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CONCERNING THE STRUCTURE OF ENNIATIN B

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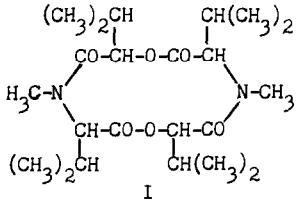
IN 1947 Plattner and collaborators[1,2,3] isolated two new antimycobacterial substances, enniatin A ($C_{24}H_{42}N_2O_6$, m.p. 121-122°, $[\alpha]_D^{20}$ -92°, c 1 in $CHCl_3$) and enniatin B ($C_{22}H_{38}N_2O_6$, m.p. 173-175°, $[\alpha]_D^{20}$ -108°, c 0.6 in $CHCl_3$) from the mycelium of a number of Fusarium strains. Based on the results of acid and alkaline hydrolysis enniatin B was ascribed structure (I).[4]



I

In the same year Cook et al.[5] obtained five closely related antibiotics from the culture medium of certain strains of Fusarium. Of the compounds isolated, one, lateritiin I ($C_{26}H_{46}N_2O_7$, m.p. 121-122°, $[\alpha]_D^{20}$ -95.6°, c 1 in EtOH) was found to possess the same properties as enniatin A. However it was later shown[6,7] that hydrolysis of lateritiin I affords the same

1 E. Gäumann, S. Roth, L. Ettlinger, Pl.A. Plattner and U. Nager, Experientia 3, 202 (1947).

2 Pl.A. Plattner and U. Nager, Experientia 3, 325 (1947).

3 Pl.A. Plattner, U. Nager and A. Boller. Helv. Chim. Acta 31, 594 (1948).

4 Pl.A. Plattner and U. Nager, Helv. Chim. Acta 31, 665 (1948).

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

6 A.H. Cook, S.F. Cox and T.H. Farmer, Nature, Lond. 162, 61 (1948).

7 A.H. Cook, S.F. Cox and T.H. Farmer, J. Chem. Soc. 1022 (1949).

products as in the case of enniatin B. These results cast some doubt on the validity of formula (I) for enniatin B.

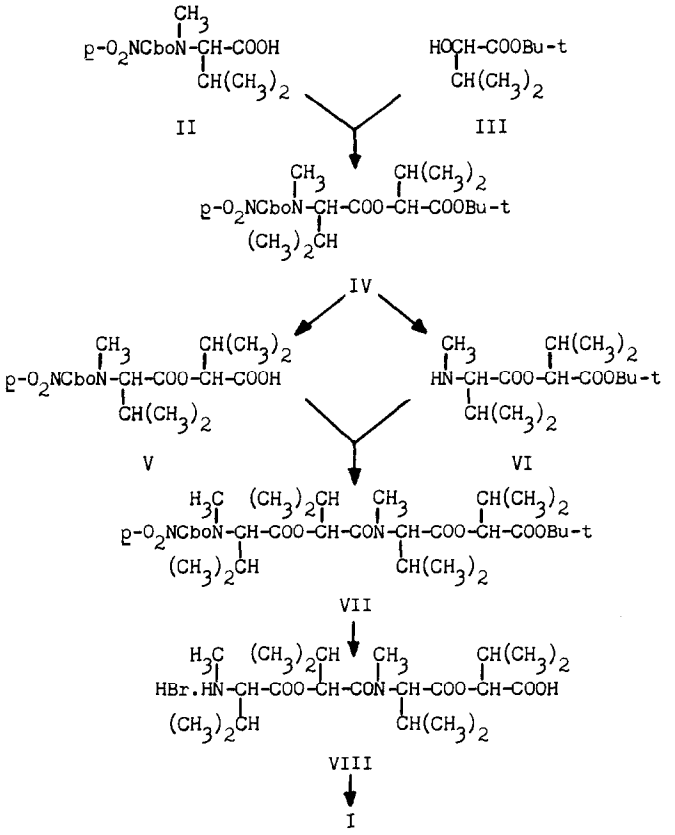
We undertook the synthesis of the cyclotetradepsipeptide (I), considered by Plattner to be enniatin B, utilizing previously developed routes[8,9] to optically active linear and cyclic depsipeptides.

Condensation of p-nitrocarbobenzoxy-N-methyl-L-valine (II) with t-butyl D-α-hydroxyisovalerate (III) by the mixed anhydride method ($PhSO_2Cl$ in pyridine; 20°; 2 hr) gave t-butyl p-nitrocarbobenzoxy-N-methyl-L-valyl-D-α-hydroxyisovalerate (IV) in 80 per cent yield. Refluxing the latter in benzene in the presence of TsOH led to p-nitrocarbobenzoxy acid (V) (yield 75 per cent). At the same time hydrogenolysis of (IV) in the presence of a Pd-catalyst gave a 70 per cent yield of the aminoester (VI). An amide bond was made between (V) and (VI) using the acid chloride method (PCl_5, Et_3N in tetrahydrofuran; -30°; 5 hr), the tetradepsipeptide (VII) being obtained in 90 per cent yield (60 per cent using carbodiimide; 20°; 15 hr). Simultaneous elimination of the C- and N-protecting groups by the action of HBr in AcOH on (VII) (20°; 8 hr) afforded the hydrobromide (VIII) (yield 70 per cent). Cyclization of this compound by the chloride method (PCl_5; Et_3N in benzene; 20°; 30 hr) or by the mixed anhydride method ($ClCOOC_2H_5$ in tetrahydrofuran; 0°; 20 hr) gave (I) in 60 and 20 per cent yields, respectively.

