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TOTAL SYNTHESIS OF SPORIDESMOLIDE I*

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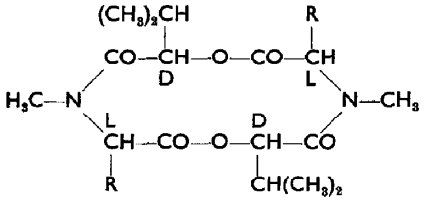
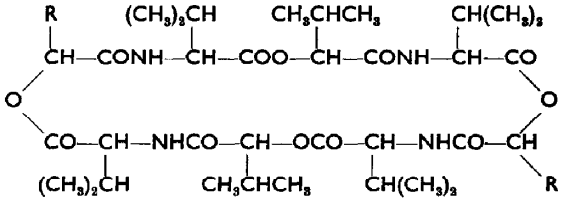
Abstract—The first total synthesis of a natural cyclodepsipeptide, namely sporidesmolide I, has been achieved and thus an unambiguous proof of its structure has been given.

IN THE course of a detailed investigation into the chemistry of depsipeptides[2–4] we described the synthesis of cyclotetradepsipeptides I and II, the structure of which in 1948 Plattner *et al.*[5] ascribed to the antibiotics enniatin A and enniatin B. Recently[6,7] we synthesized the cyclodepsipeptides III and IV the formulas of which had been assigned in 1957 to the antibiotics amidomycin[8] and valinomycin.[9] The compounds I–IV have very different properties from the corresponding antibiotics and are devoid of antimicrobial activity.



I R = $CH(CH_3)CH_2CH_3$
II R = $CH(CH_3)_2$



III (D-D-D-D-D-D-D-D) R = $CH(CH_3)_2$
IV (L-L-D-D-L-L-D-D) R = CH_3

Inasmuch as none of the cyclodepsipeptide structures proposed for this group of antibiotics has received synthetic confirmation, the question obviously arose as to whether such structures held for the other naturally-occurring compounds to which they had been assigned. In the light of this we undertook the synthesis of a number of

* Communication XIV of the series *Studies in Depsipeptide Chemistry*. For preliminary communication see.[1]

[1] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk* 1699 (1962).

[2] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, V. T. Ivanov, *Tetrahedron Letters* 301 (1962); *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk* 2154 (1962).

[3] Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, M. M. Shemyakin, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk* 1497 (1962).

[4] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, *Tetrahedron* 1963, in press.

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[6] Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, M. M. Shemyakin, *Report to the V European Symposium on Peptide Chemistry* Oxford (1962).

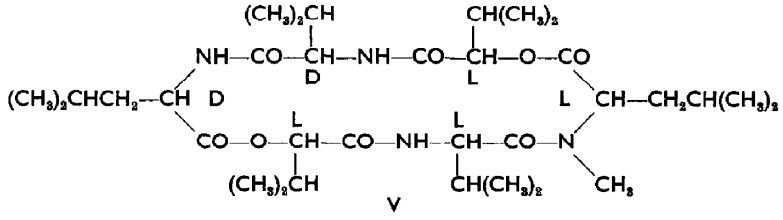
[7] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, *Tetrahedron Letters* 1963, in press.

[8] L. C. Vining, W. A. Taber, *Canad. J. Chem.* **35**, 1109 (1957).

[9] H. Brockmann, H. Geeren, *Liebig's Ann.* **603**, 216 (1957).

such cyclodepsipeptides, beginning with sporidesmolide I. This compound was isolated in 1960 by Russell[10] from a neutral, depsipeptide-containing fraction of metabolites of the pasture fungus *Pithomyces chartarum* (*Sporidesium bakeri Sydow*)[11,12] apparently the causative agent of facial eczema in ruminants.[13,14]

