SYNTHESIS OF THE PROPOSED STRUCTURE OF VISCOSIN

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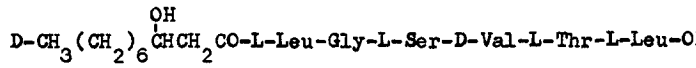
Summary - Two diastereoisomeric D-β-hydroxydecanoyl hexapeptides, (I) and (II), were synthesized. Although the structure (I) has been proposed for viscosin, neither compound was not identical with viscosin. The results therefore suggest that the structure of viscosin must be reinvestigated.

Viscosin, an acidic antibiotic active to tubercle bacillus and virus, was first isolated as colorless needles, m.p. 270 - 273° C, $[\alpha]_D$ -168.3 (c 1, C_2H_5OH) from Pseudomonas viscosa (Kochi,1951; Kochi et al.,1951; Groupé et al.,1951) and, from the results of chemical and physical examinations, the structure (I) has been proposed (Ohno et al.,1953; Toki et al.,1955, 1956).



(I)*

However, all depsipeptides containing β-hydroxy acids as hetero component hitherto isolated from bacteria and fungi have been appeared to have an even number of residues linked in a cyclic structure (Schröder et al.,1966; Russell, 1966), while no straight-chain and odd-membered depsipeptide such as (I) has yet been encountered in nature. The experimental bases for the D-configuration of valine residue in the structure (I) also seem to be insufficient.

These ambiguities prompted us to undertake synthetic study of the proposed structure (I) for viscosin.

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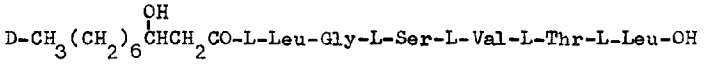
* For the nomenclature of peptide derivatives see Biochem. 5, 2485 (1966); 6, 362 (1967).

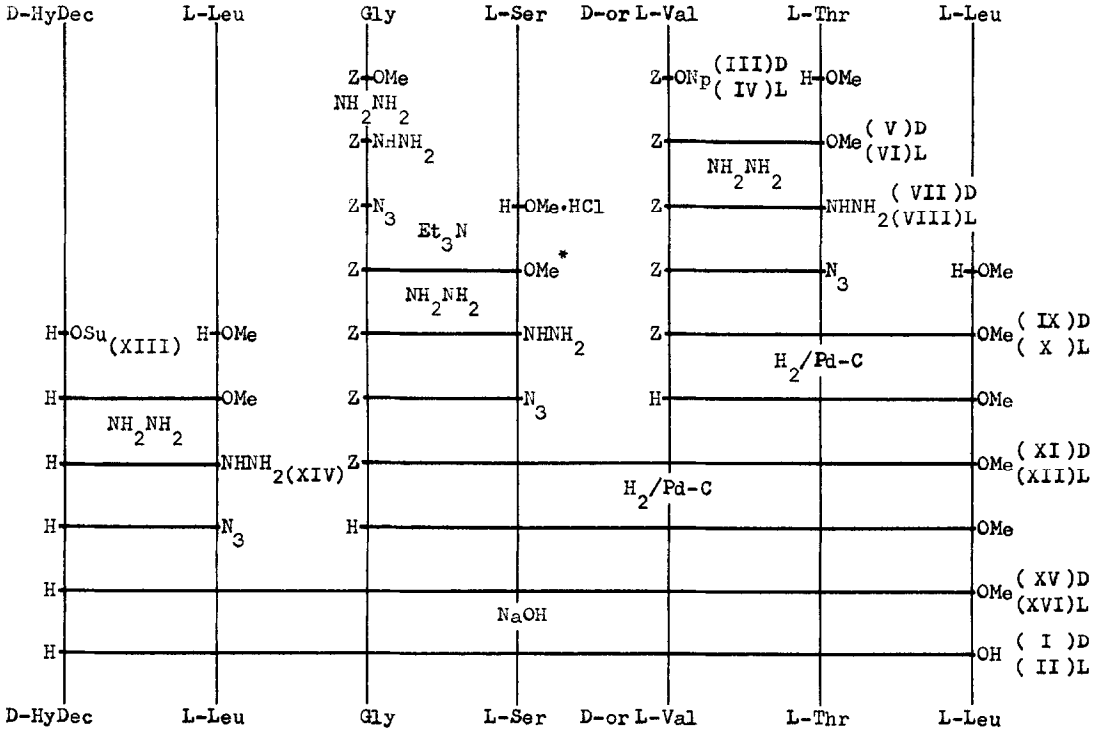
The two D-β-hydroxydecanoyl hexapeptides, (I) and its diastereoisomer (II), were synthesized by using a combination of the azide and active ester methods according to Scheme I. The intermediate, D-β-hydroxydecanoyl-L-



