Viomycin. Part I. The Structure of the Guanidine-containing Unit

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Viomycidine, a bicyclic guanidino-amino-acid obtained from viomycin by acidic hydrolysis, is shown to be an artefact derived by cyclisation from a monocyclic guanidino-carbinol unit. Several reactions of viomycin find close analogies in the chemistry of tetrodotoxin, which also contains a guanidino-carbinol fragment. The structural relationship between viomycin and capreomycin has been established.

THE antitubercular antibiotic viomycin was discovered almost simultaneously in 1951 by chemists at Pfizer and Co.,[1] who isolated it from *Streptomyces puniceus*, and at Parke Davis and Co.[2] (from *S. floridae*). Viomycin has also been isolated more recently from other *Streptomyces* species, and it is identical with vinactin A[3] from *S. vinaceus*. It finds limited clinical application for tubercular patients who have failed to respond to more classical chemotherapy. Related to viomycin, both chemically and pharmacologically, are two other antibiotics, capreomycin, isolated from *S. capreolus* by Herr and his colleagues at Eli Lilly,[4] and tuberactinomycin,

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[1] A. C. Finlay, G. L. Hobby, F. A. Hochstein, T. M. Lees, T. F. Lenert, J. A. Means, S. Y. P'an, P. P. Regna, J. B. Routein, B. A. Sobin, K. B. Tate, and J. H. Kane, *Ann. Rev. Tuberc.*, 1951, **63**, 1.

[2] Q. R. Bartz, J. Ehrlich, J. D. Mold, M. A. Penner, and R. M. Smith, *Ann. Rev. Tuberc.*, 1951, **63**, 4.

[3] R. L. Mayer, P. C. Eisman, and E. A. Konopka, *Experientia*, 1954, **10**, 335.

[4] E. B. Herr, M. E. Haney, G. E. Pittenger, and C. E. Higgens, *Proc. Indiana Acad. Sci.*, 1960, **69**, 134.

obtained from *S. verticillatus* var. *tuberacticus* by Japanese workers.[5] A discussion of the chemistry of the capreomycin group [6-8] is deferred to a later paper. Chemical work on viomycin, which is a strong base, has been greatly hampered by the difficulties of purification, partly due to the sensitivity of the antibiotic to temperature and pH. The viomycin sulphate used in our work was the commercially available grade, produced by Pfizer and Co. The precise details of the isolation have not been published, but use is made of the *O*-methyl ester [9] in the purification procedure, as this derivative is easily reconverted into viomycin by the action of boiling water. Propan-2-ol is also used for the precipitation of viomycin from aqueous solutions and was detected in certain samples. When the commercial viomycin sulphate was subjected to counter-current distribution between n-butanol and aqueous 6·4% sodium chloride it gave a single symmetrical peak of material which absorbed in the u.v. at 268 nm. The use of salts such as reineckates and helianthates and especially the sulphate (containing 1·5 mol. equiv. of H_2SO_4) has been recommended also for the purification of viomycin, and we have prepared the crystalline chloride and sulphate picrate. Our own analytical data on these salts, together with results of molecular weight determinations by several methods, have led us to adopt the molecular formula $C_{25}H_{43}N_{13}O_{10}$ (*M*, 686) for viomycin. This differs appreciably from earlier assignments [10-12] but is in good agreement with a recent *X*-ray crystallographic molecular weight determination on viomycin sulphate picrate, which gave a value of 1025, corresponding to a value of 686 for the free base.[13]

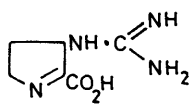
Potentiometric titration of solutions of viomycin revealed basic centres with p*K* 8·2—8·3, 10·3, and 12—12·5, the last being suggestive of a guanidine grouping which was also indicated by the positive Sakaguchi test for mono- (but not di-) substituted guanidines. Positive Fehling, ninhydrin, and biuret tests were also reported, as well as two important spectral properties. The first of these was a low-field one-proton singlet (n.m.r. in D_2O) at τ *ca.* 1·9,[12] and the second was a chromophore with absorption at 268 nm (ε 24,000) in neutral or acidic media, shifted reversibly to 285 nm (ε 15,000) in aqueous 0·1N-sodium hydroxide. The positions of these u.v. absorptions corresponded to those of certain well known pyrimidine bases, *e.g.* thymine [$\lambda_{max.}$ (H_2O) 265 nm (ε 9500); $\lambda_{max.}$ (pH 10) 291 nm (ε 5440)], and this led to some early confusion [11,12,14] about the nature of the chromophore although the intensity of absorption of viomycin was 2—3-fold greater than those of the pyrimidines. The peptide nature of the antibiotic was indicated by the results of acid hydrolysis, which gave urea, L-serine, L-αβ-diaminopropionic acid, L-β-lysine, and a guanidine-containing amino-acid, viomycidine in molar ratio 1 : 2 : 1 : 1, as well as carbon dioxide [10] and ammonia. β-Lysine has been isolated from the acid hydrolysates of other basic antibiotics, including streptothricin,[15,16] streptolin,[17] geomycin,[18] and roseothricin,[19] and has recently been shown to be involved in the metabolism of lysine.[20] The L-configuration of β-lysine has been confirmed by o.r.d. measurements.[21] We have also detected small amounts of glycine and viocidic acid (see below) in the viomycin acid hydrolysates, and have confirmed that the antibiotic does not contain a free carboxy-group.