Sporidesmolide I which Russell obtained by fractional crystallization of the neutral metabolites from chloroform–methanol solvent is a compound of molecular formula $C_{33}H_{58}N_4O_8$, m.p. 261–263°, $[\alpha]_D^{17}$ −217° (*c* 1·5, $CHCl_3$). On the basis of acid and alkaline hydrolysis of this compound as well as I.R. data, it was assigned the cyclohexadepsipeptide structure (V).[10,15]



V

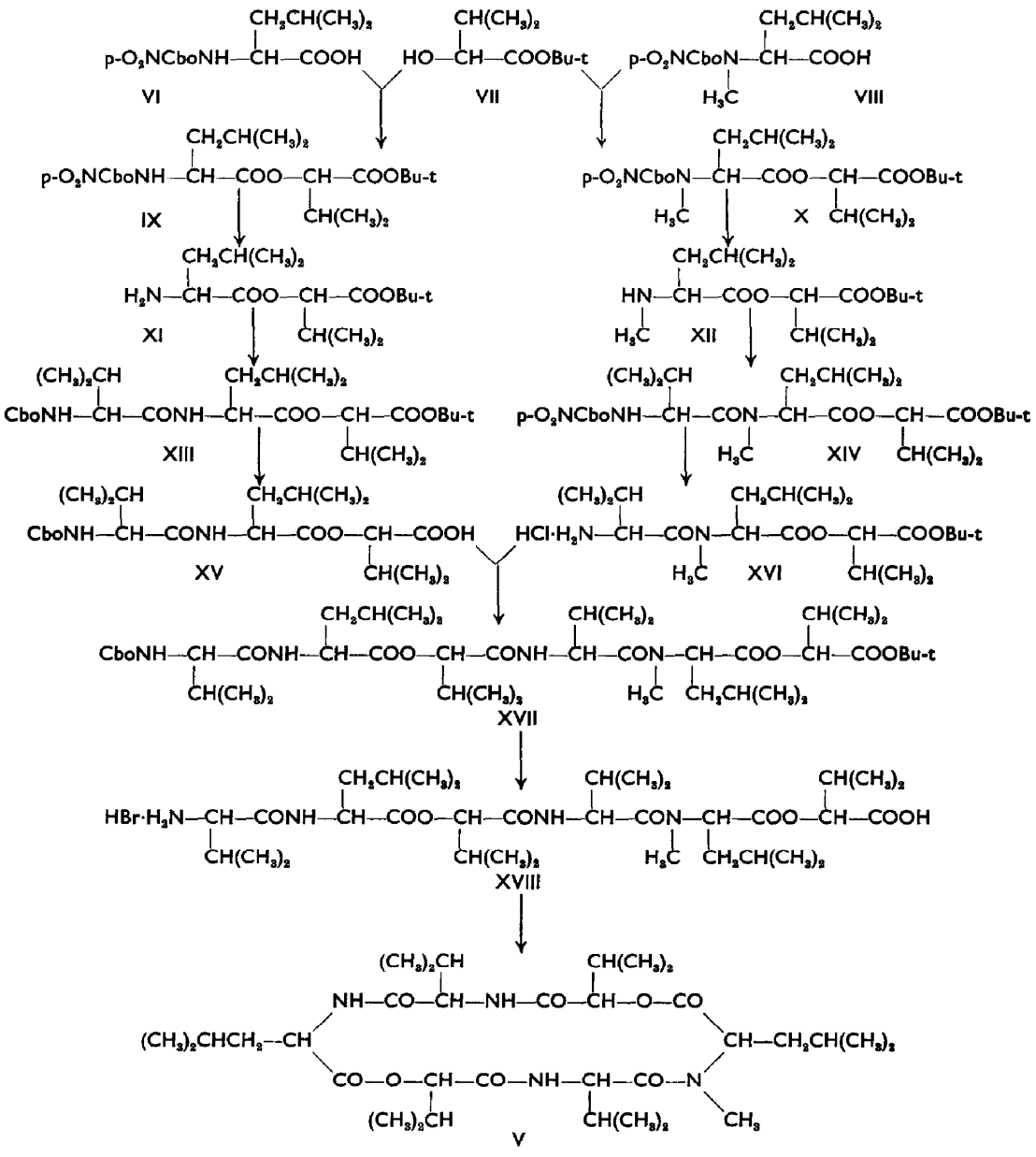
Based on the general methods we had developed for the synthesis of linear and cyclic depsipeptides[16–18] we achieved the synthesis of compound V according to Scheme I.

