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STRUCTURE OF SCLEROSPORIN, A SPOROGENIC SUBSTANCE OF SCLEROTINIA FRUCTICOLA

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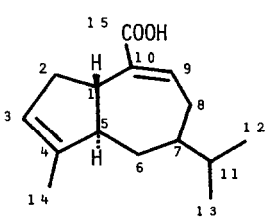
Summary: Sclerosporin, the major sporogenic substance of S. fructicola, and its related metabolite, sclerosporal, were shown to have the plain structures, 1 and 2, respectively, by using a micro-amount of the samples, and co-occurring sclerosporene probably to have the structure 3.

Sporogenesis is one of the most dramatic morphological differentiation of fungi, absolutely necessary for their life cycles. Factors influencing the sporulation have been extensively investigated with respect to morphogenesis,[2] reproduction,[3] nutritious and environmental requirements[4] etc., and also from the industrial importance that some fungal spores have been manufactured. Recently, the sporulation aroused much attention by its possible participation in the production of useful secondary metabolites.[5] One of the most important factors affected the sporulation was the effect of light and darkness,[6] by which fungi were classified into three groups, that is, the fungi requiring light for sporulation, the ones sporulatable only in darkness, and those whose sporulation occurred unrelated with light and darkness.

In our continuing research[7] on sporogenic substances, three active compounds have been isolated from Sclerotinia fructicola, which belonged to the second group of fungi above classified, producing abundant asexual arthrospores when grown in darkness, whereas poorly under illumination of light.[8] The main active compound, named sclerosporin, was capable of inducing the sporulation at 0.001 μg/ml on the mycelium grown under the light illumination.[7a] We wish now to describe the structure elucidation of sclerosporin (1), as well as its closely related metabolites, named sclerosporal (2) and sclerosporene (3), isolated from the same cultured materials. We should emphasize that the structures of these metabolites were elucidated only with a micro-amount of the samples (182 μg of 1, 194 μg of 2 and 35 μg of 3), and that sclerosporin is the first sporogenic substance clarified among the fungi which sporulated in darkness.



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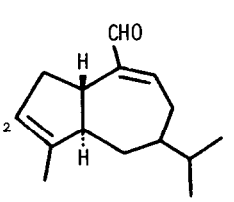

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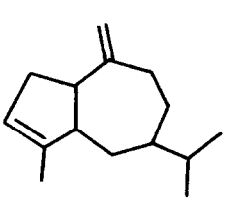

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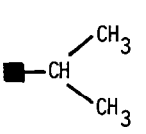
Sclerosporin, $C_{15}H_{22}O_2$ (M^+: m/e 234.1647, calcd: 234.1620); CD (MeOH): $[\theta]_{214\ nm}$ -31590, was shown from mass and ^1H nmr spectra to have three partial structures, i.e., an isopropyl group (4): δ($CDCl_3$) 0.84 and 0.92 (each 3H, doublet, J=7.1 Hz); m/e 191 ($C_{12}H_{15}O_2$, $M^+-C_3H_7$), a fragment (5): δ 2.05 (2H, triplet, J=3.9 Hz) and 7.13 (1H, triplet, J=3.9 Hz ; the δ value suggests an electron-withdrawing group (I in 5) to be attached, and the signal collapsed to a singlet upon irradiation at δ 2.05) and a fragment (6): δ 5.49 (1H, broad singlet, $W_{1/2}$=7.2 Hz), 1.69 (3H, singlet), 1.95 (2H, multiplet); the irradiation at δ 1.95 or 5.49 sharpened the protons at δ 5.49, and δ 1.69 and 1.95, respectively.



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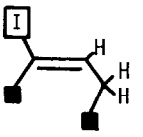

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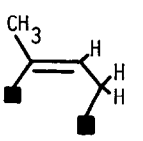

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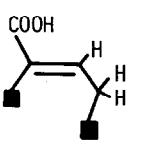

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The presence of two double bonds, suggested from spectral data, was confirmed by catalytic hydrogenation (Pd, MeOH) of sclerosporin (10 μg) to afford a tetrahydro-derivative (M^+: m/e 238), partly contaminated with the dihydro-one (M^+: 236). Two oxygens in the molecule were examined by reduction ($LiAlH_4$ in diethyl ether, 0°C) of sclerosporin (10 μg). The mass spectrum showed the highest peak at m/e 202, assignable most probably to the dehydrated ion of the carbinol product, thus, a carboxyl group was suggested. The suggestion was confirmed by methylating (diazomethane) sclerosporin (5 μg) to the methyl ester [m/e 248 (M^+), 216 (M^+-CH_3OH), 189 ($M^+-COOCH_3$) and 173 ($M^+-CH_3OH-C_3H_7$)].

