105. *Similarity of Depsipeptides from* Pithomyces chartarum *and from Pasture Samples from "Facial Eczema" Areas.*

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A mixture of non-toxic depsipeptides similar to that isolated from cultures of *Pithomyces chartarum* has been isolated from pasture samples collected during the facial eczema season.

From pasture where the disease facial eczema [1] occurs, but not from innocuous pasture, it is usually possible to isolate colourless crystalline material, which forms the basis of the so-called "beaker test." [2-4] This appeared, on preliminary chemical examination, to be a depsipeptide.[4] After the isolation of apparently similar material from cultures of *Pithomyces chartarum* (Berk. & Curt.) M.B. Ellis [5] (syn. *Sporidesmium bakeri* Syd.), the suggestion was made [6] that this organism was implicated in the ætiology of the disease. Laboratory animals dosed with these materials did not show liver lesions. Nevertheless, it seemed important to make a detailed comparison of the materials from the two sources.

By using the previous techniques [3,4,7,8] a total depsipeptide fraction was isolated from several pasture samples from different localities and from many different cultures of *P. chartarum*. Every sample sublimed in a characteristic manner and had similar optical rotation, melting point, and solubility; and the infrared spectra of all samples were the same.

After mild alkaline hydrolysis sporidesmolic acids A [9] and B [10] were obtained in identical yield from the depsipeptide fractions obtained from pasture and from cultures of the mould. On vigorous acid hydrolysis α-hydroxy-β-methylbutyric acid, valine, *N*-methylleucine, and leucine in the molar proportion 2 : 2 : 1 : 1, together with a minor component isoleucine, were obtained from depsipeptides from pasture and from the fungus.

From these results it can be concluded that the depsipeptide materials isolated from pasture and from cultures of the organism are identical. Depsipeptides are commonly associated with micro-organisms (see, *e.g.*, Done *et al.*[8]) but the structure [9,11] and physical properties of the sporidesmolides, so far as is known, are unique. Thus it is likely that the sporidesmolides found in pasture are metabolic products of *P. chartarum* growing thereon.

Experimental

M. p.s are corrected. Microanalyses were by Dr. A. D. Campbell and Dr. F. Pascher. Infrared spectra were determined on a Perkin-Elmer Infracord spectrophotometer.

Isolation of Sporidesmolides from Pasture.—(*a*) Beaker residues [3] (1·502 g.) were dissolved in a mixture of chloroform (100 ml.), water (30 ml.), and methanol (70 ml.).[12] The methanol phase was discarded and the chloroform phase processed as described previously.[8] The product (0·14 g., 9·3%) separating from 70% v/v aqueous acetic acid had $[\alpha]_D^{18}$ −198° (*c* 1·4 in chloroform), m. p. 251°, mixed m. p. with depsipeptide fraction from *P. chartarum* 255° (Found: C, 62·1; H, 9·2; N, 8·6%).

(*b*) Beaker residues [3] (0·122 g.) purified as described above provided 0·069 g. (59%) of material, m. p. 252°, mixed m. p. 258°, $[\alpha]_D^{18}$ −210° (*c* 0·7 in chloroform) (Found: O, 20·3; N, 8·5%).

[1] Done, Mortimer, and Taylor, *Res. Vet. Sci.*, 1960, **1**, 76.
[2] Perrin, *N.Z. J. Agric. Res.*, 1959, **2**, 266; Clare, Sandos, and Percival, *ibid.*, *p.* 1087.
[3] Sandos, Clare, and White, *N.Z. J. Agric. Res.*, 1959, **2**, 623.
[4] White, *N.Z. J. Agric. Res.*, 1958, **1**, 859.
[5] Ellis, *Mycol. Papers*, 1960, No. 76.
[6] Percival and Thornton, *Nature*, 1958, **182**, 1095; Thornton and Ross, *N.Z. J. Agric. Res.*, 1959, **2**, 1002; Percival, *ibid.*, 1959, **2**, 1041.
[7] Synge and White, *N.Z. J. Agric. Res.*, 1960, **3**, 907.
[8] Done, Mortimer, Taylor, and Russell, *J. Gen. Microbiol.*, 1961, **26**, 207.
[9] Russell, *Biochim. Biophys. Acta*, 1960, **45**, 411.
[10] Russell and Brown, *Biochim. Biophys. Acta*, 1960, **38**, 382.
[11] Russell, *J.*, 1962, in the press.
[12] Schwyzer and Sieber, *Helv. Chim. Acta*, 1957, **40**, 624.