Condensation of *p*-nitrobenzyloxycarbonyl-D-leucine (VI) with t-butyl L-α-hydroxyisovalerate (VII) by the mixed anhydride method (benzenesulfonyl chloride in pyridine) gave t-butyl *p*-nitrobenzyloxycarbonyl-D-leucyl-L-α-hydroxyisovalerate (IX). Similarly, condensation of *p*-nitrobenzyloxycarbonyl-N-methyl-L-leucine (VIII) with t-butyl ester (VII) led to t-butyl *p*-nitrobenzyloxycarbonyl-N-methyl-L-leucyl-L-α-hydroxyisovalerate (X). Hydrogenolysis of the diesters (IX and X) in the presence of a palladium catalyst then afforded the corresponding amino esters (XI and XII).

The amino ester (XI) was condensed with benzyloxycarbonyl-D-valine by the mixed anhydride method (ClCOOEt in tetrahydrofuran) to give the protected tridepsipeptide (XIII) and, on the other hand, the tridepsipeptide (XIV) was obtained on condensation of amino ester (XII) with *p*-nitrobenzyl-oxycarbonyl-L-valine by the acid chloride method (PCl_5 in ether). The t-butyl protecting group of XIII was then removed by means of hydrogen chloride in ether, giving the corresponding acid (XV), and the amino ester (XVI) was obtained (as the hydrochloride) on hydrogenolysis of the tridepsipeptide (XIV) in the presence of a palladium catalyst. The fragments (XV and XVI) were linked together by the chloride method: the action of PCl_5 in ether on the benzyloxycarbonyl acid (XV) gave the corresponding acid chloride, condensation of

[10] D. W. Russell, *Biochim. Biophys. Acta* **45**, 411 (1960).
[11] E. P. White, *New Zealand J. Agr. Research* **1**, 859 (1958).
[12] J. Done, P. H. Mortimer, A. Taylor, D. W. Russell, *J. Gen. Microbiol.* **26**, 207 (1961).
[13] R. H. Thornton, J. C. Percival, *Nature, Lond.* **183**, 63 (1959).
[14] J. C. Percival, *New Zealand J. Agr. Research* **2**, 1041 (1959).
[15] D. W. Russell, *J. Chem. Soc.* 753 (1962).
[16] M. M. Shemyakin, *Angew. Chem.* **71**, 741 (1959); **72**, 342 (1960).
[17] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, V. A. Oladkina, L. A. Shchukina, *Dokl. Akad. Nauk SSSR* **140**, 387 (1961).
[18] M. M. Shemyakin, *Usp. Khim.* **31**, 269 (1962); *Zh. Vsesoyuznogo Khim. Obshch.* im. D. I. Mendeleeva **7**, 471 (1962).

SCHEME I



which with the amino ester (XVI) in the presence of triethylamine led to the protected hexadepsipeptide (XVII). Simultaneous removal of the C- and N-protecting groups by treatment of XVII with hydrogen bromide in glacial acetic acid yielded the hydrobromide of D-valyl-D-leucyl-L-α-hydroxyisovaleryl-L-valyl-N-methyl-L-leucyl-L-α-hydroxyisovaleric acid (XVIII) which on cyclization by the acid chloride method finally gave the cyclohexadepsipeptide (V).

