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ISOLATION OF A NEW DEPSIPEPTIDE FROM *PITHOMYCES CHARTARUM*

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Abstract—cyclo-L-α-Oxyisovaleryl-D-allo-isoleucyl-D-leucyl-L-α-oxyisovaleryl-L-valyl-L-N-methyl-leucinate† has been isolated from the fungal tissue of *Pithomyces chartarum* growing on potato carrot medium containing 5 g/l. DL-isoleucine.

Pithomyces chartarum[1] produces a mixture of depsipeptides whose composition differs from normal[2] when the culture medium contains DL-isoleucine.[3] The abnormal depsipeptides were recrystallized and sublimed until these processes caused no further change in physical properties. The IR spectrum of the final product and that of sporidesmolide-I[2] were indistinguishable. The mass spectrum of the former showed that its mol. wt. was 652 but it also indicated contamination with sporidesmolide-I (m/*e* 638) and a new depsipeptide of m/*e* 666. The masses of the principal ions are given in the Experimental section and the spectra are shown in Figs. 1a and 1b. Structural assignments for some of these ions have been reported recently.[4] X-ray powder diagrams (Fig. 2C) also differentiated between the new depsipeptide (m/*e* 652) and sporidesmolide-I. The ratios of the clearest spacings suggested an hexagonal unit cell, the dimensions being a = 14·05 Å, *c* = 5·24 Å for sporidesmolide-I and a = 14·18 Å, *c* = 5·24 Å for the depsipeptide of m/*e* 652. This has been assumed in calculating the theoretical spacings and assigned Miller indices. All these values are given in the Experimental Section. Suitable material for single crystal analysis was not available; the samples were microscopic needle-shaped crystallites. In the case of sporidesmolide-I the needles had grown into spherulites about 1 mm in diameter. A narrow sector of such spherulite was mounted in the powder camera in such a way that the mean direction of the spherulite radius lay at an angle of about 80° to the incident beam. This gave rise to a tilted fibre diagram (Fig. 2D) from which the choice of cell axes could be verified and several more reflexions identified and measured. The unit cell of the new depsipeptide (m/*e* 652) was about 2% larger in volume than that of sporidesmolide-I. The agreement between the measured and calculated spacings is not perfect but is probably within the limits of accuracy of the measurements except, perhaps, for the 101 spacing. Measurements of the dimensions

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† Sporidesmolide—II has been used hitherto to describe depsipeptides produced by *P. chartarum* containing isoleucine residues. It is now apparent that several such compounds are produced, therefore the use of systematic nomenclature is obligatory.

[1] M. B. Ellis *Mycol. Papers* No. 76 (1960).
[2] D. W. Russell *J. Chem. Soc.* 753 (1962).
[3] W. S. Bertaud, I. M. Morice, D. W. Russell and A. Taylor *J. Gen. Microbiol.* 32 385 (1963).
[4] C. G. Macdonald, J. S. Shannon and A. Taylor *Tetrahedron Letters* 2087 (1964).

