Tetrahedron, 1963, Vol. 19, pp. 581 to 591. Pergamon Press Ltd. Printed in Northern Ireland

THE STRUCTURE OF ENNIATINS AND RELATED ANTIBIOTICS*

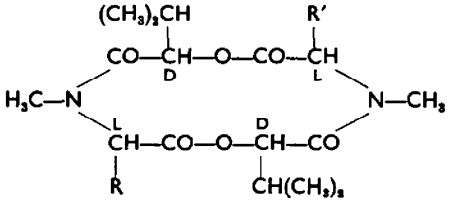
M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, V. T. IVANOV and A. A. KIRYUSHKIN
Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow

(*Received* 1 *November* 1962)

Abstract—The synthesis of cyclotetradepsipeptides the structure of which has previously been assigned to antibiotics of the enniatin group is described. It was found that these antibiotics cannot be represented by the formulas (I)–(VI).

IN 1947 Plattner *et al.* [3–5] isolated from the mycelium of a number of *Fusarium* strains two new antibiotics, enniatin A ($C_{24}H_{42}N_2O_6$; m.p. 121–122°; $[\alpha]_D^{20}$ −92°; *c* 1, $CHCl_3$) and enniatin B ($C_{22}H_{38}N_2O_6$; m.p. 173–175°; $[\alpha]_D^{21}$ −108°; *c* 0·6, $CHCl_3$) both possessing high *in vitro* activity against various mycobacteria.

Based on the results of acid and alkaline hydrolysis, the antibiotics were assigned the cyclotetradepsipeptide structures I and II, built up of D-α-hydroxyisovaleric acid and N-methyl-L-isoleueine (or N-methyl-L-valine) residues[6,7].



I R = R′ = $CH(CH_3)CH_2CH_3$; II R = R′ = $CH(CH_3)_2$;
III R = R′ = $CH_2CH(CH_3)_2$; IV R = $CH(CH_3)CH_2CH_3$,
R′ = $CH(CH_3)_2$; V R = $CH(CH_3)CH_2CH_3$, R′ = $CH_2CH(CH_3)_2$;
VI R = $CH(CH_3)_2$, R′ = $CH_2CH(CH_3)_2$.

It was found that certain of the *Fusarium* strains produce in addition to enniatins A and B an antibiotic with N-methylleucine as the amino acid component. This compound, not isolated in the pure state, was named enniatin C[8] and to it could be ascribed

* Communication No. XIII of the series, *Studies in Depsipeptide Chemistry*; and for preliminary communications see refs 1 and 2.

[1] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin and V. T. Ivanov, *Tetrahedron Letters*, No. 7, 301 (1962).
[2] Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin and M. M. Shemyakin, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk* 1497 (1962).
[3] E. Gäumann, S. Roth, L. Ettlinger, Pl. A. Plattner and U. Nager, *Experientia* **3**, 202 (1947).
[4] Pl. A. Plattner and U. Nager, *Experientia* **3**, 325 (1947).
[5] Pl. A. Plattner, U. Nager and A. Boller, *Helv. Chim. Acta* **31**, 594 (1948).
[6] Pl. A. Plattner and U. Nager, *Helv. Chim. Acta* **31**, 2192 (1948).
[7] Pl. A. Plattner and U. Nager, *Helv. Chim. Acta* **31**, 665 (1948).
[8] Pl. A. Plattner and U. Nager, *Helv. Chim. Acta* **31**, 2203 (1948).

formula III. Furthermore, a detailed chromatographic analysis of various enniatins hydrolysates led Plattner and Nager to the conclusion that enniatins with two different N-methyl-amino acids in the molecule are possible.[8] The antibiotics could, by analogy, be assigned structures IV, V or VI.

At about the same time Cook *et al.*[9] isolated five closely related antibiotics from the broth of certain *Fusarium* strains. These were named lateritiin I ($C_{26}H_{46}N_2O_7$; m.p. 121–122°; $[\alpha]_D^{20}$ −95·6°, *c* 1, C_2H_5OH), lateritiin II ($C_{26}H_{46}N_2O_7$; m.p. 125°; $[\alpha]_D^{19}$ −92°, *c* 1, C_2H_5OH), avenacein ($C_{25}H_{44}N_2O_7$; m.p. 139°; $[\alpha]_D^{19}$ −101°, *c* 1, C_2H_5OH), sambucinin ($C_{24}H_{42}N_2O_7$; m.p. 86–87°; $[\alpha]_D^{21}$ −83°, *c* 1, C_2H_5OH) and fructigenin ($C_{26}H_{44-46}N_2O_7$; m.p. 129°; $[\alpha]_D^{18}$ −103°, *c* 1, C_2H_5OH).

A number of similar properties (mixed m.p., IR spectra, X-ray diagrams, bacterial spectra) suggested that lateritiin I is identical with enniatin A. It was later found, however,[10,11] that on hydrolysis lateritiin I forms the same products as enniatin B (but not enniatin A) namely, N-methyl-L-valine and D-α-hydroxyisovaleric acid under acid conditions and D-α-hydroxyisovaleryl-N-methyl-L-valine under alkaline conditions, and the latter compound was subsequently converted to the corresponding lactone and the methyl ester (XXXIII). The same products are also formed on hydrolysis of lateritiin II, avenacein, sambucinin and fructigenin.

The discrepancy in these data cast some doubt on the validity of formulae I and II proposed by the Swiss chemists for enniatins A and B. In order to throw light on this question we undertook the synthesis of the cyclotetradepsipeptides (I)–(VI)*.