Compound V was found to have identical properties with the natural sporidesmolide I. It has: 260–261° and $[\alpha]_D^{20}$ −215° (*c* 0·5, $CHCl_3$). Direct comparison of the two substances both by us and by Dr. Russell (mixed m.p., chromatographic behaviour IR spectra, etc.) confirmed their complete identity. This was also supported by the comparative measurement of the alkaline hydrolysis rate of the synthetic and natural products (with spectrophotometric control) carried out by Dr. Russell and also by results of the analysis of their total amino acid constituents.

It thus follows that our synthetic studies confirmed the formula proposed by Dr. Russell for sporidesmolide I and the problem of its structure may now be considered to be unambiguously solved*.

EXPERIMENTAL†

O-Benzyloxycarbonyl-L-α-hydroxyisovaleric acid. This compound was prepared by the same method as that for O-benzyloxycarbonyl-D-α-hydroxyisovaleric acid;[4] m.p. 57–58° (from hexane), $[\alpha]_D^{20}$ −9·8° (*c* 0·6, C_6H_6). (Found: C, 61·83; H, 6·41. $C_{13}H_{16}O_5$ requires: C, 61·89; H, 6·39%).

t-Butyl L-α-hydroxyisovalerate (VII), prepared analogously to t-butyl D-α-hydroxyisovalerate;[4] b.p. 61–63° (1·8 mm), m.p. 30–31°, $[\alpha]_D^{20}$ −2·9° (*c* 0·8, C_6H_6). (Found: C, 61·83; H, 10·39. $C_9H_{18}O_3$ requires: C, 62·04; H, 10·41%).

p-Nitrobenzyloxycarbonyl-D-leucine. To a solution of 19·6 g (0·15 moles) of D-leucine in 75 ml of 2N NaOH was gradually added in about equal portions under cooling and shaking 49 ml of 4N NaOH and 40·3 g (0·188 moles) of *p*-nitrobenzyloxycarbonyl chloride in 50 ml of dioxane. The mixture was shaken for another 30 min, filtered and the filtrate acidified to congo with conc. HCl. The oil which separated was taken up in ethyl acetate, the solution washed with water and extracted with saturated $NaHCO_3$ solution. After acidifying the bicarbonate extract with HCl it was extracted with ethyl acetate and the extract, after drying over $MgSO_4$, was evaporated under red. press. *p*-Nitrobenzyloxycarbonyl-D-leucine (yield 41·8 g = 90%) was obtained as a yellow oil with $[\alpha]_D^{20}$ +11·4° (*c* 1·8, C_6H_6). (Found: C, 54·07; H, 5·97; N, 8·82. $C_{14}H_{18}O_6N_2$ requires: C, 54·19; H, 5·85; N, 9·03%).

t-Butyl p-nitrobenzyloxycarbonyl-D-leucyl-L-α-hydroxyisovalerate (IX). To a solution of 34·1 g (0·11 moles) of *p*-nitrobenzyloxycarbonyl-D-leucine in 150 ml of dry pyridine was added under stirring (0°, 5 min) 18·9 g (0·107 moles) of benzenesulfonylchloride. After 15 min a solution of 17·4 g (0·1 mole) t-butyl L-α-hydroxyisovalerate (VII) in 20 ml dry pyridine was added and after stirring at 0° for 2 hr and then at 20° for 3 hr the contents were poured into water and the mixture extracted with ether. The extract was washed with water, 1N HCl and saturated $NaHCO_3$ solution, and, after drying over $MgSO_4$, was evaporated. The residue was subjected to chromatography on neutral Al_2O_3 with benzene–ethyl acetate solvent (gradient elution) and the t-butyl ester (IX) was obtained as a yellow oil, yield 37·3 g (80%); $[\alpha]_D^{20}$ +7·1° (*c* 1·0, C_6H_6). (Found: C, 58·93; H, 7·16; N, 6·30. $C_{23}H_{34}O_8N_2$ requires: C, 59·21; H, 7·35; N, 6·01%).

