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THE STRUCTURE OF THE CYCLOHEXADEPSIPEPTIDE, SPORIDESMOLIDE III

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THE FUNGUS Pithomyces chartarum produces at least three depsipeptides, of which one, sporidesmolide III, is present only in small amount.[1] Sporidesmolide III is a neutral compound, $C_{32}H_{56}N_4O_8$, with absorptions due to ester carbonyl (5.72 μ), amide I (5.95, 6.09 μ), and amide II (6.45, 6.55 μ) in the infrared. A vigorous acid hydrolysate, prepared and examined by paper chromatography in four different solvent systems, as described for sporidesmolide I[1], contained valine (1.98 mol.) and leucine (1.97 mol.); α-hydroxy-β-methylbutyric acid (ca. 2 mol.) was similarly identified in three different solvent systems.

A recent paper[2] described the rationalization of the mass spectra of the cyclohexadepsipeptides sporidesmolide I (A) and sporidesmolide II (B) in terms of their chemical structures. We now report an example of the application of this rationale which, when combined with the hydrolysis results, allowed the formulation of a unique structure for sporidesmolide III.

The mass spectrum of sporidesmolide III (Fig. 1)* is very similar to the mass spectra of A and B[2] except for differences which can be ascribed to sporidesmolide III having one less methyl group than A and two less than B.

* The figures in brackets placed after the m/e values in Fig. 1 and also in the text are the shifts which the peaks display in the mass spectrum of N-deuteriated sporidesmolide III prepared by exchange with D_2O.

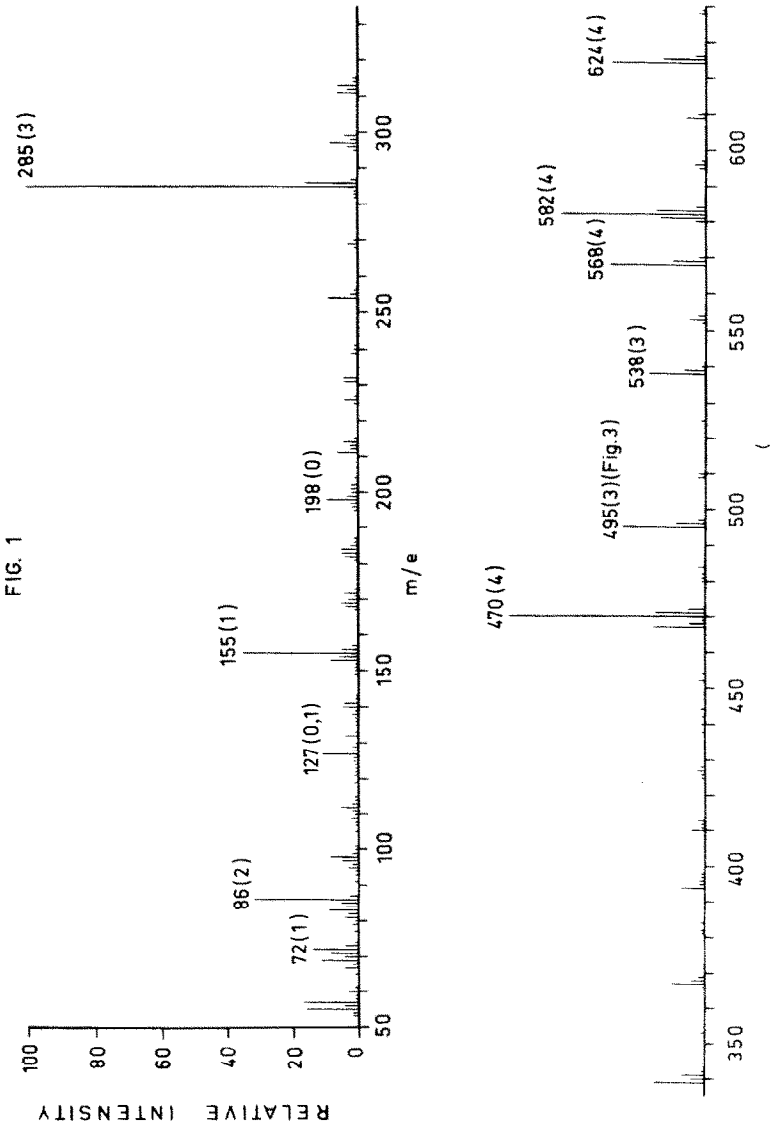
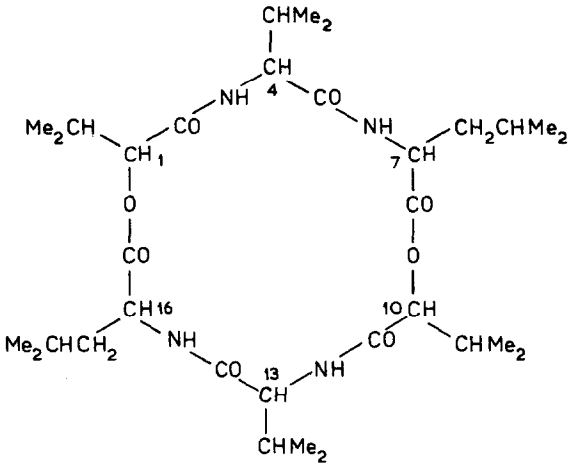