Viomycidine, $C_6H_{10}N_4O_2$, gave a positive Sakaguchi test, showed only end-absorption in the u.v. (ε_{212} 2530), and was optically active. Potentiometric titration indicated pK_a values of 1·3 (estimated), 5·5, and 12·6, and the results of vigorous hydrogenation suggested the presence of a double bond. The amino-acid yielded guanidine and glycine on permanganate oxidation, pyrrole-2-carboxylic acid on hydrolysis with barium hydroxide, and 2-aminopyrimidine and glycine on fusion with sodium hydroxide.[22] On this basis, Dyer *et al.*[22] suggested structure (I) for viomycidine, and this was accepted by Bowie, Johnson, and Thomas,[14] who pointed out that if the guanidine and carboxylic groups of (I) were to cyclise to a cytosine ring, as in (II), this would provide an explanation for the nature of the chromophore. Shortly afterwards, Dyer [12] modified his view of the structure of viomycidine to (III), and as partial hydrolyses of viomycin [14] had indicated the existence of an αβ-diaminopropionylviomycidine fragment, he suggested (IV) as the chromophoric unit of viomycin. He also criticised structure (II) on the grounds that the hydroxypyrimidine would be expected to be acidic whereas potentiometric titration of viomycin had not revealed such a group, and also acid hydrolysis of (II) would not be expected to yield optically active viomycidine as was observed. Furthermore the 2-acylaminopyrimidine fragment of the suggested viomycin

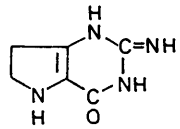
[5] A. Nagota, T. Ando, R. Izumi, H. Sakakibara, T. Take, K. Hayano, and J. Abe, *J. Antibiotics* (*Japan*), 1968, **21**, 681.
[6] E. B Herr, *Antimicrobial Agents and Chemotherapy*, 1962, 201.
[7] E. B. Herr and M. O. Redstone, *Ann. New York Acad. Sci.*, 1966, **135**, 940.
[8] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Nature*, 1971, **231**, 301.
[9] F. A. Hochstein and R. L. Miller to Chas. Pfizer and Co., Inc., U.S.P., 2,910,998 (*Chem. Abs.*, 1960, **54**, 10,246).
[10] T. H. Haskell, S. A. Fusari, R. P. Frohardt, and Q. R. Bartz, *J. Amer. Chem. Soc.*, 1952, **74**, 599.
[11] J. H. Bowie, D. A. Cox, A. W. Johnson, and G. Thomas, *Tetrahedron Letters*, 1964, 3305.
[12] J. R. Dyer, C. K. Kellogg, R. F. Nassar, and W. E. Streetman, *Tetrahedron Letters*, 1965, 585.
[13] J. Carlisle and W. A. Jones, personal communications; W. A. Jones, Ph.D. Thesis, University of London, 1963.
[14] J. H. Bowie, A. W. Johnson, and G. Thomas, *Tetrahedron Letters*, 1964, 863.
[15] H. E. Carter, W. R. Hearn, E. M. Lansford, A. C. Page, N. P. Salzman, D. Shapiro, and W. R. Taylor, *J. Amer. Chem. Soc.*, 1952, **74**, 3704.
[16] E. E. van Tamelen and E. E. Swissman, *J. Amer. Chem. Soc.*, 1952, **74**, 3713.
[17] E. E. van Tamelen and E. E. Swissman, *J. Amer. Chem. Soc.*, 1953, **75**, 2031.
[18] H. Brockmann and H. Musso, *Chem. Ber.*, 1955, **88**, 648.
[19] T. Goto, Y. Hirata, S. Hosoya, and N. Komatsu, *Bull. Chem. Soc. Japan*, 1957, **30**, 304.
[20] H. P. C. Hogenkamp, *Ann. Rev. Biochem.*, 1968, **37**, 22
[21] H. Yonehara and N. Otake, *Tetrahedron Letters*, 1966, 3...
[22] J. R. Dyer, H. B. Hayes, E. G. Miller, and R. F. Nassar, *J. Amer. Chem. Soc.*, 1964, **86**, 5363.

structure would not give a positive Sakaguchi reaction and it would no longer be strongly basic.