Condensation of *p*-nitrobenzyloxycarbonyl-N-methyl-L-isoleucine (VII), *p*-nitrobenzyloxycarbonyl-N-methyl-L-valine (VIII) or *p*-nitrobenzyloxycarbonyl-N-methyl-L-leucine (IX) with t-butyl D-α-hydroxyisovalerate (X) by the mixed anhydride method (benzenesulfonyl chloride in pyridine) affords the corresponding esters XI, XII and XIII. A benzene solution of the latter when refluxed in the presence of *p*-toluenesulfonic acid yields the *p*-nitrobenzyloxycarbonyl acids XIV, XV and XVI. On the other hand, hydrogenolysis of XI, XII and XIII in the presence of a Pd-catalyst affords the corresponding amino esters XVII, XVIII and XIX. The acid chloride method was then employed to join the fragments XIV–XVI and XVII–XIX by an amide bond. This was accomplished by reaction of the *p*-nitrobenzyloxycarbonyl acids XIV–XVI with phosphorus pentachloride to give the corresponding chlorides which were then condensed with the amino esters XVII–XIX in the presence of triethylamine, resulting in the tetradepsipeptides XX–XXV.†

The C- and N-protecting groups were simultaneously removed from the tetradepsipeptides XX–XXV by the action of hydrogen bromide in glacial acetic acid, yielding the tetradepsipeptide hydrobromides XXVI–XXXI. Cyclization of the latter by the

* Regarding methods for the synthesis of optically active depsipeptides see refs. 12 and 13.

† The tetradepsipeptide (XXI) was also prepared by condensation of XV and XVIII with the aid of N,N′dicyclohexylcarbodiimide.

[9] A. H. Cook, S. F. Cox, T. H. Farmer and M. S. Lacey, *Nature, Lond.* **160,** 31 (1947).

[10] A. H. Cook, S. F. Cox and T. H. Farmer, *Nature, Lond.* **162,** 61 (1948).

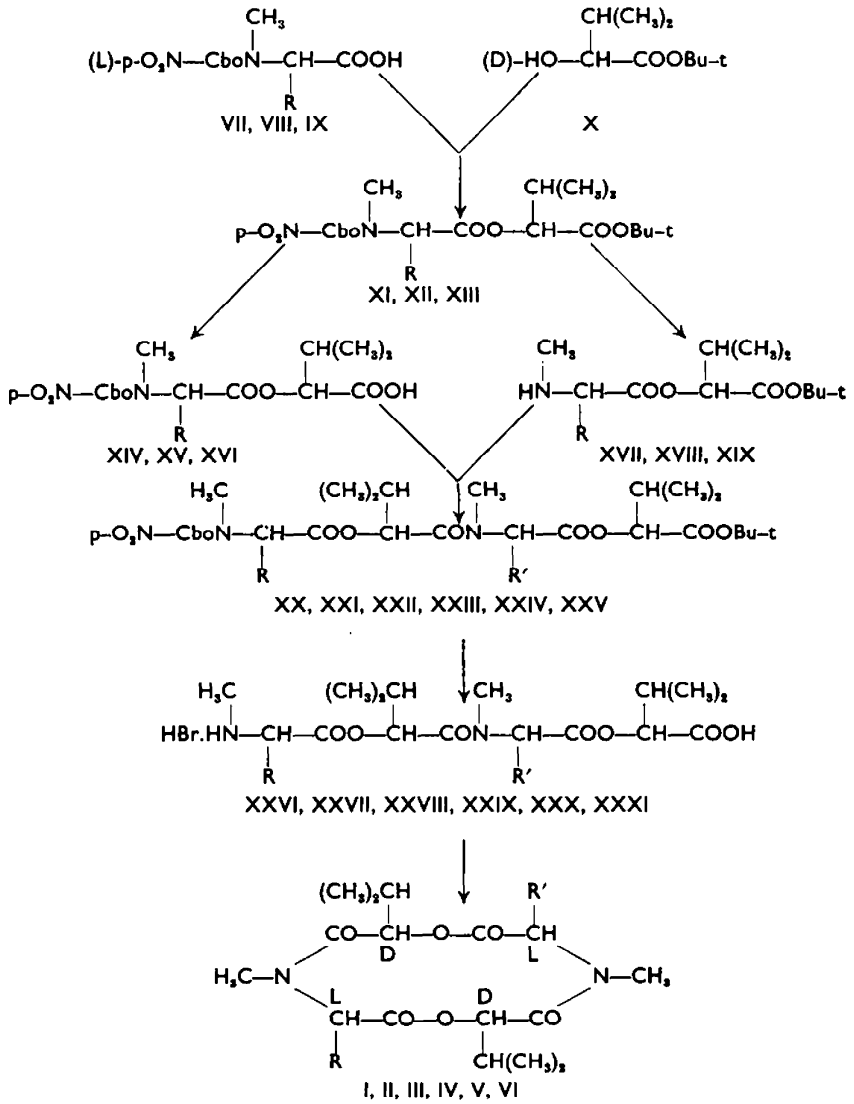
[11] A. H. Cook, S. F. Cox and T. H. Farmer, *J. Chem. Soc.* 1022 (1949).

[12] M. M. Shemyakin, *Angew. Chem.* **71,** 741 (1959), **72,** 342 (1960); *Uspekhi Khim.* **31,** 269 (1962).