RELATIVE INTENSITY
100
80
60
40
20
0
50
100
150
200
250
300
350
400
450
500
550
600
m/e
285(3)
198(0)
155(1)
127(0,1)
86(2)
72(1)
470(4)
495(3)(Fig.3)
538(3)
568(4)
582(4)
624(4)

FIG. 1

Further, the mass spectrum of the N-deuteriated compound shows that it has four active hydrogen atoms, suggesting that it may have structure C in which the N-methyl group of A is replaced by N-H at position 15.

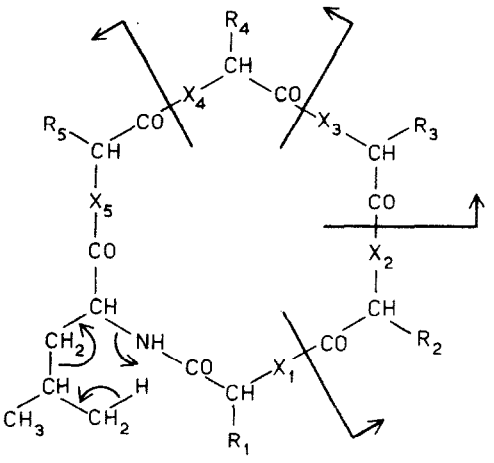


C

Ignoring isomerism due to the asymmetric centres, the hydrolysis results allow 16 hexadepsipeptide structures for sporidesmolide III, differing in the order of linkage of the residues. The ions which would be produced from these 16 structures by the reactions shown in Fig. 2, which are analogous to the reactions d, e, f, g, l, m, n, and o proposed[2] for A and B, have been compared with the spectrum of sporidesmolide III. Only in the case of structure C are all the predicted peaks (at m/e 467(2), 367(2), 254(1), and 155(0)) observed.

Peaks at m/e 582(4), 568(4), 341(2), 313(2), 285(3), and 127(0) in the spectrum of sporidesmolide III can be explained by the operation on structure C of reactions a or b, b, h or i, j or k, r or s, and p or q[2] respectively; the peaks at m/e 198(0), 86(2), and 72(1) may be due to the reactions proposed to account for the same peaks in the spectra of A and B.

FIG. 2



R_1 . . . . . R_5 are either isopropyl or isobutyl

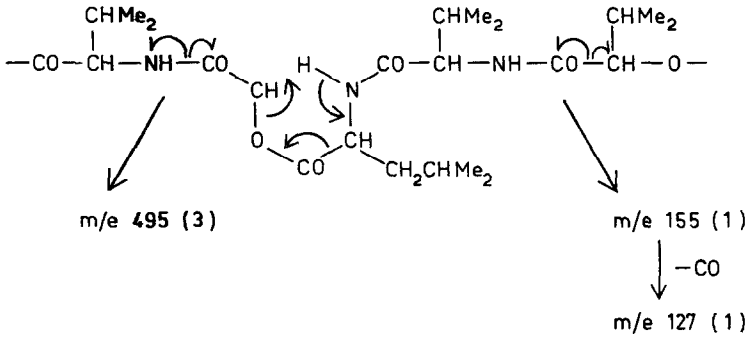
X_1 . . . . . X_5 are either O or NH

The peak at m/e 538(3) may be due to a reaction analogous to reaction c[2], but involving hydrogen transfer at a leucyl rather than at an N-methyl-leucyl residue. The ions of m/e 155(1) and 127(1) may be formed as shown in Fig. 3. The relatively small production from A and B of ions corresponding to these and to that at m/e 538(3) is presumably associated with the smaller number of N-H groups in A and B.

