the formation of formaldehyde upon ozonolysis.[2]

Conjugation of the exocyclic double bond with one of the lactone carbonyl groups is indicated by the UV absorption of canadensolide [$\lambda_{max.}$ 210 mμ ε 10,000] and by the position in its NMR spectrum of its vinyl resonances which appear as fine doublets at 3·54 τ, J = 3 c/s and 3·88 τ, J = 2·5 c/s. These collapse to singlets upon irradiation in the vicinity of a 1H multiplet at 6·0 τ, corresponding to an allylic proton.

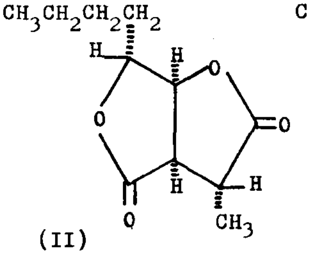
The NMR spectrum of canadensolide also shows a double doublet at 4·88 τ (H_D) and a double triplet at 5·4 τ (H_E) which were each assigned to protons geminal to an oxygen atom of the two γ-lactone functions. Spin decoupling studies established the coupling of H_C to H_D (J_{CD} = 6·5 c/s) and of H_D to H_E (J_{DE} = 4·5 c/s). H_E was shown to be also coupled to a methylene group which appeared as a 2H multiplet at 7·1 τ. Since the latter still appeared as a multiplet on irradiation in the vicinity of H_E, it was therefore in turn coupled to more than one other proton. This leads to the partial structure



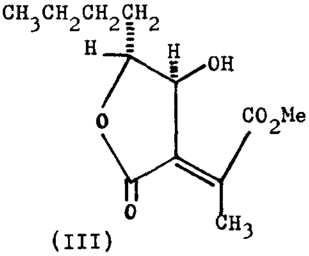
The remaining seven protons are evidently part of a <u>n</u> butyl side chain since a 3 proton triplet at 9·0 τ corresponding to a methyl group collapses to a singlet upon irradiation in the vicinity of the 4H multiplet at 8·5 τ. The structure I, apart from relative stereochemistry, follows for canadensolide. The low τ values for H_C (allylic and α to lactone carbonyl group) and H_D (geminal to lactone oxygenation) partly result from deshielding by the lactone carbonyl group to which each is β.



(I)



(II)



(III)

Models suggest that with the relative stereochemistry shown, a dihedral angle is formed between H-5 and H-6 of ca. 120°. The predicted[3] coupling constant $J_{5,6}$ is ca. 4 c/s which is close to that observed (4·5 c/s).

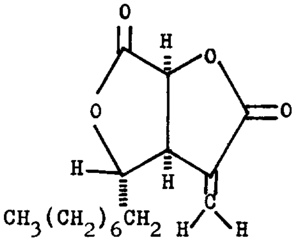
In accordance with this structure, the dilactone system opens in base to give a 1,2 diol which cleaves with sodium metaperiodate. The volatile product, <u>n</u> valeraldehyde, and its sodium borohydride reduction product, <u>n</u> pentanol, were identified by GLC. This provides chemical confirmation of the presence of the <u>n</u> butyl side chain. The latter is also indicated by the initial mass spectral breakdown pattern which appears to involve a loss of 57 mass units from the parent ion (itself of very low abundance), and of 57, 43, 29 and 15 mass units respectively from the M-44 ion.

A second metabolite, m.p. 94–94·5°, $[\alpha]_D$ -31°, produced by P. canadense, was readily recognised as a dihydro derivative of canadensolide from similarities in IR, NMR and mass spectra. The spectral features characteristic of the terminal methylene group were absent in this product and a secondary methyl group (doublet at 8·50 τ) was evident in the NMR. Spin decoupling studies as described for canadensolide, established the structure II for dihydrocanadensolide, where the dihedral angles between H-7 and H-10 (ca. 110°) and between H-5 and H-6 (ca. 120°) give predicted coupling constants $J_{7,10}$ and $J_{5,6}$ respectively, close to the values observed.

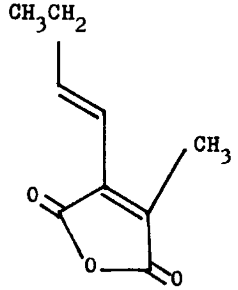
Catalytic reduction of canadensolide I gave a product which was more polar than II. The NMR spectrum showed that reduction of the terminal methylene group had been accompanied by reduction of one or both lactone rings to give a mixture of lactols. Oxidation of the mixture with Jones' reagent gave II as the major product.

Canadensolide reacts at room temperature with 3% sulphuric acid in methanol to give several products. Preparative TLC afforded one of these, a hydroxy ester III, $C_{12}H_{18}O_5$, m.p. 65–66°, which was identical with a third product isolated from broth extracts. The conjugation of lactone, double bond and ester was indicated by appropriate carbonyl absorption ($\nu_{max.}^{CHCl_3}$ 1763, 1715 cm.$^{-1}$) and by a maximum at 229 mμ (ε 15,000) in the ultraviolet. Spin decoupling studies supported structure III for this compound and established the occurrence of homoallylic coupling[4] between the vinyl methyl group (3H doublet, J = 2 c/s at 7·80 τ) and H-6 (1H broad singlet at 4·93 τ). The very small coupling constant between H-5 (a broadened triplet, J = 6 c/s at 5·86 τ) and H-6 ($J_{5,6}$ = 1 c/s) indicates that these protons have a trans relationship like the corresponding protons in canadensolide. From the relationship of this ester to canadensolide the stereochemistry about the double bond is assigned as in III. Very little hydrogen bonding however was apparent between the hydroxyl group ($\nu_{max.}^{Chf}$ 3590) and the ester ($\nu_{max.}^{Chf}$ 1715 cm.$^{-1}$). The double bond migration involved in the formation of III may be partly due to the fact that the isomeric hydroxy ester initially formed by methanolysis of canadensolide is a considerably more crowded molecule than III.

(IV)



(V)



Canadensolide bears a striking resemblance to another antifungal product avenaciolide (IV)[5] and may be closely related biogenetically to the C_9 precursor (V) of glauconic acid.[6] The structure and biosynthesis of canadensic acid; another metabolite related to canadensolide will be reported in a future communication.

References.

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