t-Butyl p-nitrobenzyloxycarbonyl-N-methyl-L-leucyl-L-α-hydroxyisovalerate (X). From 35·6 g (0·11 moles) *p*-nitrobenzyloxycarbonyl-N-methyl-L-leucine (VIII)[4] and 17·4 g (0·1 moles) *t*-butyl ester (VII) under the conditions of the previous experiment 40·8 g (85%) of the diester (X) was obtained; m.p. 46–47° (from hexane), $[\alpha]_D^{20}$ −30° (*c* 1·0, C_6H_6). (Found: C, 59·99; H, 7·59; N, 5·91. $C_{24}H_{36}O_8N_2$ requires: C, 59·98; H, 7·55; N 5·83%).

* Recently, among the metabolic products of *Sporidesmium bakeri* D. W. Russel and A. Taylor discovered still another cyclodepsipeptide to which they gave the name sporidesmolide II. This is probably an analog of sporidesmolide I, containing D-isoleucine in place of D-valine. In the light of this we synthesized cyclo-D-isoleucyl-L-α-hydroxyisovaleryl-N-methyl-L-leucyl-L-valyl-L-α-hydroxyisovaleryl-D-leucyl and also its isomer containing allo-isoleucine instead of isoleucine. The compounds have the following constants: m.p. 236–238° and 228–230, $[\alpha]_D^{20}$–201°(*c* 0·7, $CHCl_3$) and −195°(*c* 0·6, $CHCl_3$) for the first and second, respectively.

† All m.p. are uncorrected. The purity of the synthesized compounds was controlled by thin layer chromatography on alumina or silica gel.

t-Butyl-D-leucyl-L-α hydroxyisovalerate (XI). The ester (IX; 23·3 g = 0·05 moles) was dissolved in 250 ml methanol, containing 6 ml glacial acetic acid and hydrogenated (20°, 760 mm) in the presence of a palladium catalyst (from 1·5 g PdO) until the theoretical amount of hydrogen was absorbed (3 hr). The catalyst was filtered off and the filtrate was diluted with benzene, washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated. After vacuum fractionation of the residue 10 g (70%) of the amino ester (XI) was obtained; b.p. 92–94° (0·2 mm), $[\alpha]_D^{20}$ −33° (*c* 0·4, C_6H_6). (Found: C, 62·53; H, 10·26; N, 4·98. $C_{15}H_{29}O_4N$ requires: C, 62·68; H, 10·17; N, 4·87%).

t-Butyl N-methyl-L-leucyl-L-α-hydroxyisovalerate (XII). The ester (X; 24 g = 0·05 moles) under the conditions of the previous experiment gave 11·6 g (77%) of the amino ester (XII); b.p. 90–92° (0·15 mm), $[\alpha]_D^{20}$ −28° (*c* 0·8, C_6H_6). (Found: C, 63·86; H, 10·36; N, 4·48. $C_{16}H_{31}O_4N$ requires: C, 63·75; H, 10·37; N, 4·65%).

t-Butyl benzyloxycarbonyl-D-valyl-D-leucyl-L-α-hydroxyisovalerate (XIII). To a solution of 5·5 g (22 mmoles) of benzyloxycarbonyl-D-valine[19] in 50 ml of dry tetrahydrofuran 2·2 g (22 mmoles) triethylamine and 2·4 g (22 mmoles) ethyl chloroformate was added with stirring (0°, 20 min). After 10 min a solution of 5·7 g (20 mmoles) amino ester (XI) in 30 ml dry tetrahydrofurane was added (0°, 30 min) and the mixture was stirred for 2 hr at 20°, and then diluted with ether. The ether solution was washed with 1N HCl and saturated $NaHCO_3$ and then dried over $MgSO_4$ and evaporated. The residue crystallized on standing. On recrystallization from hexane 9·4 g (90%) tridepsipeptide (XIII) was obtained; m.p. 78–79°, $[\alpha]_D^{20}$ +26° (*c* 0·8, C_6H_6). (Found: C, 64·77; H, 8·47; N, 5·78. $C_{28}H_{44}O_7N_2$ requires: C, 64·59; H, 8·52; N, 5·38%).