The peak at m/e 495(3) may be due to the reaction shown in Fig. 3, while that at m/e 470(4) may be due to the loss from the molecular ion of two isobutyl side chains by reaction b and one isopropyl side chain by reaction a or b. The small size of the peaks, corresponding to these, in the spectra of A and B is unexpected. The availability of an extra N-H for hydrogen bonding in sporidesmolide III, reflected in the marked decrease in chloroform solubility

compared with **A**, may also be responsible for conformational differences which influence the ease of formation of these ions.

FIG. 3



Structure **C** possesses six asymmetrically substituted carbon atoms, 1, 4, 7, 10, 13 and 16, and may exist in forty-eight optically active and eight meso forms. The compound (223 mg, $[\alpha]_D$ -80.0 ± 1.2° (c, 1.6 in acetic acid)), hydrolysed by acid as described for **A**[1], gave α-hydroxy-β-methylbutyric acid (52 mg) that after threefold vacuum-sublimation had $[\alpha]_D$ + 18.2 ± 2.3° (c, 8 in chloroform); it was thus the L-isomer. It was further characterized by oxidation with sodium bismuthate[3] to isobutyraldehyde, isolated as the 2,4-dinitrophenylhydrazone, m.p. 183–184°, mixed m.p. 184–185°.

The hydrolysate, after extraction of the L-α-hydroxy-β-methylbutyric acid, gave on evaporation a mixture of leucine and valine hydrochlorides (176 mg), $[\alpha]_D$ - 0.3 ± 0.7° (c, 3.5 in 6N hydrochloric acid) and, in a separate experiment, 0.0° ± 1.0° (c, 1). Sporidesmolide III accordingly contains one mol. each of L-valine, D-valine, L-leucine and D-leucine.

Only two stereoisomers of C have therefore to be considered, in which the asymmetric centres 1, 4, 7, 10, 13, 16 are respectively L,D,D,L,L,L and L,L,D,L,D,L. Both of these would give on saponification a mixture of

α-hydroxy-β-methylbutyryl-valyl-leucines, the configurations being in the one case L,D,D and L,L,L, and in the other case, L,D,L and L,L,D. Of these, the L,D,D isomer is the known sporidesmolic acid A, m.p. 200-201°, $[\alpha]_D$ + 61° (in acetic acid)[1].

Sporidesmolide III (29.6 mg) consumed 1.9 equivalents of sodium hydroxide under conditions described for A[1], to give a mixture of acids (32.9 mg, m.p. 175-195°) with paper electrophoretic behaviour at pH 2.5 and 8 identical with that of sporidesmolic acid A. Leucine and valine were present in acid hydrolysates; the former amino acid was absent after Dakin-West reaction[1,4], thus confirming the amino acid sequences depicted in C. Digested with carboxypeptidase at pH 8 the mixture yielded rapidly about 1 mol. of leucine and, more slowly, a small amount of valine, suggesting strongly the presence of the L,L,L isomer. After hydrolysis had ceased the acidified digest yielded to ether an acid mixture of which the chloroform-insoluble portion (9.3 mg) was sporidesmolic acid A, $[\alpha]_D$ + 56.5° ± 2.3° (c, 1.25 in acetic acid). The infrared spectrum (paraffin mull) was indistinguishable from that of a reference sample. Recrystallized once, the sporidesmolic acid A was obtained as characteristically diamond-shaped flat prisms, m.p. and mixed m.p. 199-201°.

The sequence of the asymmetric centres 1, 4, 7, 10, 13, 16 must accordingly be L,D,D,L,L,L, which is the same arrangement as that found in A[1]. Sporidesmolide III is therefore N-desmethyl-sporidesmolide I.

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