Compound (I) was found to differ considerably in its physical, biological and certain of its chemical properties from enniatin B. The structure of the compound was established as follows: Determination of the molecular weight by three different methods gave the values 424 (cryoscopically, in benzene), 417 (isothermal distillation, in acetone) and

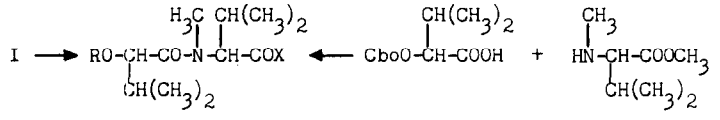
[8] M.M. Shemyakin, Angew. Chem. 71, 741 (1959); 72, 342 (1960).

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



460 (thermoelectrically, in butyl acetate) in agreement with formula (I). Optically pure D-α-hydroxyisovaleric acid (80 per cent yield) and optically pure N-methyl-L-valine (90 per cent yield) were obtained on acid hydrolysis of cyclodepsipeptide (I) (conc. HCl; 100°; 20 hr). Saponification of (I) with $Ba(OH)_2$ in aqueous methanol (35°; 30 hr) followed by treatment of the products with CH_2N_2 gave the methyl ester of D-α-hydroxyisovaleryl-N-methyl-L-valine (IXa) in 95 per cent yield. The ester was then converted into the amide (IXb) and the hydrazide (IXc). It was also prepared by a counter synthesis from D-(X) and L-(XI) with the aid of the chloride method (PCl_5; Et_3N in tetrahydrofuran; -30°; 6 hr) and subsequent hydrogenolysis of the

amide (IXd). Compounds (IXa), (IXb) and (IXc) were found to be identical with the previously described[4,10] degradation products of enniatin B.



IX X XI

(IXa): R=H, X=OCH_3; (IXb): R=H, X=NH_2; (IXc): R=H, X=$NHNH_2$;
(IXd): R=Cbo, X=OCH_3.

The structure of cyclotetradepsipeptide (I) was confirmed by its I.R.-spectrum. The substance manifests no activity against Mycobacterium phlei in concentrations up to 150 γ/ml, whereas enniatin B is active at 3 γ/ml[3].

Comp.	b.p. or m.p.	$[\alpha]_D^{20}$	Found (%)			Calculated (%)		
			C	H	N	C	H	N
I	228-229° (heptane)	+4.8° (c 0.9, $CHCl_3$)	61.81	8.98	6.71	61.94	8.98	6.57
II [a]	83.5-84° (C_6H_6-hexane	-84° (c 1.0, C_6H_6)	54.26	5.97	9.18	54.19	5.85	9.03
III	30-31° (hexane)	+2.9° (c 0.8, C_6H_6)	61.70	10.48		62.04	10.41	
IV	oil [b]	-50° (c 1.3, C_6H_6)	59.52	7.54	6.07	59.21	7.35	6.01
V	83-84° (C_6H_6-hexane)	-86° (c 0.6, C_6H_6)	55.86	6.59	7.02	55.60	6.39	6.83
VI	86-89° (0.15 mm)	+22° (c 0.7, C_6H_6)	63.01	10.05	4.73	62.68	10.17	4.87
VII	oil [b]	-97° (c 0.5, C_6H_6)	59.97	7.82	6.21	60.08	7.86	6.18
VIII	90-93° (AcOEt)	-11.5° (c 0.5, EtOH)			5.30			5.33
IXd	73.5-74° (hexane)	-83° (c 1.4, EtOH)	63.04	7.63	3.51	63.30	7.70	3.69
X	57-58° (hexane)	+9.7° (c 0.6, C_6H_6)	61.93	6.34		61.89	6.39	
XI-HCl	140-141° (acetone)	+17.5° (c 1.0, H_2O)	45.97	8.87	7.46	46.25	8.87	7.71

[a] Obtained by resolution of the racemate with the aid of L-threo-1-(p-nitrophenyl)-2-amino-1,3-propanediol.

[b] Purified by chromatography on neutral Al_2O_3 in the system AcOEt-C_6H_6.

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

It thus follows that formula (I) does not represent enniatin B and the question as to the structure of the latter still remains open. At present we are engaged in checking the structures of other natural cyclodepsipeptides, beginning with enniatin A, the structure of which was proposed by Plattner[11] in 1948.

[11] Pl.A. Plattner and U. Nager, Helv. Chim. Acta 31, 2192 (1948).