[13] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, V. A. Oladkina and L. A. Shchukina, *Dokl. Akad. Nauk SSSR* **140,** 387 (1961).

acid chloride method ($SOCl_2$, Et_3N in benzene) affords the corresponding cyclotetradepsipeptides, in yields of 70–75%.*

In all physical and in certain chemical properties (greater resistance towards acid and alkaline hydrolysis) the cyclotetradepsipeptides I–VI differ considerably from all antibiotics of the enniatin group described both by Plattner[3–5,8] and by Cook.[9]

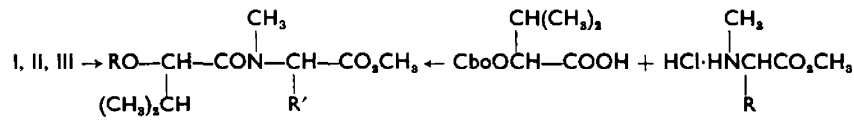


VII, XI, XIV, XVII R = $CH(CH_3)CH_2CH_3$; VIII, XII, XV, XVIII R = $CH(CH_3)_2$;
IX, XIII, XVI, XIX R = $CH_2CH(CH_3)_2$; I, XX, XXVI R = R′ = $CH(CH_3)CH_2CH_3$;
II, XXI, XXVII R = R′ = $CH(CH_3)_2$; III, XXII, XXVIII R = R′ = $CH_2CH(CH_3)_2$;
IV, XXIII, XXIX R = $CH(CH_3)_2$, R′ = $CH(CH_3)CH_2CH_3$; V, XXIV, XXX R = $CH_2CH(CH_3)_2$,
R′ = $CH(CH_3)CH_2CH_3$; VI, XXV, XXXI R = $CH(CH_3)_2$, R′ = $CH_2CH(CH_3)_2$.

* Cyclization of XXVII by the mixed anhydride method ($ClCOOC_2H_5$ in tetrahydrofuran) resulted in only a 17% yield of II.

Moreover, none of the cyclotetradepsipeptides synthesized display activity against *Mycobacterium phlei* in concentrations up to 100 γ/ml, whereas enniatin A is active in concentrations of 1–1·25 γ/ml and enniatin B at 3γ/ml.

The structure of the cyclodepsipeptides I–VI was established as follows. Analytical data and the results of molecular weight determinations (cryoscopic in benzene or isothermal distillation in acetone) are in conformity with the respective formulae. Acid hydrolysis of the cyclotetradepsipeptides I–III gives high yields of D-α-hydroxyisovaleric acid and N-methyl-L-iso-leucine, N-methyl-L-valine or N-methyl-L-leucine, respectively. The methyl esters (XXXII, XXXIII and XXXIV) are obtained on saponification of compounds I–III with barium hydroxide, followed by treatment of the products with diazomethane. The esters are also prepared by counter-synthesis, utilizing the chloride method and starting from O-benzyloxycarbonyl-D-α-hydroxyisovaleric acid (XXXV) and the methyl ester hydrochlorides of the corresponding N-methylamino acids (XXXVI, XXXVII and XXXVIII) followed by hydrogenolysis of the benzyloxycarbonyl esters (XXXIX, XL and XLI). The resultant compounds (XXXII and XXXIII) proved to be identical with the earlier described[6,7] degradation products of enniatins A and B.*



XXXII R = H, R′ = $CH(CH_3)CH_2CH_3$; XXXIII R = H, R′ = $CH(CH_3)_2$;
XXXIV R = H, R′ = $CH_2CH(CH_3)_2$; XXXVI R = $CH(CH_3)CH_2CH_3$;
XXXVII R = $CH(CH_3)_2$; XXXVIII R = $CH_2CH(CH_3)_2$; XXXIX R = Cbo,
R′ = $CH(CH_3)CH_2CH_3$; XL R = Cbo, R′ = $CH(CH_3)_2$; XLI R = Cbo,
R′ = $CH_2CH(CH_3)_2$.

It follows, therefore that the formulae I–VI proposed by Plattner *et al.* for the enniatin antibiotics do not represent these compounds and the problem of their structure still remains to be clarified.

EXPERIMENTAL

1. *Tosyl-N-methyl-L-valine*

A solution of 10·85 g (0·04 mole) tosyl-L-valine[15,16] in 80 ml 2N NaOH was shaken in a sealed tube (70°, 1 hr) with 17·8 g (0·125 mole) methyl iodide. The mixture was cooled, washed with chloroform (2 × 20 ml), acidified (to congo) with conc HCl and extracted with ether (4 × 50 ml). The extract was dried ($MgSO_4$) and evaporated. After crystallization of the residue from a 1:1 di-*n*-propyl ether–hexane mixture, 8·55 g (75%) tosyl-N-methyl-L-valine was obtained; m.p. 89–90°, $[\alpha]_D^{20}$ −66° (*c* 1·0, C_6H_6). (Found: C, 54·74; H, 6·87; N, 4·90; S, 11·20. $C_{13}H_{19}O_4NS$ requires: C, 54·71; H, 6·71; N, 4·91; S, 11·24%).

* The corresponding amide and hydrazide have also been obtained from the methyl ester (XXXIII). These derivatives were also found to be identical with the corresponding compounds obtained on degradation of enniatin B.[7,14]

[14] G. E. Hall, *Chem. & Ind.* 1270 (1960).

[15] E. W. McChesney and W. K. Swann, *J. Amer. Chem. Soc.* **59**, 1116 (1937).

[16] P. Karrer and F. C. van der Sluys Veer, *Helv. Chim. Acta* **15**, 746 (1932).

2. *Tosyl-N-methyl-L-isoleucine*

From 14·3 g (0·05 mole) tosyl-L-isoleucine[17] and 14 g (0·1 mole) methyl iodide under the conditions of experiment 1, 12 g (80%) tosyl-N-methyl-L-isoleucine were obtained as an oil, $[\alpha]_D^{20}$ −47° (*c* 1·0, C_6H_6)*. (Found: C, 56·19; H, 7·31; N, 4·89; S, 10·53. $C_{14}H_{21}O_4NS$ requires: C, 56·16; H, 7·07; N, 4·68; S, 10·70%).

