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# THE STRUCTURE AND TOTAL SYNTHESIS OF ENNIATIN B

M.M. Shemyakin, Yu.A. Ovchinnikov, A.A. Kiryushkin
and V.T. Ivanov
Institute for Chemistry of Natural Products
U.S.S.R. Academy of Sciences, Moscow

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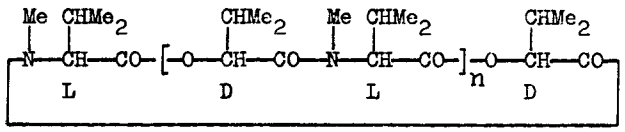
IN 1962[1,2] we described the synthesis of a cyclotetradepsipeptide (I, n=1) whose structure had been assigned by Plattner[3] in 1948 to the antibiotic enniatin B. However, it turned out that the synthetic compound differed from the naturally occuring antibiotic in physical, biological and some chemical properties. At the same time the only product of its alkaline hydrolisis was, as in the case of enniatin B[3], D-$\alpha$-hydroxyisovaleryl-N-methyl-L-valine.




(I)

[1] M.M. Shemyakin, Yu.A. Ovchinnikov, A.A. Kiryushkin, V.T. Ivanov, Tetrahedron Letters 301 (1962).

[2] M.M. Shemyakin, Yu.A. Ovchinnikov, A.A. Kiryushkin, V.T. Ivanov, Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk 2154 (1962).

[3] Pl.A. Plattner, U. Nager, Helv. Chim. Acta 31, 665 (1948).

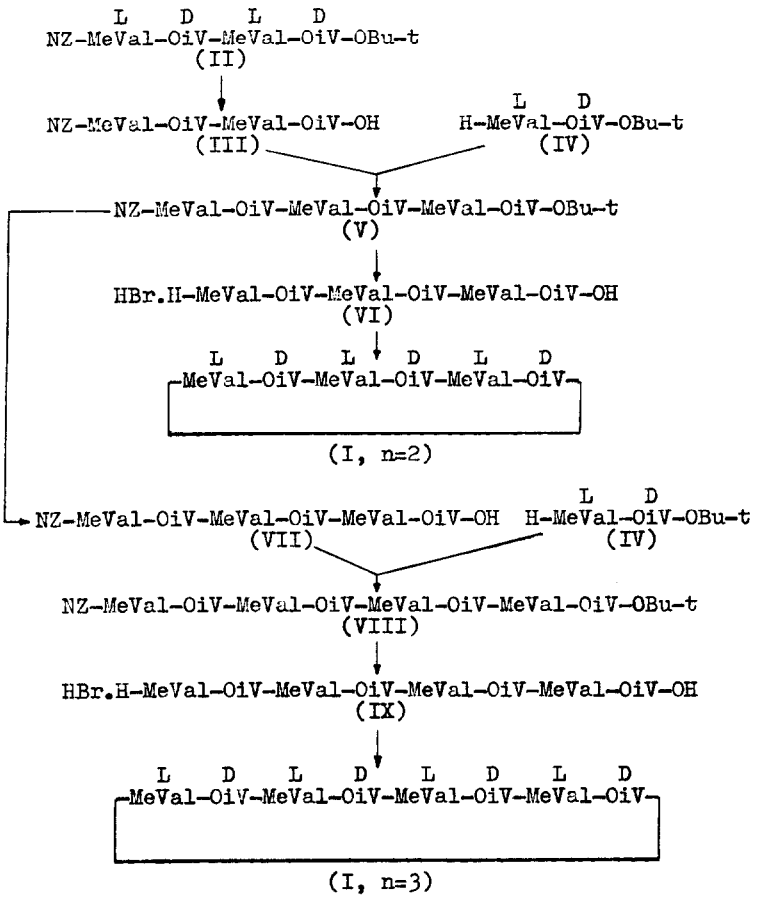
We therefore postulated[4] that only some minor difference should distinguish the chemical structures of these two substances. Their direct comparison would have been the easiest way to find the difference between these compounds and to thus solve the problem of the structure of enniatin B, but we regrettably had none of the latter at our disposal. Prof. Plattner to whom we had sent our synthetic sample very kindly sent us in August 1962 its IR and NMR spectra together with those of enniatin B taken under identical conditions. The IR spectra of both compounds were very similar in the region of the C=O, $N-CH_3$ and C-O frequencies, exhibiting differences only in the finger print region. The NMR spectra proved to be identical. Our assumption of the close relationship between these two compounds was therefore confirmed by their spectral characteristics. We considered the most plausible explanation of the similarity and differences of these compounds to lie in the assumption that enniatin B is a larger ring cyclopolymer homolog of compound (I, n=1). This belief was strengthened when in September 1962 at the First International Symposium on Pharmaceutical Chemistry (Florence) Prof. Prelog privately communicated to one of us (M.M. Shemyakin) that according to his data the molecular weight of enniatin B had been inaccurately determined and was probably too low. We therefore undertook the synthesis of the nearest cyclopolymer homologs of compound

[4] Yu.A. Ovchinnikov, V.T. Ivanov, A.A. Kiryushkin, M.M. Shemyakin, Report to the V European Symposium on Peptide Chemistry, Oxford, England, September 1962.