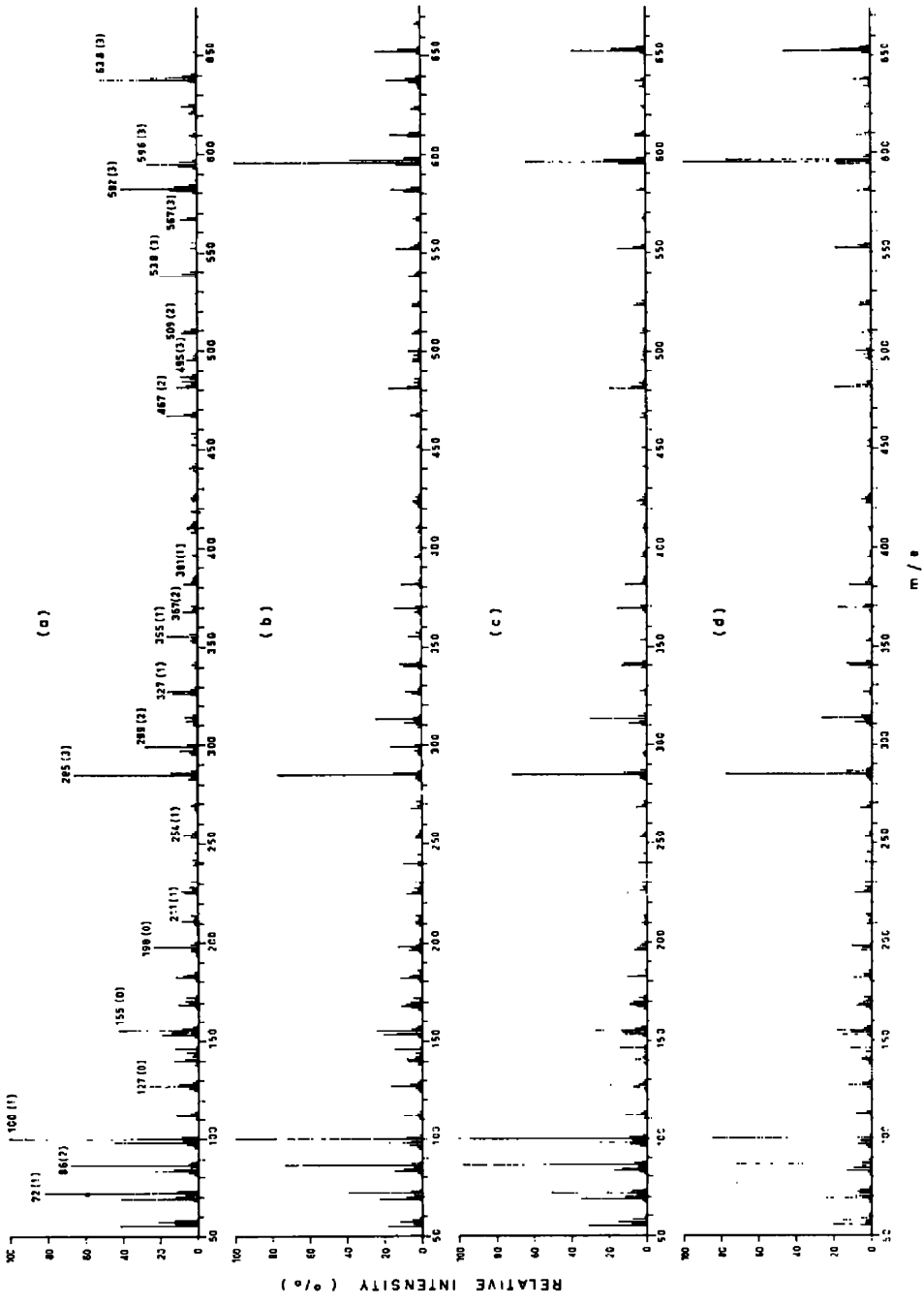
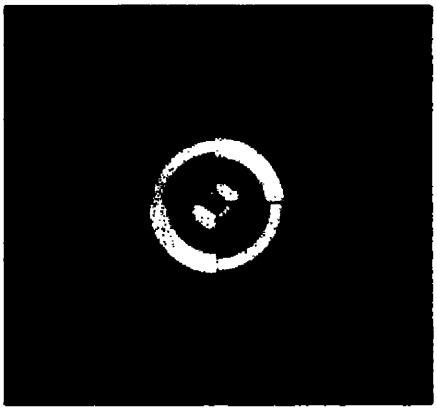
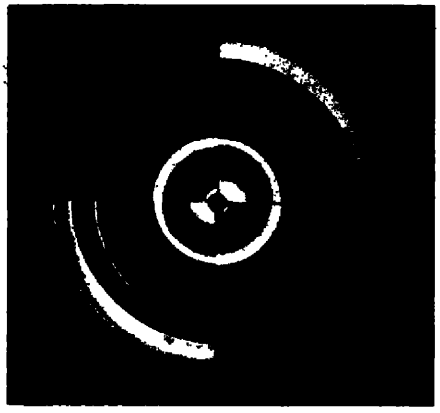


FIG. 1a Sporidesmolide—I. FIG. 1b Depsipeptide (=I) from cultures of *P. chartarum* grown on medium enriched with DL-isoleucine. FIG. 1c Synthetic depsipeptide (=I with D-isoleucine residue). FIG. 1d Synthetic depsipeptide (=I with D-allo-isoleucine residue).