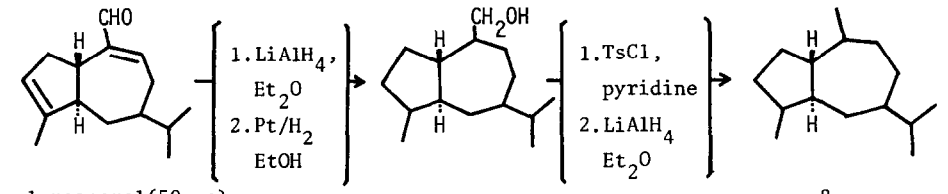
In parallel with these studies, we searched for any metabolic precursors of sclerosporin in the cultured materials, and as the results, two new metabolites, sclerosporal (2, 194 μg) and sclerosporene (3, 35 μg), were isolated. Sclerosporal: colorless oil ; $C_{15}H_{22}O$ (M^+: m/e 218.1672, calcd: 218.1671); CD (n-hexane): $[\theta]_{225\ nm}$ -48000; ^1H nmr ($CDCl_3$) δ 0.84 (3H, doublet, J=7.1 Hz), 0.93 (3H, doublet, J=7.1 Hz), 1.69 (3H, singlet), 1.2-2.4 (9H), 2.60 (1H, broad doublet, J=12.4 Hz), 5.52 (1H, broad singlet), 6.81 (1H, triplet, J=5.1 Hz), 9.40 (1H, singlet). The ^1H nmr closely resembled that of sclerosporin only with difference that the former showed an aldehydic proton (δ 9.40), and a slightly shielded (Δ 0.32 ppm) olefinic proton compared with that (δ 7.13) of the latter compound. The UV, λ_{max} (n-hexane) 228 nm (ε 9760), was characteristic of a disubstituted α,β-unsaturated aldehyde.

In order to correlate sclerosporal with sclerosporin, the former (20 μg) was converted to the methyl ester upon oxidation (Jones reagent) followed by methylation (diazomethane). The mass spectrum was identical with that of the methyl ester of sclerosporin. Also, the both compounds were reduced ($LiAlH_4$) to the carbinol derivatives, whose mass spectra were identical each other, concluding that they had the same structure, except for the functional group to be either carboxyl in 1 or aldehyde in 2, hence, the α,β-unsaturated carboxyl group (7) was shown to be present in sclerosporin.

The carbon skeleton of these metabolites was established, after several attempts, by successful transformation of sclerosporal (50 μg) into the hydrocarbon (8) through four

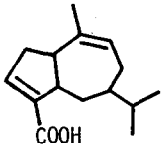
steps of reaction, as shown in Scheme I. The product (8), when analyzed by GC-MS, showed the mass spectrum essentially indistinguishable from those of authentic stereoisomers of guaiane.[9]

Scheme I.



sclerosporal(50 μg) 8

From partial structures (4, 5, 7) combined with the guaiane skeleton, two possible structures (1 and 10) were deduced for sclerosporin. The structure (1) to be correct was obtained from mass spectra of sclerosporin, its methyl ester and sclerosporal, whose characteristic peaks appeared at m/e 135 ($C_8H_7O_2$), 149 ($C_9H_9O_2$) and 119 (C_8H_7O), respectively, besides the commonly observed tropylium ion at m/e 91 (C_7H_7), were clearly ascribed to the tropyliums bearing carboxyl (11), methoxycarbonyl (12) and formyl groups (13), respectively.



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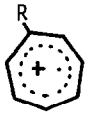

11, R=COOH

12, R=$COOCH_3$

13, R=CHO

The structure (1) was further supported by double resonance experiments on sclerosporin, which showed the C-2 methylene protons (δ 1.95) to be coupled with both C-3 vinyl (δ 5.49) and C-1 methine protons (δ 2.60), the last one was identified based on its higher field shifting by reduction of carboxyl to carbinol.

The plain structures of sclerosporin and sclerosporal were thus established. The relative configuration of the ring juncture (C-1 and C-5) was indicated to be trans from the J value (12.4 Hz),[10] however, the other relative as well as the absolute stereochemistry of sclerosporin remained still unclear.

Sclerosporene was suggested to have the structure (3), on the basis that (i) its catalytic hydrogenation product showed the mass spectrum identical with that of the hydrocarbon (8), (ii) its base peak at m/e 105, differing from that (m/e 91) of sclerosporin, coupled with a lack of the typical fragment ions (m/e 93 and 107) of the $\Delta^{1(10)}$ guaiane skeleton, indicated that the double bond must be located at the exocyclic $\Delta^{10(15)}$ position.

Our further studies on sclerosporin whether it induces similar arthrospores on other related fungi, and also on other new sporogenic substances inducing different types of asexual spores such as conidia, are now under investigation.

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References and Notes

(1) A research fellow from Noda Institute for Scientific Research, Nada-shi, Chiba-Ken 278.

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(9) The mass spectra of four stereoisomers of cis-guaianes were compared with that of the hydrocarbon product (8). All of these spectra were essentially indistinguishable, however, the minor difference only in intensity of a few weak peaks was observed in the spectra between the hydrocarbon (8) and cis-guaianes, and also in those among stereoisomers of cis-guaianes. The relative stereochemistry of sclerosporin was indicated from the $C_{1,5}$-J value (12.4 Hz) to be trans.

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