3. N-*Methyl-L-valine hydrobromide*

A solution of 4 g (14 moles) tosyl-N-methyl-L-valine in 30 ml hydrogen bromide-saturated glacial acetic acid was heated in a sealed tube at 70° for 2 hr. The mixture was then poured into water, washed with ether and the aqueous solution evaporated *in vacuo* to dryness. The solid residue was recrystallized from acetone to give 2·3 g (77%) N-methyl-L-valine hydrobromide; m.p. 183–184°; $[\alpha]_D^{20}$ + 23° (*c* 0·5, C_2H_5OH). (Found: C, 33·83; H, 6·47; N, 6·95; Br. 37·93. $C_6H_{14}NO_2Br$ requires: C, 33·98; H, 6·65; N, 6·61; Br, 37·68%).

4. N-*Methyl-L-isoleucine hydrobromide*

From 5·7 g (0·02 mole) tosyl-N-methyl-L-isoleucine, 3·16 g (70%) N-methyl-L-isoleucine hydrobromide was prepared under the conditions of experiment 3; m.p. 178–179°; $[\alpha]_D^{20}$ +40° (*c* 0·5, C_2H_5OH). (Found: C, 37·20; H, 7·17; N, 6·10; Br, 35·10. $C_7H_{16}NO_2Br$ requires: C, 37·18; H, 7·13; N, 6·19; Br, 35·34%).

5. N-*methyl-L-leucine hydrobromine*

From 5·7 g (0·02 mole) tosyl-N-methyl-L-leucine,[18] 3·3 g (73%) N-methyl-L-leucine hydrobromide was prepared under conditions of experiment 3; m.p. 168–169° (from acetone); $[\alpha]_D^{20}$ +28° (*c* 0·6, C_2H_5OH). (Found: C, 36·97; H, 7·30; N, 6·38; Br, 34·89. $C_7H_{16}NO_2Br$ requires: C, 37·18; H, 7·13; N, 6·19; Br, 35·34%).

6. p-*Nitrobenzyloxycarbonyl-N-methylamino acids* (VII, VIII, IX)

To a solution of 0·1 mole N-methylamino acid hydrobromide in 55 ml 4N NaOH, 25 ml 4N NaOH and a solution of 26·9 g (0·125 mole) *p*-nitrobenzyloxycarbonyl chloride in 35 ml dioxane were added gradually in approximately equal portions under vigorous shaking and cooling in ice. The mixture was shaken for another hr at 0–5°, diluted with water, acidified with conc HCl and the oil which formed extracted with ether. The ether solution was washed with water and extracted with a saturated solution of $NaHCO_3$. The bicarbonate solution was then acidified with conc HCl, extracted with ether and the ether solution after drying ($MgSO_4$) was evaporated. The residue after recrystallization from dipropyl ether or dipropyl ether–heptane mixture afforded the corresponding *p*-nitrobenzyloxycarbonyl acids (VII, VIII or IX) in 85–90% yields.

Comp.	Molecular formula	m.p.	$[\alpha]_D^{20}$	Analysis					
				Found %			Required %		
				C	H	N	C	H	N
VII	$C_{15}H_{20}O_6N_2$	73–74°	−66° *c* 0·7, C_6H_6	55·67	6·24	8·57	55·55	6·22	8·64
VIII	$C_{14}H_{18}O_6N_2$	83·5–84°	−84° *c* 1·0, C_6H_6	54·26	5·97	9·13	54·19	5·85	9·03
IX	$C_{15}H_{20}O_6N_2$	99–100°	−24° *c* 0·7, C_6H_6	55·59	6·21	8·64	55·55	6·22	8·64

* Purified by chromatography on silica gel with benzene–ether solvent system.

[17] P. G. Katsoyannis and V. du Vigneaud, *J. Amer. Chem. Soc.* **76**, 3113 (1954).
[18] E. Fischer and W. Lipschitz, *Ber. Dtsch. Chem. Ges.* **48**, 360 (1915).

7. *O-Benzyloxycarbonyl-D-α-hydroxyisovaleric acid* (XXXV)

To a solution of 118 g (1·0 mole) D-α-hydroxyisovaleric acid[19] in 800 ml dry pyridine 188 g (1·1 mole) benzyloxycarbonyl chloride was added under stirring (−20°, 1 hr). The mixture was stirred for another 2 hr at 0°, left to stand overnight at 20°, diluted with water (300 ml) and evaporated *in vacuo*. The residue was dissolved in 1·5 l. ether, the ether solution washed with 2N HCl (2 × 200 ml) and extracted with a saturated solution of $NaHCO_3$ (6 × 200 ml). The extract was acidified to congo with conc HCl and the oily product which separated extracted with ether. The ether solution after drying ($MgSO_4$) was evaporated, giving 207 g (82%) benzyloxycarbonyl acid (XXXV); m.p. 57–58° (from petroleum ether), $[\alpha]_D^{20}$ +9·7° (*c* 0·6, C_6H_6). (Found: C, 61·93; H, 6·34. $C_{13}H_{16}O_5$ requires: C, 61·89; H, 6·39%).

8. *t-Butyl D-α-hydroxyisovalerate* (X)

A mixture of 126 g (0·5 mole) benzyloxycarbonyl acid (XXXV), 1 l. methylene chloride and 25 g TsOH was saturated with isobutylene at 5–10° (until the volume was increased by 500 ml). The solution was left to stand for 70 hr at 20°, diluted with ether, washed with 10% Na_2CO_3 solution and then with water and, after drying ($MgSO_4$) evaporated *in vacuo*. The resultant t-butyl O-benzyloxycarbonyl-D-α-hydroxyisovalerate (142 g, 92%) was dissolved in 800 ml methanol and hydrogenated in a current of hydrogen (20°, 40 hr) in the presence of a Pd-catalyst (from 3 g PdO). After filtering off the catalyst and evaporating the filtrate *in vacuo* the residue was dissolved in 500 ml ether. The ether solution was washed with a saturated solution of $NaHCO_3$, dried ($MgSO_4$) and evaporated. After fractionation of the residue *in vacuo* 56 g (64%) *t*-butyl ester (X) was obtained; b.p. 42–43° (0·5 mm); m.p. 30–31°; $[\alpha]_D^{20}$ +2·9° (*c* 0·8, C_6H_6). (Found: C, 61·70; H, 10·48. $C_9H_{18}O_3$ requires: C, 62·04; H, 10·41%).

