Tetrahedron Letters No, 13, pp. 1087-1090, 1970. Pergamon Press. Printed in Great Britain.

THE REVISED STRUCTURE OF VISCOSIN, A PEPTIDE ANTIBIOTIC[1]

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(Received in Japan 7 January 1970; received in UK for publication 17 February 1970)

Viscosin, an acidic antibiotic active to tubercle bacillus and virus, was isolated as colorless needles, m.p. 270 - 273°, $[\alpha]_D^{29}$-168.3 (c 1, EtOH), from Pseudomonas viscosa[2] and the structure (I) had first been proposed by Ohno et al[3].

D-$CH_3(CH_2)_6CH(OH)CH_2CO$-L-Leu-Gly-L-Ser-D-Val-L-Thr-L-Leu-OH (I)

We have previously reported chemical synthesis of the acyl hexapeptide (I) and found that (I) was not identical with viscosin[4]. The present communication describes the corrected structure (IV) for the antibiotic.

Quantitative amino acid analysis of viscosin revealed the presence of 1 mole each of glutamic acid, allothreonine, valine and isoleucine, 2 moles of serine, and 3 moles of leucine. Saponification of viscosin with a dilute solution of sodium hydroxide in aqueous ethanol followed by acidification yielded colorless crystals, m.p. 170 - 175°, $[\alpha]_D^{20}$-10.0 (c 1, EtOH), Anal. Found: C, 55.92; H, 8.39; N, 10.83, Calcd. for $C_{54}H_{97}N_9O_{17}H_2O$: C, 55.80; H, 8.59; N, 10.83; it has the same amino acid composition as that of viscosin, however, unlike viscosin, it does not exhibit ester or lactone carbonyl absorption band (1739 cm^{-1}) in its IR spectrum, indicating that the lactone linkage in viscosin was cleaved to form an open chain N-acyl nonapeptide, which was designated as viscosic acid. Titration in 90% methanol with sodium methoxide indicated that viscosin was monobasic and viscosic acid dibasic acid. Permethylation of viscosic acid by methyl iodide and silver oxide in dimethylformamide[5] gave a product of sufficient volatility for mass spectral analysis[6]. The mass spectrum of permethylated viscosic acid gave a molecular ion at m/e

1353 and peaks due to the loss of methanols at M-32, M-64 and M-96, confirming the expected molecular weight of nona-N-methylviscosic acid dimethyl ester tetra-O-methyl ether. Fragmentations took place principally at the peptide bonds, with accompanying peaks due to the loss of methanols from the corresponding fragment ions (Table I); the resulting peaks determined the entire amino acid sequence of viscosic acid. Both hydrazinolysis[7] and digestion with carboxypeptidase A[8] revealed that isoleucine was located in the C terminal of viscosic acid. Thus the above results led unequivocally to the structure (II) for viscosic acid.

$CH_3(CH_2)_6CH(OH)CH_2CO$-Leu-Glu-*allo*-Thr-Val-Leu-Ser-Leu-Ser-Ile-OH (II)

Permethylated viscosin gave a mass spectrum very similar to that of the corresponding derivative of viscosic acid, showing that viscosin had the same constituents and sequence as those of viscosic acid. Furthermore the mass spectrum of the perdeuteriomethylated viscosin methyl ester, which was derived

Table I Fragmentation of permethylviscosic acid (i) and perdeuteriomethylviscosin methyl ester (ii)*

(i) Permethylviscosic acid

Mass units	157	129	113	127		
Dec(OMe)-MeLeu	MeGlu(OMe)	Me*allo*-Thr(OMe)	-MeVal	-MeLeu		
m/e 280(65.3)	437(23.1)	534(2.1)	647(2.2)	774(1.2)		
312(100)	469(44.6)	566(5.0)	679(5.0)	806(2.5)		
		598(2.5)	711(0.8)	838(1.2)		

115	127	115	158	
MeSer(OMe)	-MeLeu	MeSer(OMe)	MeIle-OMe	
857(0.3)	984(0.5)	1099(0.2)	1257(0.1)	[M-96]
889(0.6)	1016(0.9)	1131(0.2)	1289(0.2)	[M-64]
921(0.5)	1048(0.5)	1163(0.2)	1321(0.2)	[M-32]
953(0.3)			1353(0.1)	[M]

(ii) Perdeuteriomethylviscosin methyl ester (partial mass spectrum)

Mass units	160	135	116	130
Dec(OCD_3)-CD_3Leu	CD_3Glu(OMe)	CD_3*allo*-Thr(OCD_3)	-CD_3Val	-CD_3Leu
m/e 283(28.1)	443(7.8)	543(2.4)	659(2.0)	789(0.7)
318(100)	478(28.8)	578(6.0)	694(3.9)	824(1.7)

* Relative abundances are shown in parentheses

from viscosin by action of diazomethane and subsequent treatment with deuteriomethyl iodide and silver oxide, clearly exhibited peaks containing the methyl ester of the glutamic acid residue (Table I). Viscosin showed pKa 6.1 (in 90% methanol), which is in the expected region for α-glutamyl peptides[9], and also the mass spectrum of viscosic acid dimethyl ester (prepared with diazomethane) gave peaks arising from the elimination of CO and/or methanal from the glutamic acid ester residue (e.g. at m/e 367, 382, 478 and 577), suggesting the occurrence of α-glutamyl peptide linkage[10]. These facts indicated that the glutamic acid residue in viscosin has a free γ-carboxyl group and is not involved in the lactone linkage, thus leading to the conclusion that the C terminal amino acid of viscosic acid is engaged in the lactone bond of viscosin. The second bridgehead of the lactone bond could be any of the hydroxy acid or three hydroxyamino acids. Viscosin and viscosic acid were respectively oxidized with chromic acid in acetic acid[11] and the amino acids of the oxidation products were analyzed. The product from viscosin gave allothreonine as a hydroxyamino acid (Ser, 0; allo-Thr, 0.9 in molar ratios), while the product from viscosic acid gave none of hydroxyamino acid (Ser, 0.2; allo-Thr, 0.1); thus obviously the hydroxyl group of the allothreonine is involved in the lactone bond.

In order to determine the configuration of the constituent amino acids, they were isolated from an acid hydrolysate of viscosin by ion exchange chromatography (resin: Dowex 50W X4, in H^+cycle)[12]. From the degree of their susceptibility to L-glutamic acid decarboxylase or D-amino acid oxidase[13] it was concluded that two of serine, glutamic acid, allothreonine and valine residues possess D-configuration, and three leucine and isoleucine residues have L-configuration.

Since structure and D-configuration[14] of the hydroxy acid has been already established by Ohno et al.[3], we now can propose the structure (III) to viscosic

D-$CH_3(CH_2)_6CH(OH)CH_2$CO-L-Leu-D-Glu-D-allo-Thr-D-Val-L-Leu-
(III) D-Ser-L-Leu-D-Ser-L-Ile-OH

D-$CH_3(CH_2)_6CH(OH)CH_2$CO-L-Leu-D-Glu-D-allo-Thr-D-Val-L-Leu-D-Ser
(IV) L-Ile←D-Ser←L-Leu←

acid, and the structure (IV) to viscosin. The structure (IV) indicates that viscosin is an unusual cyclodepsipeptide containing a remarkable number of D-amino acids, and a β-hydroxy fatty acid moiety which does not participate in the formation of the lactone ring.

Acknowledgements. We kindly thank Dr. S. Yamaoka (Sumitomo Chemicals Co.) for supplying us with samples of viscosin and Dr. K. Harada (Tanabe Pharmaceutical Co.) for amino acid analyses.

References and Notes

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