t-Butyl p-nitrobenzyloxycarbonyl-L-valyl-N-methyl-L-leucyl-L-α-hydroxyisovalerate (XIV). To a solution of 7·1 g (24 mmoles) *p*-nitrobenzyloxycarbonyl-L-valine[20] in 50 ml dry ether, cooled to 0°, 8·3 g (40 mmoles) finely powdered PCl_5 was added. The mixture was stirred for 1 hr at 0°, the excess PCl_5 filtered off and the filtrate evaporated *in vacuo*. The resultant chloride was dissolved in 40 ml dry ether and the solution added (−40°, 1 hr) to a solution of 6·02 g (20 mmoles) amino ester (XII) and 2·5 g (25 mmoles) triethylamine in 60 ml dry ether. The mixture was stirred for another 2 hr at 20°, washed with 1N HCl and then saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was subjected to chromatography on neutral Al_2O_3 with benzene–ethyl acetate as solvent (gradient elution). In this way 9·9 g (85% yield) of tridepsipeptide (XIV) was obtained as a yellow oil, $[\alpha]_D^{20}$ −42° (*c* 0·7, C_6H_6). (Found: C, 60·31; H, 7·89; N, 7·26. $C_{29}H_{45}O_9N_3$ requires: C, 60·08; H, 7·83; N, 7·25%).

Benzyloxycarbonyl-D-valyl-D-leucyl-L-α-hydroxyisovaleric acid (XV). A solution of 5·2 g (0·01 mole) of the tridepsipeptide (XIII) in 50 ml dry ether was saturated at 20° with hydrogen chloride and allowed to stand for 15 hr, then washed with water and extracted with 5% $NaHCO_3$. The bicarbonate extracts were acidified to congo with conc. HCl and extracted with ether. The ether solution was washed with water, dried over $MgSO_4$ and evaporated. In this way 3·5 g (75% yield) benzyloxycarbonyl acid (XV) was obtained as a colorless oil, $[\alpha]_D^{20}$ −26° (*c* 0·8, C_6H_6). (Found: C, 62·14; H, 7·88; N, 6·08. $C_{24}H_{36}O_7N_2$ requires: C, 62·05; H, 7·81; N, 6·03%).

Hydrochloride of t-butyl L-valyl-N-methyl-L-leucyl-L-α-hydroxyisovalerate (XVI). The tridepsipeptide (XIV; 5·8 g = 0·01 mole) was dissolved in 50 ml methanol containing 1·46 g (0·04 moles) hydrogen chloride and hydrogenated (20°, 750 mm) in the presence of a palladium catalyst (from 0·5 g PdO) until the theoretical amount of hydrogen had been absorbed (3 hr). The catalyst was filtered off and the filtrate evaporated *in vacuo*. The residue was treated with dry ether, *p*-toluidine hydrochloride was filtered off and the filtrate again evaporated. The semi-solid residue was dissolved with slight heating in 15 ml of di-n-propyl ether and the solution allowed to stand overnight at 0°. The crystals were filtered off and dried *in vacuo* over P_2O_5, giving 3·1 g (71%) of the amino ester hydrochloride (XVI), m.p. 143–146°, $[\alpha]_D^{20}$ −24° (*c* 0·7, EtOH), R_f 0·74 (toluene:sec-butanol:AcOH:H_2O = 2:4:1:4 v/v). (Found: Cl, 8·18; neutralization equivalent 0·1N NaOH 412. $C_{21}H_{41}O_5N_2Cl$ requires: Cl, 8·11%; 437).

t-Butyl benzyloxycarbonyl-D-valyl-D-leucyl-L-α-hydroxyisovaleryl-L-valyl-N-methyl-L-leucyl-L-α-hydroxyisovalerate (XVII). To a solution of 2·32 g (5 mmoles) of the benzyloxycarbonyl acid (XV) in 20 ml dry ether, cooled to 0°, 2·1 g (10 mmoles) of finely powdered PCl_5 was added. The mixture was stirred for 1 hr at 0° and, after filtering off the excess PCl_5, the filtrate was evaporated *in vacuo*.