9. *t-Butyl esters* (XI, XII, XIII)

To a solution of 0·12 mole *p*-nitrobenzyloxycarbonylamino acids (VII, VIII or IX) in 100 ml dry pyridine 19·4 g (0·11 mole) benzenesulfonyl chloride was added with stirring (0°, 10 min). After 15 min a solution of 17·4 g (0·1 mole) t-butyl ester (X) in 20 ml dry pyridine was added; the mixture was stirred for 2 hr at 0° and then for 3 hr at 20°, following which it was poured into water and extracted with ether. The extract was washed with 1N H_2SO_4, water, saturated $NaHCO_3$ solution and, after drying with $MgSO_4$, evaporated. The residue was subjected to chromatography on a neutral Al_2O_3 column and the corresponding t-butyl esters (XI, XII or XIII) obtained as oily products in 75–80% yields on elution with benzene.

Comp.	Molecular formula	$[\alpha]_D^{20}$	Analysis					
			Found %			Required %		
			C	H	N	C	H	N
XI	$C_{24}H_{36}O_8N_2$	−57° *c* 0·4, C_6H_6	60·28	7·47	5·63	59·98	7·55	5·83
XII	$C_{23}H_{34}O_8N_2$	−50° *c* 1·3, C_6H_6	59·52	7·54	6·07	59·21	7·35	6·01
XIII	$C_{24}H_{36}O_8N_2$	−37° *c* 0·5, C_6H_6	60·30	7·48	6·08	59·98	7·55	5·83

10. p-*Nitrobenzyloxycarbonyl acids* (XIV, XV, XVI)

A mixture of 50 mmoles *t*-butyl ester (XI, XII or XIII) and 2 g TsOH was refluxed for 1·5 hr in 100 ml benzene and then diluted with 400 ml ether, washed with water and extracted with saturated $NaHCO_3$ solution (4 × 100 ml). The extract was acidified (to congo) with conc HCl and the oil

[19] L. A. Shchukina, R. G. Vdovina, Yu. B. Shvetsov and A. V. Karpova, *Izv. Acad. Nauk SSSR, Otdel. Khim. Nauk* 310 (1962).

formed, extracted with ether. The extract was dried ($MgSO_4$) and evaporated. The residue on recrystallization from a mixture of hexane and benzene or dipropyl ether gave the corresponding *p*-nitrobenzyloxycarbonyl acids (XIV, XV or XVI) in 70–80% yields.

Comp.	Molecular formula	m.p.	$[\alpha]_D^{20}$	Analysis					
				Found %			Required %		
				C	H	N	C	H	N
XIV	$C_{20}H_{28}O_8N_2$	63–64°	−71° *c* 0·6, C_6H_6	56·61	6·59	6·81	56·59	6·65	6·60
XV	$C_{19}H_{26}O_8N_2$	83–84°	−86° *c* 0·6, C_6H_6	55·83	6·52	7·02	55·60	6·39	6·83
XVI	$C_{20}H_{28}O_8N_2$	oil*	−35° *c* 1·0, C_6H_6	56·49	6·59	6·75	56·59	6·65	6·60

* Purified by chromatography on silica gel with the aid of benzene–ether solvent system.

11. *Amino esters* (XVII, XVIII, XIX).

A solution of 50 mmoles *p*-nitrobenzyloxycarbonyl ester (XI, XII or XIII) in 250 ml methanol containing 6 ml glacial acetic acid was hydrogenated (20°, 750 mm Hg) in the presence of a Pd-catalyst (from 0·5 g PdO) until the theoretical amount of hydrogen was taken up (3 hr). After filtering off the catalyst and diluting the filtrate with benzene the solution was washed with a saturated $NaHCO_3$ solution, dried ($MgSO_4$) and evaporated. The residue was fractionated *in vacuo* to give the corresponding amino esters (XVII, XVIII or XIX) as oily products in 70–75% yields.

Comp.	Molecular formula	b.p.	$[\alpha]_D^{20}$	Analysis					
				Found %			Required %		
				C	H	N	C	H	N
XVII	$C_{16}H_{31}O_4N$	90–92° (0·15 mm)	+23° *c* 1·0, C_6H_6	63·53	10·30	4·69	63·75	10·37	4·65
XVIII	$C_{15}H_{29}O_4N$	86–89° (0·15 mm)	+22° *c* 0·7, C_6H_6	63·01	10·05	4·73	62·68	10·17	4·87
XIX	$C_{16}H_{31}O_4N$	90–92° (0·15 mm)	+16° *c* 1·0, C_6H_6	63·78	10·29	4·83	63·75	10·37	4·65

12. *Tetradepsipeptides* (XX, XXI, XXII, XXIII, XXIV and XXV)

(a) To a solution of 15 mmoles *p*-nitrobenzyloxycarbonyl acid (XIV, XV or XVI) in 50 ml of dry ether cooled to 0° was added 5·2 g (25 mmoles) of finely ground PCl_5. The mixture was stirred for 2 hr at 0°, the solution decanted from the unreacted PCl_5 and evaporated *in vacuo*. The acid chloride residue was dissolved in 20 ml dry tetrahydrofuran and the resultant solution added (−40°, 1 hr) to a solution of 14 mmoles of the corresponding amino esters (XVII, XVIII or XIX) and 1·5 g (15 mmoles) of triethylamine in 30 ml dry tetrahydrofuran. The mixture was stirred for 1 hr, left to stand overnight at 20° and diluted with ether (200 ml). After washing with 1N H_2SO_4 (2 × 40 ml), water and saturated $NaHCO_3$ the solution was dried ($MgSO_4$) and evaporated. The residue was chromatographed on a neutral Al_2O_3 column and after elution with a benzene–ethyl acetate mixture the respective tetradepsipeptides (XX, XXI, XXII, XXIII, XXIV or XXV) were isolated as yellow oils* (yield 90–95%).