A



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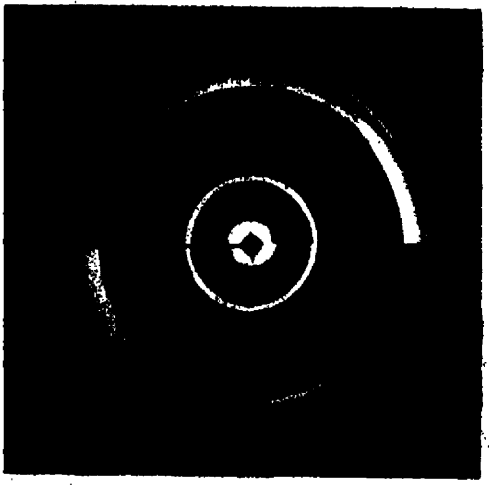

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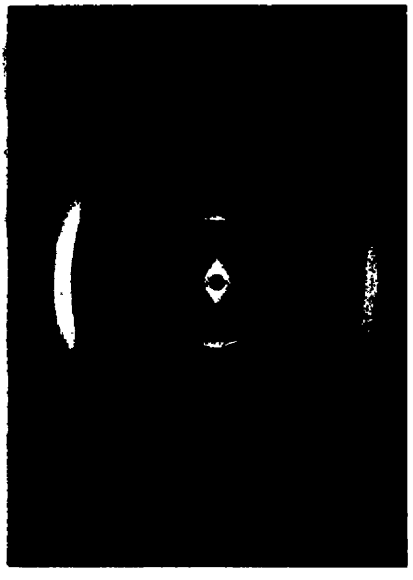

D

FIG. 2C Top right Bottom left = sporidesmolide—I
Top left Bottom right = I from cultures of *P. chartarum* grown on medium enriched with DL-isoleucine.

FIG. 2D Fibre diagram of Sporidesmolide—I

FIG. 2A Top right Bottom left = I from cultures of the mould.
Top left Bottom right = I (synthetic)

FIG. 2B Top right Bottom left = I (synthetic)
Top left Bottom right = D-isoleucine analogue of I

of a Courtauld model of sporidesmolide-I indicated that one molecule would fit into the unit cell. From the mol. wt. and unit cell dimensions the density of sporidesmolide-I was calculated to be 1·183 g cm^{-3}. This value was higher than the measured value which lay between 1·10 and 1·13 g cm^{-3}. The two discrepancies indicate that the suggested hexagonal unit cell is only an approximation.

Equimolecular proportions of D-isoleucine, L(?D)-N-methyl-leucine,[5] D-leucine and L-valine were obtained after acid hydrolysis of the depsipeptide of m/*e* 652. α-Hydroxyisovaleric acid was the only α-hydroxy acid present in the hydrolysate. Professor Shemyakin and Dr. Ovchinnikov sent us samples of cyclo-L-α-oxyisovaleryl-D-isoleucyl-D-leucyl-L-α-oxyisovaleryl-L-valyl-L-N-methyl-leucinate and its D-*allo*-isoleucine(I) analogue which they had synthesized. The mass spectra (Fig. 1c 1d) and the X-ray powder diagrams (Fig. 2A 2B) of the synthetic compounds were identical with the corresponding properties of the natural depsipeptide of m/*e* 652. The X-ray powder diagrams and the mass spectra of the two synthetic depsipeptides were indistinguishable. However our colleagues in Moscow were able to show that the natural depsipeptide of m/*e* 652 contained a D-*allo*-isoleucine residue.[6]

