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VIOMYCIN. FURTHER DEGRADATIVE STUDIES

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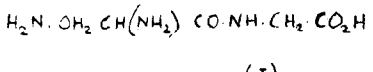
In an earlier Communication (1), we suggested a structure for the chromophore of the antibiotic viomycin which was later (2) incorporated into an extended partial structure for the antibiotic itself. Our later work has necessitated a substantial amendment of our earlier views; in particular, the substance described as "peptide A" has proved to be a mixture and consequent structural deductions made earlier are therefore invalidated. We also discount another formulation (3) of the viomycin chromophore. A recent account (4) of the amino-acid sequence in viomycin prompts us to describe some of our more recent findings.

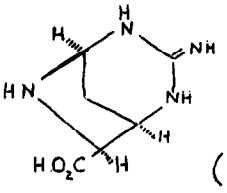
In contrast to earlier proposals (1,2) we now suggest that the guanidine unit of viomycin, which appears in the amino-acid viomycidine after acid hydrolysis is unlikely to be concerned with the chromophore. Kinetic studies have shown that the rate of loss of the chromophore on hydrolysis is equivalent to the rate of production of urea and this implies that urea is a part of the chromophoric system. The remainder of the molecule after removal of the urea, desureaviomycin, shows no absorption in the ultraviolet (cf. 3), although, after addition of alkali, it has $\lambda_{max.}$ 272 mμ (cf. 4).

With regard to the location of the guanidine fragment, we find that mild hydrolysis of viomycin with N/10 aqueous sodium hydroxide gives a high yield of 2-aminopyrimidine as well as a mixture of peptides. Chromatography of the peptide mixture gave a crystalline dipeptide which on hydrolysis gave glycine and αβ-diaminopropionic acid. Hydrolysis of the bis-2,4-dinitrophenyl derivative of the dipeptide yielded glycine and the bis-2,4-dinitrophenyl derivative of αβ-diaminopropionic acid (5). Thus the dipeptide is αβ-diaminopropionylglycine (6) (I).

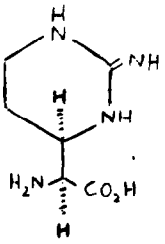
Acid hydrolysis of the above peptide mixture gave glycine as well as β-lysine, αβ-diaminopropionic acid, and serine but no viomycidine. Thus the viomycidine obtained from acid hydrolysis of viomycin is replaced by 2-aminopyrimidine and glycine in the alkaline hydrolysis product (7). This is evident in structure (II) recently deduced by Büchi and Rayleigh (8) for viomycidine. We have also shown that hydrogenation of viomycin, followed by total acid hydrolysis, affords no viomycidine but instead α(2-iminohexahydro-4-pyrimidyl)glycine (III). This amino-acid also results from total acid hydrolysis of the closely related capreomycin group of antibiotics (9,10). We have confirmed the gross structure of (III) by synthesis and this will be described in a later communication. Attempts to convert viomycidine itself into (III) have so far been unsuccessful, (8).



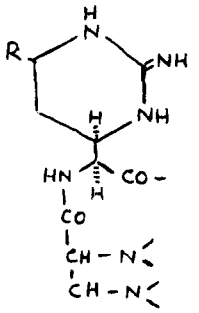
(I)



(II)



(III)

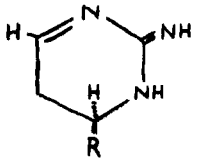


(IV)