* The depsipeptide (XXII) crystallized on standing; m.p. 87–88° (from hexane).

Comp.	Molecular formula	$[\alpha]_D^{20}$	Analysis					
			Found %			Required %		
			C	H	N	C	H	N
XX	$C_{36}H_{57}O_{11}N_3$	−73° *c* 0·5, C_6H_6	61·39	7·95	6·20	61·08	8·12	5·93
XXI	$C_{34}H_{53}O_{11}N_3$	−97° *c* 0·5, C_6H_6	59·97	7·82	6·21	60·08	7·86	6·18
XXII	$C_{36}H_{57}O_{11}N_3$	−65° *c* 0·8, C_6H_6	61·35	8·06	6·13	61·08	8·12	5·93
XXIII	$C_{35}H_{55}O_{11}N_3$	−90° *c* 0·8, C_6H_6	60·40	7·93	6·06	60·59	7·99	6·06
XXIV	$C_{36}H_{57}O_{11}N_3$	−39° *c* 0·9, C_6H_6	61·08	8·13	5·97	61·08	8·12	5·93
XXV	$C_{35}H_{55}O_{11}N_3$	−75° *c* 0·8, C_6H_6	60·50	7·92	6·04	60·59	7·99	6·06

(b) To a solution of 282 mg (0·686 mmole) *p*-nitrobenzyloxycarbonyl acid (XV) in 2 ml methylene chloride 142 mg (0·686 mmole) N,N′-dicyclohexylcarbodiimide was added and then, on cooling with ice and stirring, a solution of 197 mg (0·686 mmole) amino ester (XVIII) in 2 ml methylene chloride. The mixture was allowed to stand 20 hr at 20° and then filtered. The filtrate was diluted with ether (20 ml), washed with 1N H_2SO_4 (2 × 5 ml), water and saturated $NaHCO_3$ (3 × 10 ml), and, after drying ($MgSO_4$), evaporated. The residue was chromatographed on a neutral Al_2O_3 column and on elution with a benzene–ethyl acetate mixture, 280 mg (60%) of the tetradepsipeptide (XXI) with $[\alpha]_D^{20}$ −97° (*c* 0·5, C_6H_6) obtained.

13. *Tetradepsipeptide hydrobromides* (XXVI, XXVII, XXVIII, XXIX, XXX and XXXI)

A solution of 10 mmoles of the tetradepsipeptides (XX, XXI, XXII, XXIII, XXIV or XXV) in 40 ml 35% glacial acetic acid solution of hydrogen bromide was allowed to stand at 20° for 9 hr and then evaporated *in vacuo*. The residue was dissolved in 20 ml ether and the solution extracted with water (3 × 50 ml). The resultant aqueous solution was evaporated to dryness *in vacuo* and dry toluene was twice added to the residue and then evaporated *in vacuo*. The corresponding hydrobromides (XXVI, XXVII, XXVIII, XXIX, XXX and XXXI) were obtained as amorphous powders in 70% yields.

14. *Cyclotetradepsipeptides* (I, II, III, IV, V and VI)

(a) A solution of 7 mmoles of the hydrobromides (XXVI, XXVII, XXVIII, XXIX, XXX or XXXI) in 15 ml thionyl chloride was allowed to stand for 30 min at 20°; the excess thionyl chloride distilled off *in vacuo* and dry toluene added to the residue and the latter again evaporated *in vacuo*. The resultant acid chloride was dissolved in 400 ml dry benzene and the solution added dropwise under stirring (20°, 10 hr) to 2 l. dry benzene, simultaneously with a solution of 18 mmoles triethylamine in 400 ml dry benzene. The mixture was left to stand overnight, following which 5 ml triethylamine was added and after 2 hr the mixture evaporated to dryness. The residue was taken up in ether, the ether solution washed with 1N H_2SO_4, water, saturated $NaHCO_3$ and again with water, and then dried ($MgSO_4$) and evaporated. The semicrystalline residue was chromatographed on a neutral Al_2O_3 column and on elution with benzene and then a mixture of benzene and ethyl acetate (with gradual increase in concentration of the latter from 3 to 30%) the respective cyclotetradepsipeptides (I, II, III, IV, V or VI) were obtained in 70–75% yields. They were recrystallized from *n*-heptane.

(b) To a solution of 22 mg (0·2 mmole) ethyl chloroformate in 2 l. dry tetrahydrofuran were added under stirring (6 hr, 0°) a solution of 109 mg (1 mmole) ethyl chloroformate in 200 ml dry tetrahydrofuran and simultaneously a solution of the triethylammonium salt of N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleric acid obtained from 526 mg (1 mmole) of the

Comp.	Molecular formula (Mol. W)	$[\alpha]_D^{20}$	R_f (C_5H_5N-iso-$C_5H_{11}OH$-H_2O 1:1:2)	Mol. W. (titr. 0·1 N NaOH)	Analysis	
					Found %	Required %
					Br	Br
XXVI	$C_{34}H_{45}O_7N_3Br$ (553·7)	−27° *c* 0·9, C_2H_5OH	0·87	552	15·11	14·67
XXVII	$C_{32}H_{41}O_7N_3Br$ (525·5)	−11·5° *c* 0·5, C_2H_5OH	0·77	524	15·18	15·21
XXVIII	$C_{34}H_{45}O_7N_3Br$ (553·7)	−10·5° *c* 0·9, C_2H_5OH	0·85	558	14·75	14·67
XXIX	$C_{33}H_{43}O_7N_3Br$ (539·6)	−31° *c* 0·9, C_2H_5OH	0·85	544	15·24	15·05
XXX	$C_{34}H_{45}O_7N_3Br$ (553·7)	−21° *c* 1·0, C_2H_5OH	0·92	560	15·12	14·67
XXXI	$C_{33}H_{43}O_7N_3Br$ (539·6)	−9° *c* 0·8, C_2H_5OH	0·84	546	15·49	15·05

