Sporidesmolic acid B, a hydroxyacyldipeptide from *Sporidesmium bakeri*

Certain antibiotics[1–4] possess cyclic structures containing α-hydroxy- and α-amino acids linked by alternate ester and amide bonds. Alkaline degradation of these and similar[5] compounds gives hydroxyacylamino acids. We have isolated an hydroxyacyldipeptide from the fungus *Sporidesmium bakeri*, by saponification of a fraction which is believed to be neither toxic nor antibiotic.

Felts of sporing cultures, from which sporidesmin[6] had been removed, were dried from the frozen state and continuously extracted with methanol. The residue left after evaporation of the methanol was partitioned between the two phases of the system chloroform–methanol–water (10:7:3) and the material obtained by concentrating the lower phase extracted with ether. The insoluble residue was separated and extracted with chloroform; the extract was filtered, decolorized with charcoal and evaporated to dryness. The residue had m.p. 256–259° with prior sintering, and was obtained in a yield of 6.9 g/kg dry fungus. It was saponified in a large excess of methanolic NaOH. Removal of the methanol and acidification (HCl) gave a precipitate of "saponification acid"[7], about half of which dissolved in chloroform leaving a crystalline residue of sporidesmolic acid A. This possesses no free amino group, and yields on hydrolysis α-hydroxyisovaleric acid, valine, leucine and possibly N-methylvaline; it is probably a mixture.

The portion of "saponification acid" soluble in chloroform, termed sporidesmolic acid B, was obtained in a yield of 2.75 g/kg dry fungus. It crystallized from aqueous acetic acid as needles, m.p. 165–166° (corr.), $[\alpha]_D^{16°}$—108° in acetic acid (*c*, 4), readily soluble in organic solvents but sparingly soluble in water. A 1% solution in aq. tetrahydrofuran had a pH of about 3; the acid dissolved in aq. $NaHCO_3$ and was precipitated unchanged on acidification. It produced no coloration with ninhydrin, migrated towards the anode on paper electrophoresis at pH 8 but was immobile at pH 2.5. The infrared spectrum showed peaks characteristic of amide, hydroxyl and carboxyl, but no absorption ascribable to ester carbonyl. (Found: C, 59.30; H, 9.35; N, 8.08. Calc. for $C_{17}H_{32}O_5N_2$: C, 59.24; H, 9.37; N, 8.13. Eq. wt. (by titration) 344, 347; calc.: 346.) Methyl ester (diazomethane), m.p. 105–106° (corr.). (Found: C, 60.16; H, 9.89; N, 7.55. Calc. for $C_{18}H_{34}O_5N_2$: C, 60.32; H, 9.56; N, 7.81. Mol. wt. (isopiestic, chloroform) 379; calc.: 359.)

Acid hydrolysis (6 *N* HCl, 110°, 72 h) furnished a mixture of α-hydroxyisovaleric acid, valine and N-methylleucine, each identified by paper chromatography in three or more different solvent systems using reference compounds as markers. Partial acid hydrolysis (conc. HCl, 37°) for 24 h liberated N-methylleucine and no other ninhydrin-positive substance. A trace of valine was present after 48 h. Ether extraction of a total acid hydrolysate and addition of cyclohexylamine to the ethereal extract gave cyclohexylammonium L-α-hydroxyisovalerate, $[\alpha]_D^{20°}$—9° in water (*c*, 4). An authentic sample had $[\alpha]_D^{20°}$—8.4°. The amino acids in the hydrolysate were separated by chromatography on cellulose powder in the system *tert*-butanol–aq. ammonia (s.g. 0.90)–water (20:1:4)[3]. N-methylleucine had $[\alpha]_D^{20°}$ + 20° in water (*c*, 1.3) (*cf.* ref.8 for L-isomer, + 20.4°); valine had $[\alpha]_D^{20°}$ + 47° in acetic acid (*c*, 2), (*cf.* ref.9 for L-isomer, + 62°). Although the valine was partly racemized, all the acids are clearly of the L-configuration. Sporidesmolic acid B is thus L-α-hydroxyisovaleryl-L-valyl-N-methyl-L-leucine.