(*c*) The substance described previously,[4] m. p. 260° (0·233 g.), in chloroform (2 ml.) was treated with carbon tetrachloride (10 ml.) and left overnight. The crystals that had separated (47·6 mg.; m. p. 260—261°) recrystallised from methanol as colourless needles, m. p. 254—258° (24·4 mg.) (Found: C, 61·9; H, 9·15%). The mother-liquors were evaporated to dryness and the residue (0·195 g.) recrystallised from 70% (v/v) aqueous acetic acid. The product had m. p. 258—259°, $[\alpha]_D^{23\cdot8}$ −94·5° (*c* 1·7 in acetic acid), −209° (*c* 0·24 in chloroform) (Found: C, 61·8; H, 9·2; O, 20·2; N, 8·8; S, 0·0%).

Isolation of Total Sporidesmolide Fraction from P. chartarum.—The material obtained by the process described by Done *et al.*[8] separated from 70% (v/v) aqueous acetic acid as colourless needles, m. p. 257°, $[\alpha]_D^{20}$ −205° (*c* 1 in chloroform), −98° (*c* 1 in acetic acid) (Found: C, 61·9, 61·8; H, 8·9, 9·15; O, 20·1, 20·2; N, 8·5, 9·15%).

Alkaline Hydrolysis of Depsipeptides from Pasture.—The material obtained as in (*a*) above (0·064 g.) was dissolved in boiling methanol (7 ml.), cooled, and treated with 5N-sodium hydroxide (1·8 ml.), followed after 15 min. by water (7 ml.). The bulk of the methanol was removed *in vacuo* at 40° and the residual solution acidified with 6N-hydrochloric acid (2 ml.). After 16 hr. at 0° the crystalline precipitate was collected, washed with water, and dried (KOH) *in vacuo*. The product was shaken for 1 hr. with chloroform (1 ml.), the precipitate {0·019 g.; m. p. 198—200°, $[\alpha]_D^{18}$ +56° (*c* 0·58 in acetic acid), mixed m. p. with sporidesmolic acid A [9,11] 201—202°} was collected, and the filtrate and washings were evaporated to dryness. The residue (0·028 g.), recrystallised from 25% (v/v) aqueous acetic acid, had m. p. 165—167°, $[\alpha]_D^{18}$ −109° (*c* 0·45 in acetic acid), mixed m. p. with sporidesmolic acid B 167°. The infrared spectrum of the product was identical with that of sporidesmolic acid B from sporidesmolide I.[11]

Acid Hydrolysis of Depsipeptides from Pasture.—The material obtained as in (*a*) or (*c*) above (0·0554 g.) was heated with concentrated hydrochloric acid (2 ml.) at 120° for 18 hr. (subsequently equal parts of hydrochloric and acetic acids were used). The cooled solution was extracted with diethyl ether 21 hr. and the extract dried (Na_2SO_4) and evaporated to dryness. The residue (7 mg.), on chromatography on paper [t-butyl alcohol (100 ml.), 4·25N-ammonia (25 ml.), Chlorophenol Red], showed an acid spot indistinguishable from that from α-hydroxy-β-methylbutyric acid. The aqueous phase from the above extraction was evaporated to dryness *in vacuo* (KOH) and the residue (0·0616 g.) dissolved in water (5 ml.). After chromatography on paper with the solvent system butan-1-ol (80 ml.), acetic acid (10 ml.) and water (100 ml.) ninhydrin-staining spots indistinguishable from those of valine, leucine, and *N*-methyl-leucine were observed.

The amino-acids were also estimated quantitatively as previously described,[13] with the results given in the Table. Results are expressed as molar proportions, *N*-methyl-leucine being assigned the value 1·0.

	Sample	Valine	*N*-Methyl-leucine	Leucine	Isoleucine
Pasture	(*a*)	2·1	1·0	0·9	0·2
,,	(*b*)	1·8	1·0	1·0	0·2
,,	(*c*)	2·1	1·0	1·0	0·2
Sporidesmolides from *P. chartarum*		2·0	1·0	1·0	0·2

Acid Hydrolysis of Sporidesmolic Acid A and B from Pasture.—The acid (0·003 g.) was heated at 110° in a sealed tube with 6N-hydrochloric acid (0·2 ml.) for 24 hr. The resulting solution was evaporated to dryness (KOH) *in vacuo* and the residue applied to Whatman No. 3MM paper. The chromatogram was developed with a solution of t-butyl alcohol (100 ml.) in 4·25N-ammonia (25 ml.). Valine and leucine were obtained from sporidesmolic acid A together with a trace of isoleucine. Valine and *N*-methyl-leucine were obtained from sporidesmolic acid B.

Dakin–West Degradation of Sporidesmolic Acid A and B from Pasture.—This was carried out as described by Russell.[11] Only valine was detected on the chromatograms.

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[13] Russell, *J. Chromatog.*, 1960, **4**, 251.