However, structure (III) was also open to many objections. Thus, it would be expected to undergo



(I)



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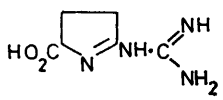

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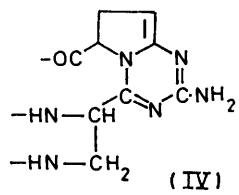

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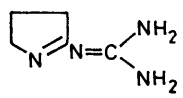

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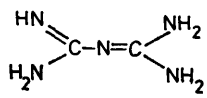


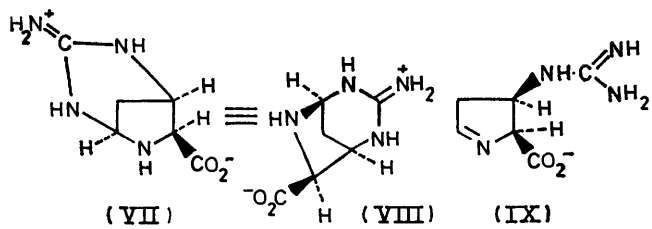
(VI)

hydrolytic ring cleavage and moreover synthetic experiments have shown that 2-guanidino-Δ^1-pyrroline, obtained by the action of guanidine on the imino-ether, exists almost entirely as (V), *i.e.* it absorbed at 241 nm (ε 9670) and the salt at 242·5 nm (ε 10,580) [*cf.* biguanide (VI), 230 nm (ε 8900), and the corresponding hydrochloride, 230 nm (ε 12,360)].

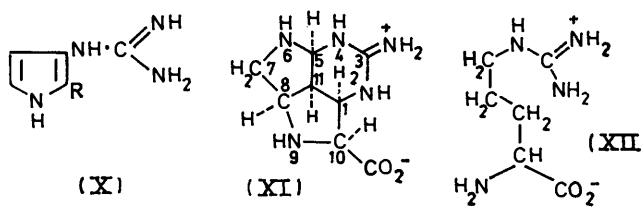
Other criticisms of the suggested structures (II) and (IV) for the chromophore of viomycin have been made more recently,[23] but in 1968 we showed[24] that the chromophore was a unit quite separate from the guanidine-containing entity. The nature of the chromophore will be discussed in the following paper.

In 1966, a detailed study[25] of viomycidine by Büchi and Raleigh showed that the structure should be amended to (VII) ≡ (VIII) [*i.e.* from the cyclisation of (IX)], a conclusion reached on both physical and chemical grounds. They found that the action of mercury(II) acetate on viomycidine gave 3-guanidinopyrrole (X; R = H) and that a similar reaction with viomycidine methyl ester gave methyl 3-guanidinopyrrole-2-carboxylate (X; R = CO_2Me). These observations were paralleled recently by hydrogenolysis of viomycidine to 3-guanidinoproline, converted into 3-aminoproline by barium hydroxide.[26] The n.m.r. spectrum of viomycidine showed the presence of an ABX system as well as three methine protons, which indicated the existence of the bicyclic ring system. Detailed interpretation of the n.m.r. spectrum led to the assignment of relative stereochemistry,[25] which was verified in the absolute configuration later determined by *X*-ray structural studies, first on a minor acid hydrolysis product of viomycin, viocidic acid (XI),[27,28] and then on viomycidine itself.[29,30] The relationship between viomycidine (VIII) and arginine (XII) is obvious, and the positive Sakaguchi reaction shown by viomycidine is explicable by the ring opening to (IX) brought about by the alkaline conditions of the reaction.

Viocidic acid (XI) was purified by chromatography of the hydrolysate on Dowex 50 exchange resin and was isolated as the dipicrate, m.p. 173—175°. The corresponding dihydrochloride monohydrate, $C_8H_{17}N_5O_3Cl_2$, m.p. 210—212°, was optically active and the i.r. spectrum indicated a disubstituted guanidine grouping (bands at 1692 and 1585 cm^{-1}).[31] It gave a yellow ninhydrin reaction and a negative Sakaguchi test and showed no u.v. absorption. Titration indicated pK_a values of 1·3, 5·8, 6·8, and >12·5. The dihydrochloride was stable to treatment with acid (12N-hydrochloric acid at 100° for 48 h); the 2,4-dinitrophenyl derivative of viocidic acid was also prepared but on acidic hydrolysis it underwent considerable degradation and no products were identified.