(I, n=1), namely the cyclohexadepsipeptide (I, n=2) and the cyclooctadepsipeptide (I, n=3), with the purpose of ascertaining whether one of them might not possess the structure of enniatin B. We carried out the synthesis according to Scheme I.

Treatment of tert.-butyl p-nitrobenzyloxycarbonyl-N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovalerate (II)[2] with hydrogen chloride in ether (20°, 20 hr) gave the corresponding acid (III) in 90% yield. Condensation of (III) with tert.-butyl N-methyl-L-valyl-D-α-hydroxyisovalerate (IV)[2] by the acid chloride method ($PCl_5$, $Et_3N$ in tetrahydrofuran, -30°, 5 hr) lead with 85% yield to the protected hexadepsipeptide (V). Simultaneous removal of the C- and N-protecting groups by treatment of the hexadepsipeptide with HBr in AcOH (20°, 3 hr) and subsequent cyclization of the resultant hydrobromide (VI) by the acid chloride method ($SOCl_2$, $Et_3N$ in benzene, 20°, 30 hr) afforded cyclo-N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleryl (I, n=2) in 60% yield. On the other hand, treatment of the hexadepsipeptide (V) with HCl in ether gave the p-nitrobenzyloxycarbonyl acid (VII) in 92% yield. This was condensed with the amino ester (IV) by the acid chloride method to the protected octadepsipeptide (VIII) (yield 80%). Treatment of (VIII) with HBr in AcOH transformed it into the hydrobromide (IX) and the latter on cyclization by the acid chloride method afforded the cyclooctadepsipeptide (I, n=3) in 65% yield. The constants

Scheme I




OiV = $-OCH(CHMe_2)CO-$; MeVal = $-N(Me)CH(CHMe_2)CO-$;

NZ = p-$O_2NC_6H_4CH_2OCO-$.

and analytic data of the compounds are presented in Table 1.

Table I

| Comp. | $[\alpha]_D^{20}$ | Analysis | | | | | | Mol. weight (Found) |
|---|---|---|---|---|---|---|---|---|
| | | Found % | | | Calculated % | | | |
| | | C | H | N | C | H | N | |
| III | -110° (c 0.65, $C_6H_6$) | 57.8 | 7.3 | 6.8 | 57.8 | 7.3 | 6.7 | |
| V | -120° (c 0.65, $C_6H_6$) | 60.8 | 8.1 | 6.2 | 60.6 | 8.1 | 6.3 | |
| VII | -120° (c 1.0, $C_6H_6$) | 58.8 | 7.7 | 6.6 | 58.8 | 7.7 | 6.7 | |
| VIII | -125° (c 1.3, $C_6H_6$) | 60.6 | 8.2 | 6.3 | 60.8 | 8.3 | 6.3 | |
| I, n=2[x] | -110° (c 0.8, $CHCl_3$) | 61.9 | 9.0 | 6.6 | 61.9 | 9.0 | 6.6 | 630 |
| I, n=3[x] | -106° (c 1.3, $CHCl_3$) | 62.0 | 9.0 | 6.6 | 61.9 | 9.0 | 6.6 | 840 |

[x] Compound (I, n=2) has m.p. 168-169° (from hexane); compound (I, n=3) has m.p. 177-179° (from hexane); the other compounds are amorphous and were purified by chromatography on alumina or silica gel in the system benzene - ethyl acetate.

The results of molecular weight determinations (cryoscopic in benzene) and analytic data of the synthetic cyclodepsipeptides (I, n=2) and (I, n=3) are in complete accord with the respective formulas. One of the compounds, namely the cyclohexadepsipeptide (I, n=2) proved to be identical in properties with enniatin B (m.p., specific rotation, biological activity). Comparison of the IR spectra of the synthetic product with that of enniatin B sent us by Plattner also confirmed their identity.

It is noteworthy that in contrast to the cyclotetradepsipeptide (I, n=1) and a number of its analogs, enti-

rely devoid of activity[5], the cyclooctadepsipeptide (I, n=3) is almost as active against Mycobacterium phlei as enniatin B (data of Dr. I.D. Ryabova).

We have thus completely established the structure of enniatin B and have carried out its total synthesis.

[5] M.M. Shemyakin, Yu.A. Ovchinnikov, V.T. Ivanov, A.A. Kiryushkin, Tetrahedron (1963) in press.