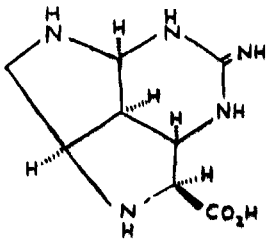
Our results find a logical explanation in the existence of the unit (IV; R = OH) in viomycin. Alternative dihydropyrimidine ring structures such as (V) are discounted by the absence of a signal in the n.m.r. spectrum of viomycin corresponding to a methine proton at C_1. It is also relevant that viomycin hydrochloride readily forms an O-methyl derivative on heating with methanol (11). Viomycidine is therefore an artefact formed by reaction of the guanidine-carbinol system with the amino group of the glycine fragment. On the other hand,

base-catalysed elimination of water, glycine and αβ-diaminopropionic acid from (IV; R = OH) yields 2-aminopyrimidine. Several of these reactions are paralleled in the properties of tetrodotoxin (12). Another degradation product of viomycin which gives further support to the existence of the unit (IV; R = OH) in the antibiotic is an acid, viocidic acid, isolated as its crystalline dihydrochloride, $C_8H_{13}N_5O_2.2HCl.H_2O$, and which also forms a crystalline dihydrobromide and dipicrate. The complete structure and absolute stereochemistry of the dihydrobromide have been determined by X-ray crystallography. The salt crystallised from aqueous ethanol in the orthorhombic system, space group $P2_12_12_1$ with four units of $C_8H_{13}N_5O_2.2HBr.3H_2O$ per unit cell of dimensions a = 8.17, b = 12.17, c = 15.22 Å. Initial co-ordinates for the two bromine atoms were obtained from the three dimensional Patterson function. The remaining atoms were located by three dimensional electron density distributions. Two successive Fourier calculations gave the molecular structure and the co-ordinates were refined by one round of structure factor and Fourier calculations. The present R value is 12.1% for 1434 independent reflections and the results establish that the structure and stereochemistry are as shown in (VI). The absolute configuration was determined by Bijvoet's method (13) based on the anomalous dispersion of CuK_α radiation by the two bromine atoms. The absolute stereochemistry as shown gives R = 12.1% for the estimated hkl data, while the opposite configuration gives R = 12.3%. The mode of formation of (VI) from (IV; R = OH) will be discussed in detail in the full Paper, but it should be noted that the relative stereochemistry of viomycidine (II) (8), deduced largely by interpretations of n.m.r. spectra, is confirmed by structure (VI).

End group analysis of viomycin reveals, as reported by other workers (3,4), that the only free amino groups are those of β-lysine. The sequence of the remaining amino-acids and the nature of the chromophore of viomycin are at present under investigation.



(V)



(VI)

The capreomycin group of antibiotics (9) bears many resemblances to viomycin. Thus one member of the group, capreomycin IB ($\lambda_{max.}$ 266 mμ; ε, 24,050; cf. viomycin, $\lambda_{max.}$ 268 mμ; ε, 24,500) on total hydrolysis gives αβ-diaminopropionic acid, alanine, β-lysine and α(2-iminohexahydro-4-pyrimidyl)glycine (III) (ratio 2:1:1:1). Partial acid hydrolysis resulted in the formation of a number of dipeptides which were isolated but not fully characterised. One of these dipeptides however was stated to be αβ-diaminopropionyl-α(2-iminohexahydro-4-pyrimidyl)glycine. We have also obtained this dipeptide, in the form of its crystalline dihydrochloride, $C_9H_{18}N_6O_2.2HCl.H_2O$, which indicates that the unit (IV; R = H; stereochemistry unspecified) is present in capreomycin IB. As the hexahydropyrimidine ring in capreomycin lacks the 6-hydroxyl group of viomycin the formation of 2-aminopyrimidine and glycine from alkaline hydrolysis is precluded, as has been observed. Furthermore hydrolysis of capreomycin with N/10 hydrochloric acid at 100° has now been shown to release one equivalent of urea to give desureacapreomycin which, like desureaviomycin, does not show ultraviolet absorption in [illegible] solution. Once again the rate of production of urea corresponds

to the rate of loss of the chromophore. It therefore seems probable that viomycin and capreomycin contain the same chromophoric unit. The n.m.r. spectra of both viomycin and capreomycin show a low field signal at τ 1.9 (D_2O).

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8. We thank Professor Büchi for sending us this information prior to its publication.
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