(VII) (VIII) (IX)



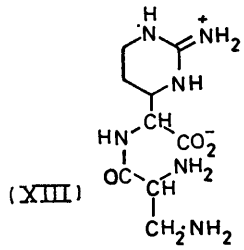
(X) (XI) (XII)

It seemed possible that the guanidine group, being near to a peptide bond, might be exerting a strong stabilising effect, but this idea was dispelled by an examination of a related dipeptide (XIII) from capreomycin. The isolation of (XIII) from capreomycin had been described by Herr and Redstone,[7] who had also stated that acidic hydrolysis gave the amino-acids αβ-diaminopropionic acid and capreomycidine (XIV). The structure and

[23] T. Kitagawa, Y. Sawada, T. Miura, T. Ozasa, and H. Tamiyama, *Tetrahedron Letters*, 1968, 109.
[24] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Tetrahedron Letters*, 1968, 5901.
[25] G. Büchi, personal communication, J. A. Raleigh, Ph.D. Thesis, Massachussetts Institute of Technology, 1966.
[26] G. Gallina, V. Koch, and A. Romeo, *Tetrahedron Letters*, 1969, 3055.
[27] B. W. Bycroft, D. Cameron, L. R. Croft, A. W. Johnson, T. Webb, and P. Coggon, *Tetrahedron Letters*, 1968, 2925; B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Experientia*, 1971, **27**, 501.
[28] P. Coggon, *J. Chem. Soc.* (*B*), 1970, 838.
[29] J. C. Floyd, J. A. Bertrand, and J. R. Dyer, *Chem. Comm.*, 1968, 998.
[30] T. Takita and M. Maeda, *J. Antibiotics*, 1969, **22**, 34.
[31] T. Goto, K. Nakanishi, and M. Ohashi, *Bull. Chem. Soc. Japan*, 1957, **30**, 723.

absolute stereochemistry of viocidic acid (XI) was established [28] by *X*-ray crystallographic study of the dihydrobromide. The molecular formula $C_8H_{13}N_5O_2$ indicated that it was an artefact produced by secondary reactions during the acid hydrolysis of viomycin and the mechanism of its formation will be discussed in the following paper.

Viocidic acid may be regarded as a proline derivative, which accords with the yellow colour it gives with ninhydrin.[32] In the n.m.r. spectrum of the dihydrochloride in D_2O, the proton at C-5 (XI) absorbs at a lower field (τ 4·25) than any other proton because C-5 is attached to two electronegative nitrogen atoms, and the signal is split to a doublet by the C-11 proton, although the magnitude of the coupling constant (8·5 Hz) accords with the conclusion from *X*-ray crystallography that the dihedral angle between the planes H–C(5)–C(11) and C(5)–C(11)–H is small.



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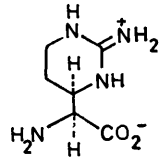
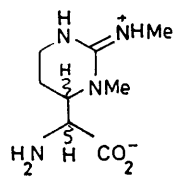


(XIV)

Certain reactions of viomycin led us to the view that viomycidine (VIII), like viocidic acid (XI), was not present as an integral part of the viomycin molecule and was also an artefact. Thus, we observed that when viomycin was hydrolysed by base under mild conditions, 2-aminopyrimidine was obtained in high yield. No viomycidine was detected in this hydrolysate although viomycidine was stable under these mild basic conditions. We therefore deduced that both 2-aminopyrimidine and viomycidine were derived from the same unit of viomycin. It had in fact already been reported [22] that fusion of viomycidine with sodium hydroxide gave 2-aminopyrimidine, pyrrole-2-carboxylic acid, and glycine in unspecified yields.

Secondly, hydrogenation of viomycin hydrochloride over a platinum catalyst gave a colourless glass after removal of solvent. This product was hydrolysed with acid and, after neutralisation, was separated on an Amberlite CG 400 resin to yield the normal hydrolysis products except that alanine was also obtained [33] and that viomycidine was replaced by capreomycidine (XIV). Capreomycidine, after crystallisation from aqueous ethanol, was identical with the product isolated from capreomycin. This important observation provided the first structural and stereochemical correlation of viomycin and capreomycin and suggested that viomycin also contained a cyclic guanidine system based on 4-(2-iminohexahydropyrimidyl)glycine. This ring system had previously been recognised in capreomycidine (XIV), as a unit (XV) of stendomycin,[34] and in pseudoblastidone (XVI) from blasticidin S.[35] The streptothricin group of antibiotics contain a unit of streptolidine (XVII),[36,37] a related cyclic guanidine.