The problem of identity of the depsipeptide of m/*e* 652 with 'sporidesmolide-II', postulated to be present in the total sporidesmolide fraction by Russell[2] remains open. Our attempts to isolate depsipeptides other than sporidesmolide-I from this mixture have been unsuccessful. After some of the sporidesmolide-I had been removed by crystallization the residue was subjected to a 500 stage counter-current distribution in the solvent system formic acid–benzene. No resolution into fractions (on the basis of wt.) was observed after this procedure. From the tail fractions of the distribution sporidesmolide-I was obtained. The leading fractions of the distribution were combined and recrystallized to constant optical rotation. The product gave valine, leucine, isoleucine and N-methyl-leucine after hydrolysis. Its mass spectrum showed molecular ions at m/*e* 666 (10%) and at m/*e* 652 (30%) and fragment ions at m/*e* 299 (100%, cf. Fig. 1c 1d) and at m/*e* 285 (25%). The figures in brackets are the relative abundance of these ions. Hence the leading fractions from the distribution contain at least two depsipeptides. The ratio of abundance of the ions of m/*e* 652 and m/*e* 285 in the mass spectrum of the recrystallized leading fractions of the distribution is approximately 1, whilst the ratio of the same ions in the spectrum of I is 3 (Fig. 1b). Thus the depsipeptide(s) of m/*e* 652 in the leading fractions of the distribution differ from I. We conclude that the total sporidesmolide fraction is more complex mixture than has hitherto been supposed.

EXPERIMENTAL

A flat film vacuum camera and CuK_α radiation were used for the X-ray powder diagrams. The beam was collimated to 0·25 mm diam and specimen to film distances of 40 mm and 80 mm were used. Accurate calibration was obtained in each case by means of a silver diagram. Mass spectra were obtained using an Atlas CH4 instrument. IR spectra were measured on a Perkin–Elmer '137' spectrophotometer. Depsipeptides were hydrolysed and the amino acids obtained were estimated and their configuration determined as described previously.[5,7] Optical rotations were measured in $CHCl_3$ (*c*, 0·25).

[5] H. Herrmann, R. Hodges and A. Taylor *J. Chem. Soc.* 4315 (1964).
[6] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov and A. A. Kiryushkin *Tetrahedron Letters* 1927 (1963).
[7] D. W. Russell, R. L. M. Synge, A. Taylor and E. P. White *J. Chem. Soc.* 554 (1962).

Sporidesmolide—I. Principal spacings (Å), calculated spacings (Å) and assigned Miller indices given in this order (the latter two values in parentheses), d = 12·10 (12·17, 100); 7·02 (7·03, 110); 6·08 (6·08, 200); 4·60 (4·60, 120); 4·05 (4·06, 300); 3·53 (3·52, 220); 3·37 (3·38, 130); 3·04 (3·04, 400); 2·82, (2·79, 230); 2·66 (2·65, 140); — (2·43, 500); — (2·35, 330); 2·31 (2·30, 240); 2·18 (2·18, 150); 2·01 (2·03, 600); 5·22 (5·24, 001); 4·87 (4·81, 101); 4·23 (4·20, 111); 3·97 (3·96, 201); 3·45 (3·46, 121); 3·19 (3·22, 301); 2·96 (2·92, 221); 2·80 (2·84, 131) (Fig. 2C).

