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VIOMYCIN I. THE AMINO ACID SEQUENCE OF VIOMYCIN

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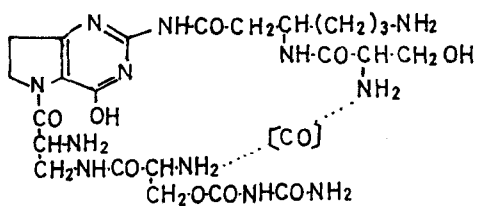
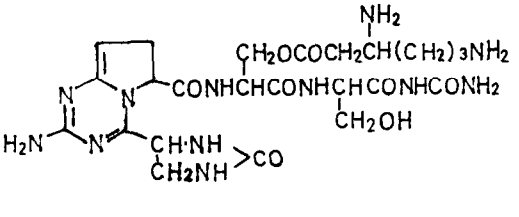
Many attempts have been made to elucidate the structure of viomycin, a strongly basic tuberculostatic streptomyces antibiotic (1-7). These studies led Bowie, Cox, Johnson and Thomas (6) to present I and more recently Dyer, Kellogg, Nassar and Streetman (7) to propose II as the structure of viomycin. This report describes our determination of the amino acid sequence of viomycin.



I



II

Complete acid hydrolysis of viomycin yielded the amino acids L-serine, L-α, β-diaminopropionic acid (dapa), L-β-lysine and viomycidine[*1] (ratio: 2:1:1:1) and urea, carbon dioxide and ammonia, as reported by others (5,6).

Partial hydrolysis of viomycin was achieved with 1 N hydrochloric acid at 100° for six hours. The hydrolysates were separated by column chromatography on active charcoal, eluting with water, 5% acetic acid and 5% acetic acid-20% phenol. From the water eluate L-serine and urea were obtained.

The 5% acetic acid eluate was rechromatographed on an Amberlite CG-120 column and β-lysine and peptide III isolated, $[\alpha]_D^{27°}$ -2° (0.1% in H_2O) ;

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*1 Amino acid analyse were carried out, using an Hitachi Amino acid analyser KLA-3, with 50 cm. Column.

Rf*2: 0.6; R*3:0.8; no ultraviolet absorption; positive Sakaguchi reaction.

The 5% acetic acid-20% phenol eluate was chromatographed on a cellulose powder column and 3 peptides isolated: Peptide Ia, $[\alpha]_D^{27^\circ}$ -43° (1% in H_2O); Rf: 0.25; R: 0.98; λmax 269.5 m μ (log ε 3.21); positive Sakaguchi reaction. Peptide Ib, $[\alpha]_D^{12^\circ}$ -49.5°(1% in H_2O) ; Rf: 0.11; R: 0.88; λmax 285 m μ (log ε 3.93); positive Sakaguchi reaction. Peptide II, $[\alpha]_D^{17^\circ}$ -38°(1% in H_2O); Rf: 0.5; R: 0.95; λmax 268 m μ (log ε 4.12); positive Sakaguchi reaction.

Dyer et. al. (7) reported the hydrolysis of viomycin with 0.1 N hydrochloric acid at 90° for 8 hours to give urea and desureaviomycin. Following the same hydrolysis and chromatographing the hydrolysates on an active charcoal column we obtained urea *4 and peptide Ic*5, $[\alpha]_D^{16^\circ}$ -37° (1% in H_2O); Rf: 0; R: 0.9; λmax 272 m μ (log ε 3.95); positive Sakaguchi reaction.

Peptide III gave L-serine, L-dapa and viomycidine on complete hydrolysis. Hydrazinolysis of peptide III gave viomycidine as a free amino acid. Hydrolysis of the bis-2,4-dinitrophenyl peptide III (λmax 365 m μ, log ε 4.44) yielded viomycidine, 2,4-dinitrophenylserine and mono-2,4-dinitrophenyldapa.