Comp.	Molecular formula (Mol. W)	m.p.	$[\alpha]_D^{20}$	Mol. weight (Cryosc. in C_6H_6)	Analysis					
					Found %			Required %		
					C	H	N	C	H	N
I	$C_{24}H_{42}O_6N_2$ (454·6)	215–216°	+13·5° *c* 0·8, $CHCl_3$	461	63·59	9·50	6·09	63·41	9·31	6·16
II	$C_{22}H_{38}O_6N_2$ (426·5)	228–229°	+4·8° *c* 0·9, $CHCl_3$	424*	61·81	8·98	6·71	61·94	8·98	6·57
III	$C_{24}H_{42}O_6N_2$ (454·6)	156–157°	+35° *c* 1·0, $CHCl_3$	441	63·41	9·35	6·21	63·41	9·31	6·16
IV	$C_{23}H_{40}O_6N_2$ (440·6)	206–207°	−9° *c* 0·8, $CHCl_3$	439	62·48	9·09	6·36	62·70	9·15	6·36
V	$C_{24}H_{42}O_6N_2$ (454·6)	164–165°	−24° *c* 0·9, $CHCl_3$	449	63·57	9·21	5·88	63·41	9·31	6·16
VI	$C_{23}H_{40}O_6N_2$ (440·6)	164–165°	+22° *c* 0·8, $CHCl_3$	430	62·84	9·26	6·41	62·70	9·15	6·36

* Isothermal distillation in acetone gave 417 and the thermoelectrical method in butyl acetate, 460

hydrodromide (XXVII) and 202 mg (2 mmole) of triethylamine in 200 ml dry tetrahydrofuran. The mixture was stirred for 30 hr at 20° and then 0·5 g of triethylamine was added and the stirring continued for another 3 hr. The mixture was then evaporated *in vacuo* and treated as in Experiment 14a; cyclodepsipeptide (II) identical with the substance obtained in the previous experiment was isolated in a yield 73 mg (17%).

15. *Acid hydrolysis of cyclotetradepsipeptides* (I, II and III)

A solution of 0·45 mmole of the cyclodepsipeptides (I, II or III) in 5 ml conc HCl was heated in a sealed tube at 100° for 20 hr. The mixture was diluted with water (5 ml) and extracted with ether (10 × 5 ml). The ether solution was extracted with saturated $NaHCO_3$ (5 × 5 ml), dried ($MgSO_4$) and evaporated, yielding 50–55% of the original unreacted depsipeptide.* The acidic aqueous solution was evaporated to dryness *in vacuo* and the residue dissolved in 10 ml water. To this solution was

* In the case of the cyclodepsipeptide (III) acid hydrolysis goes to completion.

added 200 mg freshly precipitated Ag_2CO_3 and the mixture shaken for 2 hr. The precipitate of silver salts was filtered off, the filtrate saturated with hydrogen sulfide, boiled for 10 min, the precipitate then filtered, the filtrate evaporated *in vacuo* and the solid residue dried over P_2O_5. After twofold sublimation *in vacuo* the corresponding N-methylamino acid was obtained in 85–90% yield.

N-Methylamino acid	$[\alpha]_D^{20}$ in 5N HCl	R_f tert.-Amyl alcohol–water
N-Methyl-L-isoleucine	+40·0°, *c* 1·0	0·30
N-Methyl-L-valine	−28·5°, *c* 0·85	0·18
N-Methyl-L-leucine	+23·5°, *c* 0·7	0·36

The bicarbonate extract was acidified to congo with conc HCl, saturated with $(NH_4)_2SO_4$ and extracted with ether (10 × 5 ml). The ethereal solution was dried ($MgSO_4$) and evaporated; on sublimation of the residue *in vacuo* D-α-hydroxyisovaleric acid was obtained in 75–80% yield; m.p. 67–68°; $[\alpha]_D^{20}$ −13·5° (*c* 1·2, CH_3COOH), −19° (*c* 1·0, $CHCl_3$); cf.[11,19]

16. *Alkaline hydrolysis of the cyclotetradepsipeptides* (I, II *and* III)

To a solution of 0·7 mmole of the cyclotetradepsipeptides (I, II or III) in 10 ml methyl alcohol, 5 ml of 0·35 N aqueous $Ba(OH)_2$ was added. The mixture was allowed to stand for 30 hr at 35°, diluted with water, washed with ether, acidified (to congo) with conc HCl and extracted with ether (10 × 10 ml). The ether extract was washed with water, dried and then at 0° an ether solution of diazomethane was added until a stable yellow colour appeared. After 1 hr the excess diazomethane was decomposed with acetic acid, the ether solution washed with saturated $NaHCO_3$, dried ($MgSO_4$) and evaporated. The residue was recrystallized from hexane, giving 85–90% yields the respective methyl esters (XXXII, XXXIII or XXXIV), identical with those described in Experiment 19.

17. *Methyl esters of the* N-*methylamino acid hydrochlorides* (XXXVI, XXXVII *and* XXXVIII)

A solution of 0·1 mole of the N-methylamino acid hydrobromide in 1 l. methyl alcohol was saturated with hydrogen chloride. After 24 hr it was evaporated *in vacuo*, the residue was dissolved in 1 l. methyl alcohol saturated with HCl and left for 24 hr and the operation repeated. The solid residue obtained on evaporation was carefully dried *in vacuo* over P_2O_5 and the corresponding hydrochlorides (XXXVI, XXXVII or XXXVIII) were isolated in quantitative yields and recrystallized from acetone.