(II)

leucine hydrazide (XIV) was prepared by the following two routes. (i) DL-β-Hydroxydecanoyl-L-Leu-OMe was prepared from ethyl DL-β-hydroxydecanoate (Adicks et al.,1943) and L-Leu-OMe by means of the azide method, and converted into the corresponding hydrazide. The methanol soluble fraction of the hydrazide was collected, and purified by the fractional crystallization from

SCHEME I Synthetic Scheme of β-Hydroxydecanoyl Hexapeptides



* Harris et al.,(1951).

methanol-ether mixture to afford (XIV). (ii) The compound (XIII) was synthesized by reaction of N-hydroxysuccinimide (Anderson et al.,1964) with D-β-hydroxydecanoic acid [obtained by the cinchonidine resolution of the racemic acid (Cartwright,1957)], and the product was converted to (XIV) as described in Scheme I. The compounds thus obtained by the two alternative routes were identical in m.p. and ORD curves. The yields and constants of the synthesized compounds are given in Table I.

TABLE I Yields and Constants of Compounds Synthesized*

Compound	Yield (%)	M.P.(°C)	$[\alpha]_D$
V	86	152 - 153 , AcOEt - Petr.ether	+ 9°, c 1.0 , EtOH
VI	86	141 - 142 , AcOEt - Petr.ether	- 16°, c 1.0 , EtOH
VII	90	230 - 231 , MeOH	- 10°, c 2.5 , 90% AcOH
VIII	94	239 - 240 , MeOH	- 20°, c 2.5 , 90% AcOH
IX	90	179 - 180 , AcOEt	- 29°, c 1.1 , EtOH
X	83	194.5- 195.5, AcOEt	- 40°, c 1.1 , EtOH
XI	71	222.5- 223.5, AcOEt	- 45°, c 0.55, MeOH
XII	74	242.5- 243.5, AcOEt	- 57°, c 0.55, MeOH
XIII	83	93 - 94 , AcOEt - Petr.ether	+ 3°, c 1.0 , AcOEt
XIV**	16	144 - 145 , MeOH - Et_2O	- 36°, c 5.0 , AcOH
XIV***	68	146 - 147 , MeOH - Et_2O	- 34°, c 5.0 , AcOH
XV	63	211 - 213 , MeOH - Et_2O	- 16°, c 1.0 , DMF
XVI	42	227.5- 229 , MeOH - Et_2O	- 12°, c 1.0 , DMF
I	86	194 - 196 , EtOH - H_2O	- 15°, c 0.67, EtOH
II	84	203.5- 206.5, EtOH - H_2O	- 24°, c 0.67, EtOH

* All compounds listed here gave satisfactory elementary analyses.
** Prepared by (i).
*** Prepared by (ii).

The hydroxyacylpeptides (I) and (II) were found to be homogeneous, respectively, by their thin layer chromatography in various solvent systems. However, both the melting point and the optical rotation of the two depsipeptides were apparently different from those of the natural viscosin. In

order to assure the correctness of the structure of our synthesized compounds and to obtain clearer information about discrepancies with viscosin, they were subjected to the following analyses. Quantitative amino acid analysis of (I) gave the expected amino acid ratios corresponding to the theoretical values (Thr 1.03, Ser 1.01, Gly 0.92, Val 1.01, and Leu 2.00 in molar ratios), but viscosin did not. The infrared spectrum of (I) showed absorptions due to carboxyl (1722 cm^{-1}), amide carbonyl groups (1642, 1658 cm^{-1}), and the amide II (1535, 1545 cm^{-1}), but in viscosin, additional bands (1171, 1273, 1739 cm^{-1}) attributable to ester or lactone groups were observed; the latter bands suggest that viscosin could possess a cyclic structure as well as other depsipeptides containing β-hydroxy acids. Thin layer chromatography of (I) on silica gel showed a blue-violet spot [Rf 0.87 in solvent I (n-BuOH : AcOH : H_2O = 4 : 1 : 5 v/v) or Rf 0.20 in solvent II (isoBuOH : 3% NH_3 = 3 : 1 v/v)] when developed by the modified Rydon's reagent (Mazur et al.,1962), while viscosin gave a yellowish green spot (Rf 0.77 in solvent I or Rf 0.34 in solvent II). The mass spectrum of the methyl ester (XV) exhibited the expected molecular ion peak at m/e 772, and the fragmentation pattern characteristic of its amino acid sequence, but the spectra of viscosin and of its methyl ester (methylated with diazomethane) could not be interpreted from the proposed structure. (Details of these results will be reported elsewhere.)

We have further prepared, in a manner similar to that described above, sixteen homologs of (I) and (II) containing C_6 - C_{18} β-hydroxy fatty acids (L- or D-β-hydroxyacyl hexapeptides), however, all compounds were different from viscosin.

All acylpeptides thus synthesized, including (I) and (II), showed only comparable antimicrobial activity with n-decanoic acid against all the microorganisms so far tested.

In view of the above results, it is concluded that viscosin does not have the proposed structure.

Further work on the elucidation of a structure for viscosin is in progress.

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