For comparison, the two possible mono-2,4-dinitrophenyldapa acids were synthesized by the modified copper complex method (8) from L-dapa, which was made from L-aspartic acid by a Schmidt reaction (9). Thus, we obtained α-amino-β-2,4-dinitrophenylamino propionic acid, m.p.205°; anal. calcd. for $C_9H_{10}N_4O_6$: C, 40.00; H, 3.55; N, 20.74; found: C, 39.54; H, 3.55; N, 20.08; $[\alpha]_D^{25^\circ}$ + 77.11° (0.82% in 0.2 N HCl); and the hygroscopic β-amino- α -2,4-dinitrophenylaminopropionic acid from acid hydrolysis of β-benzoylamino- α -2,4-dinitrophenylaminopropionic acid m.p. 236-238°; anal. calcd. for $C_{16}H_{14}N_4O_7$: c, 51.34; H, 3.77; N, 14.65; found: C, 51.61; H, 3.77; N, 14.65.

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*2 Rf values were obtained on PPC with Toyoroshi No.51 paper and a solvent of (t-BuOH:HOAc: H_2O, 2:1:1) for development.

*3 Electrophoresis was carried out with a Toyo-C type (500V, 3-5 mA) instrument for 3-5 hrs. with Toyoroshi No.51 paper and pyridine: HOAc:H_2O,(5:0.2:95) (pH 6.5) buffer solution.
R values were obtained with reference to viomycin defining the electophoresis distance of viomycin as 1, under the above conditions.

*4 Urea was detected by the urease method (10).

*5 The amino acid components, N-terminal amino acid and C-terminal amino acid of peptide Ic are identical to those of desureaviomycin which was obtained by Dyer et. al.(7). They report that desureaviomycin has no ultraviolet absorption, but the peptide Ic has ultraviolet absorption.
c.f. Ultraviolet absorvance of viomycin was followed in 0.1 N HCl at 100°. The ultraviolet absorvance decreased gradually and after 6 hrs. it had disapeared. But a lyophilized product of this solution showed ultraviolet absorption.

The mono-2,4-dinitrophenyl dapa obtained from peptide III could be assigned the α-amino-β-2,4-dinitrophenylaminopropionic acid structure by comparison of its Rf and R values with those of the corresponding synthetic compound: Rf_1 : 0.76 (n-butanol: acetic acid: water, 2:1:1) and Rf_2: 0.53 (n-butanol : t-butanol : pyridine: acetic acid : water, 15:4.5:10:3:12) on PPC and R_1: 0.47 (ethanol 20 ml. containing 20 drops of acetic acid) and R_2 : 0.53 (Phenol: ethanol: water, 15:4:1) on TLC using Kieselgel G. In contrast the isomeric β-amino - α - 2,4-dinitrophenylaminopropionic acid showed Rf_1: 0.82; Rf_2: 0.74; R_1: 0.20; R_2: 0.14.

Thus, the amino acid sequence of peptide III is determined as seryl-dapayl-viomycidine, where the β-amino group of dapa is free.

On complete acid hydrolysis peptide II and each member of the peptide I group (Ia, Ib, Ic) gave L-serine, L-dapa, L-β-lysine and viomycidine (ratios 2:1:1:1) respectively.*6 The same amino acids were obtained from the acid hydrolysis of viomycin.

Hydrazinolysis of viomycin did not give a free amino acid, *7 but hydrazinolysis of peptide II and of the peptide I group (Ia, b, c) gave L-serine as a free amino acid.

Acid hydrolysis of the bis-2,4-dinitrophenyl derivatives of viomycin and of the peptide I group gave all of the amino acids described above except β-lysine. Bis-2,4-dinitrophenyl-β-lysine was obtained in 80% yield from the viomycin derivative. Similarly it was obtained from each of the peptide I group derivatives and identified by PPC and TLC.