S. bakeri is believed to be associated with facial eczema in sheep[10,11]: a powerful hepatotoxin, sporidesmin, has been isolated from cultures of the fungus[6]. A non-toxic metabolite with unusual properties has also been described[7]. Known for historical reasons as the "beaker test" substance, it may readily be isolated from felts of sporing cultures by the technique here outlined. Different samples have yielded on saponification up to 55 % of their weight of sporidesmolic acid B. Present evidence suggests that the "beaker test" substance is a mixture of related substances.

WHITE[7] has noted the similarity of the "beaker test" substance to the amidomycin group of antibiotics[1–4]. All have similar infrared spectra, contain hydroxy- and amino-acids, and differ from true peptides by their ease of saponification to yield acids with a terminal hydroxyacyl group. To describe such substances the term "peptolide" is suggested, the definition embracing derivatives of 2,5-dioxomorpholine such as the *Pseudomonas tabaci* toxin[5].

The "beaker test" substance differs from the peptolide antibiotics in possessing an α-hydroxyisovaleryl residue with the L-configuration, and in having a true dipeptide sequence. It may be significant that no antibiotic property has yet been shown for it. In fact, while direct evidence is lacking, its function may be that of a water-insoluble coat upon the fungal spores. The yield is greater from high-sporing than from low-sporing strains[12]. Electron micrographs of intact spores[13] show an apparently crystalline covering which is absent from spores which have been washed with organic solvents: the "beaker test" substance is known to be at least partly removed from dried fungal material by brief washing with acetone[14]. Experiments are planned to determine the effect of such treatment on spore viability, and to follow the appearance of the "beaker test" substance and of spores in growing cultures of *S. bakeri*. Biological examples are not wanting in which compounds of similar basic structure fulfil diverse roles, and peptolides may prove to be another example of such biochemical versatility.

We wish to thank Dr. R. L. M. SYNGE, F.R.S. and Dr. A. TAYLOR for helpful discussions, and Mr. J. G. FRASER for reference samples of hydroxy- and N-methyl-amino acids. Analyses and molecular weight determinations were by Dr. A. D. CAMPBELL of the University of Otago and infrared spectra by Mr. C. WATSON of Dominion Laboratory, Wellington.

Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North (New Zealand)

D. W. RUSSELL
M. E. BROWN

[1] P. A. PLATTNER AND U. NAGER, *Helv. Chim. Acta*, 31 (1948) 665. 2192.
[2] A. H. COOK, S. F. COX AND TH. FARMER, *J. Chem. Soc.*, (1949) 1022.
[3] H. BROCKMANN AND H. GEEREN, *Ann. Chem.*, 603 (1957) 216.
[4] L. C. VINING AND W. A. TABER, *Can. J. Chem.*, 35 (1957) 1109.
[5] D. W. WOOLLEY, G. SCHAFFNER AND A. C. BRAUN, *J. Biol. Chem.*, 215 (1955) 485.
[6] R. L. M. SYNGE AND E. P. WHITE, *Chem. & Ind. (London)*, 8 (1959) 1546.
[7] E. P. WHITE, *New Zealand J. Agr. Research*, 1 (1958) 859.
[8] M. BLANCHARD, D. E. GREEN, V. NOCITO AND S. RATNER, *J. Biol. Chem.*, 155 (1944) 421.
[9] J. P. GREENSTEIN, S. M. BIRNBAUM AND M. C. OTLEY, *J. Biol. Chem.*, 204 (1953) 307.
[10] R. H. THORNTON AND D. J. ROSS, *New Zealand J. Agr. Research*, 2 (1959) 1002.
[11] J. C. PERCIVAL, *New Zealand J. Agr. Research*, 2 (1959) 1041.
[12] R. T. J. CLARKE AND D. W. RUSSELL, unpublished results.
[13] W. S. BERTAUD, personal communication.
[14] J. SANDOS, N. T. CLARE AND E. P. WHITE, *New Zealand J. Agr. Research*, 2 (1959) 623.

Received January 6th, 1960