Comp.	Molecular formula	m.p.	$[\alpha]_D^{20}$	Analysis: Found % C	H	N	Cl	Required % C	H	N	Cl
XXXVI	$C_8H_{18}O_2NCl$	154–155°	+39° *c* 0·7, C_2H_5OH	49·10	9·23	7·20	18·32	49·10	9·27	7·16	18·11
XXXVII	$C_7H_{16}O_2NCl$	140–141°	+17·5° *c* 1·0, H_2O	45·97	8·87	7·46	19·70	46·25	8·87	7·71	19·51
XXXVIII	$C_8H_{18}O_2NCl$	127–128°	+30° *c* 1·0, C_2H_5OH	49·29	9·40	7·22	18·04	49·10	9·27	7·16	18·11

18. *Benzyloxycarbonyl esters* (XXXIX, XL *and* XLI)

To a solution of 10·1 g (0·04 mole) benzyloxycarbonyl acid (XXXV) in 50 ml dry ether 12·5 g (0·06 mole) PCl_5 was added at 0° and the mixture stirred at that temp for 2 hr. The supernatant liquor was decanted off and evaporated *in vacuo*. The residual acid chloride was dissolved in 50 ml dry tetrahydrofuran and under stirring were added (−40°, 2 hr) to a solution of 0·03 mole of the hydrochlorides (XXXVI, XXXVII or XXXVIII) and 0·07 mole triethylamine in 50 ml dry tetrahydrofuran. The mixture was stirred for 2 hr at 0° and left to stand overnight. It was then diluted with ether and the ether solution washed with water, 1 N H_2SO_4 and saturated $NaHCO_3$, dried ($MgSO_4$) and evaporated. The residue was recrystallized from hexane, to give the corresponding benzyloxycarbonyl ester (XXXIX, XL or XLI) in 90–95% yields.

Comp.	Molecular formula	m.p.	$[\alpha]_D^{20}$	Analysis					
				Found %			Required %		
				C	H	N	C	H	N
XXXIX	$C_{31}H_{31}O_6N$	84–85°	−85° *c* 0·8, C_6H_6	64·27	7·94	3·74	64·10	7·94	3·56
XL	$C_{29}H_{29}O_6N$	73·5–74	−83° *c* 1·4, C_2H_5OH	63·04	7·63	3·51	63·30	7·70	3·69
XLI	$C_{31}H_{31}O_6N$	42–43°	−25·4° *c* 1·0, C_6H_6	64·32	7·90	3·68	64·10	7·94	3·56

19. *Methyl esters* (XXXII, XXXIII *and* XXXIV)

The benzyloxycarbonyl esters (XXXIX, XL or XLI; 5 mmoles in 40 ml ethanol) were hydrogenated with hydrogen (5 hr, 20°, 750 mm Hg) in the presence of a palladium catalyst (from 50 mg of PdO). On recrystallization from hexane the corresponding methyl esters (XXXII, XXXIII or XXXIV) were isolated in 90–95% yields, cf.[6,7]

Comp.	Molecular formula	m.p.	$[\alpha]_D^{20}$	Analysis					
				Found %			Required %		
				C	H	N	C	H	N
XXXII	$C_{18}H_{25}O_4N$	97–98°	−111° *c* 1·1, $CHCl_3$	60·38	9·70	5·53	60·20	9·72	5·40
XXXIII	$C_{12}H_{23}O_4N$	68·5–69°	−132° *c* 1·5, $CHCl_3$	58·88	9·41	5·71	58·75	9·45	5·71
XXXIV	$C_{18}H_{25}O_4N$	68–69°	−40° *c* 0·8, $CHCl_3$	60·20	9·73	5·51	60·20	9·72	5·40

20. D-α-*Hydroxyisovaleryl*-N-*methyl*-L-*valinamide*

A solution of 1 g (4·07 mmoles) methyl ester (XXXIII) in 20 ml methyl alcohol was saturated with ammonia and left 40 hr at 20°. The mixture was evaporated to dryness and the residue, recrystallized from a benzene–hexane (1:1) mixture. The yield of amide was 780 mg (83%); m.p. 109–110°, $[\alpha]_D^{20}$ −169° (*c* 1·3, C_2H_5OH); cf.[14] (Found: C, 57·33; H, 9·53; N, 12·04. Calc. for $C_{11}H_{22}O_3N_2$: C, 57·36; H, 9·63; N, 12·17%).

21. D-α-*Hydroxyisovaleryl*-N-*methyl*-L-*valinohydrazide*

A mixture of 1 g (4·07 mmoles) methyl ester (XXXIII), 0·3 g (6 mmoles) hydrazine hydrate and 7 ml ethyl alcohol was refluxed for 6 hr. It was then evaporated to dryness and the residue recrystallized from a 1:1 benzene–hexane mixture. The yield of hydrazide was 850 mg (85%); m.p. 142–144°; $[\alpha]_D^{20}$ −163° (*c* 1·0, C_2H_5OH); cf.[7] (Found: C, 53·96; H, 9·44; N, 17·38. Calc. for $C_{11}H_{22}O_3N_2$: C, 53·85; H, 9·45; N, 17·13%).

Note added in proof—On the basis of Plattner's spectral data which showed the cyclotetradepsipeptide (II) and enniatin B to be chemically very similar, we made the assumption that the latter is a cyclopolymer homolog of II. This belief was further strengthened when Prof. V. Prelog recently informed one of us that enniatin B had been incorrectly assigned too low a molecular weight. Accordingly, using the above described methods, we synthesized the corresponding cyclohexa- and cyclooctadepsipeptides, of which the first proved to be identical with enniatin B. Hence enniatin B is cyclo-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl.