

A Fungal Fusicoccane with Ophiobolane-type Functionalities: Structure Determination by N.M.R. Spectroscopy aided by Biosynthetic Incorporation of [1,2-¹³C₂]Acetate

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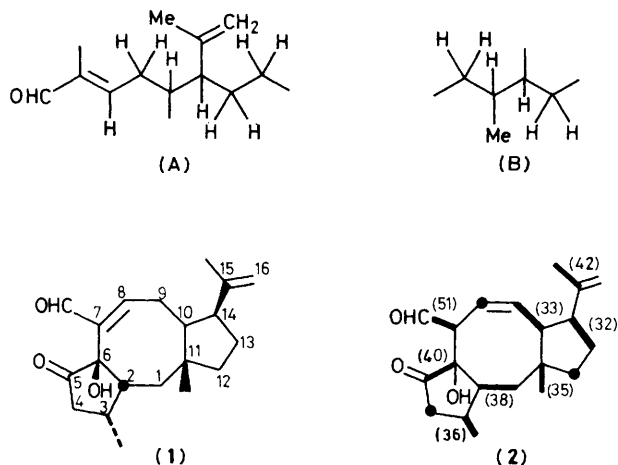
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The structure of a diterpene aldehyde isolated from a fungus provisionally identified as *Cercospora traversiana* Saccardo was elucidated as (1) mainly by extensive ¹H and ¹³C n.m.r. studies and confirmed by the labelling patterns of the substance enriched with [1,2-¹³C₂]acetate.

A fungus recently isolated as a pathogen from a standing crop of fenugreek (*Trigonella foenum-graecum* L.) was identified as *Cercospora traversiana*,¹ a species for which no chemical work has been reported previously. Extraction of the potato-dextrose media on which the fungus was grown *in vitro* furnished a colourless substance which crystallized readily from EtOAc or C₆H₆ solutions of the extracts. The compound, m.p. 234–235 °C; λ_{max} 234 nm (ε 13 000); i.r. (CHCl₃) 3530, 2855, 2720 (w), 1740 (s, CO in a five-membered ring), 1678 (s, α,β-unsaturated CHO) cm⁻¹, had the constitution C₂₀H₂₈O₃ by microanalysis and precise mass determination. The ¹H n.m.r. spectra in C₆H₆ or CD₂Cl₂ readily revealed the presence of a conjugated aldehyde group, a trisubstituted olefinic bond, an isopropenyl group, a secondary and a tertiary methyl group, and an exchangeable hydroxy proton. A series of decoupling experiments and a 300 MHz 2D COSY experiment led directly to part structures (A) and (B). The ¹³C n.m.r. spectrum confirmed and extended these findings since it contained carbonyl absorptions at δ_c 220.6 and 193.6, characteristic of a five-membered ketone and conjugated

aldehyde, respectively, with a quaternary carbinyl peak at δ_c 79.1 as well as signals for three methyl (17.0, 18.5, 22.6), five methylene (25.2, 28.5, 42.8, 43.9, 44.0), four methine (30.9, 49.8, 51.2, 54.9), and a quaternary carbon (44.4); the olefinic signals appeared at δ_c 113.8 (=CH₂), 167.3 (=CH-), 146.2, and 148.3 (=C). A heteronuclear correlation experiment served to identify the specific proton absorptions associated with each individual protonated carbon. To accommodate these data in a plausible formulation which would incorporate four intact isopentenyl units, we were led to structure (1) for this new metabolite.

To confirm this deduction, the fungus was grown on a potato-dextrose broth (1.5 l), to which [1,2-¹³C₂]acetate (0.041 M, 150 ml) was added in equal portions on days 10 and 13. After extraction of the culture filtrate on day 16, the aldehyde (13 mg) was isolated by crystallization and chromatography of the mother liquors. The ¹³C n.m.r. spectrum showed significant incorporation of the labelled acetate with enrichment of 4.4% as determined by the relative intensities of the ¹³C–¹³C satellites exhibited by the paired carbons



incorporated as intact acetate units; the labelling pattern is shown in (2) with the ^{13}C - ^{13}C coupling constants for intact acetate units in parentheses. Further confirmatory evidence was obtained from a 75 MHz ^{13}C 2D COSY experiment which, because of the enrichment level, revealed direct bondings for C-2 with C-3 and C-6, C-4 with C-3 and C-5, C-8 with C-7 and C-9, C-10 with C-11 and C-14, C-13 with C-12, and C-15 with C-14 and C-16.

Structure (1) has the carbon skeleton of the relatively rare fusicoccin-cotylenin groups of diterpenes but lacks the sugar moiety attached *via* oxygen to these molecules at C-9. Hitherto the fusicoccins have been isolated only as glycosides from *Fusicoccum amygdali* and the closely related cotylenins as glycosides from an unidentified *Cladosporium*. Both groups of compounds have received considerable attention as phytotoxins and plant growth regulators.²⁻⁴ Fusicoccins without attached sugars have been isolated recently from liverworts^{5,6} and a higher plant;⁷ these fusicoccins, however, also differ from the classical fungal compounds in oxygenation and unsaturation patterns. Aldehyde (1) differs yet again in this respect but, except for the absence of hydroxy at C-3, is identical with that of the tricyclic moiety of the sesterterpenoid

ophiobolanes.^{3,4} Another interesting feature is the labelling pattern of the isopropenyl group which differs from that for many sesquiterpenes since C-16 arises from C-2 of a mevalonate unit rather than the isopropenyl methyl carbon. It is also noteworthy that despite extensive prior chemical work on other *Cercospora* spp.⁸ none has been reported to furnish a diterpene. The most characteristic, but not invariably observed, products of these fungi are either cercosporin and related pigments or dothistromin.^{9,10} Therefore, although at present there is no other reason to doubt the identity of our fungus, it has been resubmitted for a more rigorous mycological examination.

The ^1H - ^1H coupling and nuclear Overhauser enhancement data leading to the relative stereochemistry shown in (1) will be discussed in a full paper.

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