Acid hydrolysis of the tri-2,4-dinitrophenylpeptide II (λ max 370 m μ, log ε 4.31) gave free serine and viomycidine and the derivatives bis-2,4-dinitrophenyl-β-lysine and α-amino-β-2,4-dinitrophenylaminopropionic acid. Therefore, the amino acid sequence of peptide II is:

β-lysyl-seryl-diamino(-β-NH_2)propionyl-viomycidyl-serine where the β amino group of dapa is free and the amino acid sequence of the peptide I group is:

β-lysyl-seryl-dapayl(β-NH-)-viomycidyl-serine

where the β-amino group of dapa is tied up. From these results one concludes that the amino acid sequence of viomycin is :

β-lysyl-seryl-dapayl(-β-NH-)-viomycidyl-seryl-urea III

where the β-amino group of dapa is not free. This sequence is not consistent with structure I, proposed by Bowie et. al.(5,6) or with structure II, proposed by Dyer et. al. (7) for viomycin.

The first group obtained on partial hydrolysis of viomycin with 6 N hydrochloric acid a pep-

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*6 The amino acid sequence of the peptide I group are identical but each peptide shows different ultraviolet absorption and different Rf values on PPC and R values on electrophoresis. Each peptide shows a single spot on PPC in different solvent systems and on electrophoresis.

*7 The same result was obtained by Dyer et. al. (7).

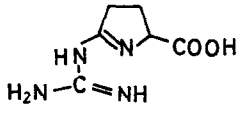
tide A (dapayl-viomycidine), a peptide B (β -seryl-β-lysine) and a small amount of a peptide C (on hydrolysis it gave viomycidine and β-lysine). With 0.1 N lithium hydroxide they obtained a peptide D (seryl-β-lysyl-viomycidine). In addition to these peptides they report that hydrolysates of a 2,4-dinitrophenyl derivative of viomycin yielded mono- α -2,4-dinitrophenyldapa and mono- α -2,4-dinitrophenyl-β-lysine. From these results they deduce the amino acid sequence and present structure I for viomycin.

Dyer et. al. (7) pointed out that hydrolysis of bis-2,4-dinitrophenylviomycin gave only bis-2,4-dinitrophenyl-β-lysine, consistent with our results. In addition they reported that they obtained serine and viomycinic acid from the action of carboxypeptidase on desureaviomycin (c.f. *5) and postulate a serine-serine-urea unit in viomycin. On treatment of viomycin with water they obtained a compound (which gave serine and lysine on hydrolysis) which they presumed to correspond to peptide B of Bowie et. al. (6) but for which they proposed a seryl ester of β-lysine.

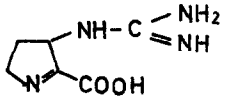
From these results and the structure of peptide A obtained by Bowie et. al. the amino acid sequence was deduced and structure II proposed for viomycin.

Examining the action of carboxypeptidase A [*8] on peptide Ic, which we assume to be identical with the above desureaviomycin, we could not obtain serine nor viomycinic acid. Since we could demonstrate the seryl-dapaylviomycidine group in viomycin and since viomycin contains two seryl units, one can deduce the presence of a viomycidyl-seryl-urea unit and exclude a viomycidyl-seryl-seryl-urea unit. Thus the amino acid sequence in viomycin must be that shown in III.

For the structure of viomycidine Dyer et. al. (4) presented IV which was supported by Bowie et. al. (5) but subsequently changed to V (7).



IV



V

Bowie et. al. (6) reported that their peptide A shows ultraviolet absorption at 275 mμ (ε 5100) and assigned structure VI to it whereas Dyer et. al. (7) presented VII for peptide A. These

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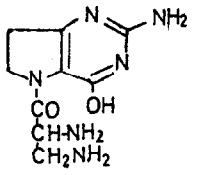
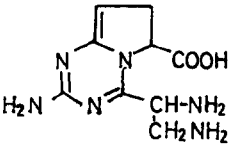
*8 Bought from Sigma chemical company.

structures were incorporated into the proposals I and II for viomycin.



VI



VII

Both structures VI and VII are not expected to show the positive Sakaguchi reaction found with viomycin and peptide A. Structures I and II also fail to explain the instability of the chromophore of viomycin (c.f.*5). The structure of the chromophore of viomycin is currently under investigation. An explanation for the release of the urea unit from viomycin under very mild conditions will be presented later.

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