Cyclo-L-α-*oxyisovaleryl*-D-*allo-isoleucyl*-D-*leucyl*-L-α-*oxyisovaleryl*-L-*valyl*-L-N-*methyl-leucinate* (I). Fungal tissue (350 g) from the culture of *Pithomyces chartarum* (isolate C[9]) on potato carrot medium[10] (50 l.) containing DL-isoleucine (California Corporation for Biochemical Research, 250 g), was dried at 20°/5 mm, ground to a powder and continuously extracted with MeOH. The extract was dissolved in a mixture of MeOH (2 l.), $CHCl_3$ (2·85 l.) and water (0·6 l.), the lower layer was collected, evaporated and the residue digested with ether. The precipitate was dissolved in $CHCl_3$ (charcoal), filtered, the filtrate evaporated and the residue (0·12 g) recrystallized from MeOH as colourless needles (51·4 mg) m.p. 205°, $[\alpha]_D^{23}$ −201°. Further recrystallization of this material from MeOH gave 37 mg m.p. 225°, $[\alpha]_D^{23}$ −220°. This crop of crystals was heated at 160–170°/0·01 mm for 5·5 hr. The sublimate (10·5 mg, m.p. 227–227·5°, $[\alpha]_D^{20}$ −222°) on hydrolysis gave valine, leucine, N-methyl-leucine and isoleucine in equal proportions. The residue (25 mg m.p. 227–227·5°, $[\alpha]_D^{20}$ −228°) gave L-valine, D-leucine, N-methyl-leucine and D-isoleucine in equal proportions. The *cyclodepsipeptide* (I) separated from MeOH as clusters (very characteristic of the purest samples) of minute colourless needles, m.p. 228°, $[\alpha]_D^{23}$ −228°. (Found: C, 62·42; H, 9·17; N, 8·05; O, 19·73. $C_{34}H_{60}N_4O_8$ requires: C, 62·56; H, 9·26; N, 8·59; O, 19·61; $C_{33}H_{58}N_4O_8$ requires: C, 62·01; H, 9·15; N, 8·77; O, 20·04%). ν_{max} (KBr) 1755, 1675, 1520, 1460, 1390, 1380 cm^{-1}; principal spacings (see above, in Å) 12·25 (12·28, 100); 7·11 (7·09, 110); 6·14 (6·14, 200); 5·20 (5·24, 001); 4·87 (4·82, 101); 4·65 (4·64, 120); 4·26 (4·21, 111); 4·10 (4·09, 3·99, 300, 201); 3·49 (3·48, 121, 130, 220); — (3·07, 400); 2·83 (2·86, 131) (Fig. 2C).

Counter-current distribution of the total sporidesmolide fraction. The total sporidesmolide fraction[2] (5 g) was recrystallized from $CHCl_3$–MeOH (1:9), the mother liquors evaporated and the residue recrystallized from $CHCl_3$–MeOH (1:9). The mother liquors were evaporated and the residue (1·95 g) dissolved in the bottom phase (25 ml) of an equilibrated mixture of formic acid (d 1·22, 3 l.) and benzene (3 l.). The solution was placed in the first tube of an hundred stage counter-current distribution apparatus containing bottom phase (25 ml) in each tube. The top phase was then admitted to the first tube after each transfer. The phases were equilibrated for 5 min, settled for 3 min and allowed to drain for 30 sec after each transfer. After 100 transfers the top phase from the 100th tube was led back into tube 1 and similarly at each transfer thereafter. After 510 transfers had been made depsipeptides were found in 70 tubes and the wt. of depsipeptide in every 5th tube (all were weighed) was as follows: 100, 4 mg; 95, 4·5 mg; 90, 5 mg; 85, 10 mg; 80, 15 mg; 75, 36 mg; 70, 56 mg; 67, 74 mg; 65, 50 mg; 60, 49 mg; 55, 31 mg; 50, 24 mg; 45, 18 mg; 40, 10 mg; 35, 6 mg; 30, 4 mg. Fractions 30–50 were bulked (since no isoleucine was found in the hydrolysates) and recrystallized from MeOH to constant m.p. and optical rotation giving sporidesmolide—I, m.p. 260–261°, $[\alpha]_D^{19}$ −211°, m/e 638 (ions of greater mass not observed). Fractions 80–87 were combined (87 mg) and after 4 recrystallizations from MeOH colourless needles were obtained (10 mg), m.p. 216–218°, $[\alpha]_D^{20}$ −224°, m/e 666, 652, 299, 285.

[8] J. S. Shannon, *Tetrahedron Letters* 801 (1963).

[9] J. M. Dingley, J. Done, A. Taylor and D. W. Russell *J. Gen. Microbiol.* **29** 127 (1962).

[10] J. Done, P. H. Mortimer, A. Taylor and D. W. Russell *J. Gen. Microbiol.* **26** 207 (1961).