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[20] W. H. McGregor, F. H. Carpenter, *J. Org. Chem.* **26**, 1849 (1961).

The acid chloride obtained was dissolved in 20 ml dry tetrahydrofuran and the solution was added gradually together with a solution of 1·5 g (15 mmoles) of triethylamine in 20 ml of tetrahydrofuran to a solution of 2·62 g (6 mmoles) of the amino ester hydrochloride (XVI) in 30 ml dry tetrahydrofuran. The mixture was stirred for 2 hr at 20°, diluted with ether, washed with water, 1N HCl, and 5% $NaHCO_3$. It was then dried over $MgSO_4$ and evaporated. The residue was subjected to chromatography on neutral Al_2O_3 with benzene–ethyl acetate as solvent (gradient elution) and 2·54 g (60% yield) of the protected hexadepsipeptide (XVII), m.p. 122–124° (from hexane), $[\alpha]_D^{20}$ −28° (*c* 0·8, C_6H_6) was obtained. (Found: C, 63·74; H, 8·87; N, 6·59. $C_{45}H_{74}O_{11}N_4$ requires: C, 63·80; H, 8·80; N, 6·61%).

Hydrobromide of D-*valyl*-D-*leucyl*-L-α-*hydroxyisovaleryl*-L-*valyl*-N-*methyl*-L-*leucyl*-L-α-*hydroxyisovaleric acid* (XVIII). The hexadepsipeptide (XVII; 0·85 g = 1 mmole) was dissolved at 20° in 10 ml 35% hydrogen bromide in glacial acetic acid and the resultant solution allowed to stand 30 min at 20°, and then evaporated to dryness. The residue was treated with dry ether. The amorphous precipitate was filtered off, washed with dry ether and dried *in vacuo*. The hydrobromide (XVIII), $[\alpha]_D^{20}$ −55° (*c* 0·8, EtOH) was obtained in a yield of 0·62 g (84%). (Found: Br, 11·3; neutralization equivalent 0·01N NaOH 359. $C_{33}H_{61}O_9N_4Br$ requires: Br, 10·80%; 369).

Sporidesmolide I. A solution of 369 mg (0·5 mmoles) of the hydrobromide (XVIII) in 5 ml thionyl chloride was allowed to stand at 20° for 30 min and then evaporated *in vacuo*. The resultant chloride was dissolved in 50 ml dry methylene chloride and the solution added with stirring, together with a solution of 150 mg (1·5 mmoles) triethylamine in 50 ml dry benzene (20°, 5 hr) to 1 l. dry benzene. The mixture was allowed to stand overnight at 20° and then evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution washed with 1N HCl, saturated $NaHCO_3$ solution and water, after which it was dried over $MgSO_4$ and evaporated. The semi-crystalline residue was chromatographed on neutral alumina with benzene–chloroform as solvent (gradient elution) and 144 mg (45%) cyclohexadepsipeptide (V) obtained. On recrystallization from di-n-butyl ether and twofold sublimation m.p. 260–261°, $[\alpha]_D^{20}$ −215° (*c* 0·5, $CHCl_3$); cf.[10]

Mixed m.p. determination with a sample of the natural product gave no depression.

Acknowledgement—We wish to express our thanks to Dr. D. W. Russell (Twyford Laboratory, London) for his sample of sporidesmolide I and for his proof of the identity of the synthetic and natural compounds.