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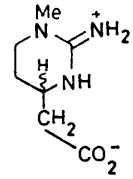

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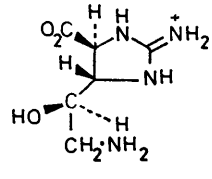


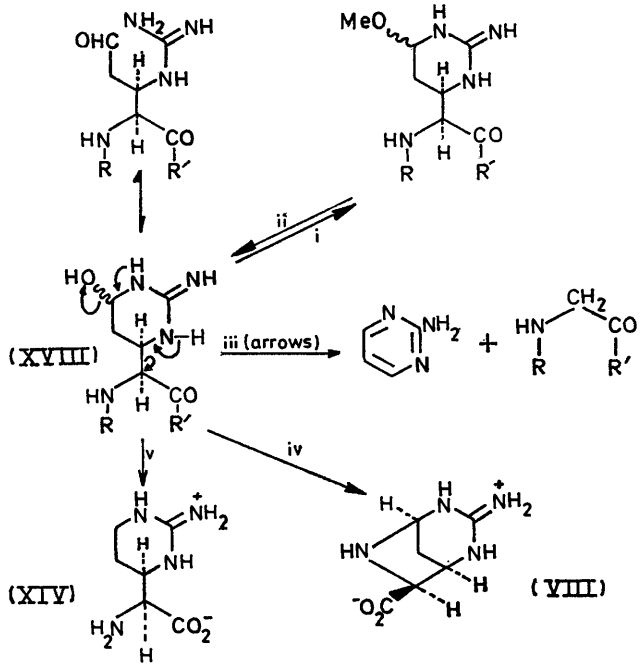
(XVII)

The formation of capreomycidine from viomycin provided further evidence that viomycidine was an artefact, and we therefore required a formulation of a viomycin unit which would yield (i) capreomycidine on hydrogenolysis and hydrolysis, (ii) viomycidine on acidic hydrolysis, and (iii) 2-aminopyrimidine on alkaline hydrolysis; of the various possibilities we have shown that the guanidine-carbinol system (XVIII) is correct.[27] As with the related carbinolamines, the hydroxy-function of the guanidino-carbinols is susceptible to nucleophilic substitution or to easy removal by dehydration. Thus, an intramolecular substitution of the hydroxy-group of the guanidino-carbinol by the amino-group (XVIII) leads to viomycidine after hydrolysis, and a relatively ready hydrogenolysis of the hydroxy-group accounts for the formation of capreomycidine. The breakdown of the guanidino-carbinol to 2-amino-pyrimidine and glycine is viewed as a retro-Michael reaction and dehydration promoted by the developing aromatic system. The formation of the *O*-methyl derivative of viomycin by warming the hydrochloride with methanol has found application in the purification of the antibiotic (above), as the methoxy-derivative is hydrolysed easily by hot water. Similar reactions with ethanol, propan-1-ol, or propan-2-ol failed to give the corresponding alkoxyviomycins, possibly because of poor solubility. The formation of alkoxy-derivatives of the parent 2-amino-4-hydroxy-1,4,5,6-tetrahydropyrimidine has also been reported.[38]

The guanidino-carbinol structure also occurs in the puffer fish neurotoxin, tetrodotoxin [39–41] (XIX), and a close parallel exists between the above reactions of viomycin and those of tetrodotoxin, *e.g.* the formation

[32] A. W. Johnson and D. J. McCaldin, *J. Chem. Soc.*, 1958, 817.
[33] Part II, following paper.
[34] D. W. Thomas, E. Lederer, M. Bodanski, J. Izdebski, and I. Muramatsu, *Nature*, 1968, **220**, 580.
[35] N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Agric. Biol. Chem.*, 1966, **30**, 132.
[36] H. E. Carter, C. C. Sweeley, E. E. Daniels, J. E. McNary, C. P. Schaffner, C. A. West, E. E. van Tamelen, J. R. Dyer, and H. A. Whaley, *J. Amer. Chem. Soc.*, 1961, **83**, 4296.
[37] J. H. Bowie, E. Bullock, and A. W. Johnson, *J. Chem. Soc.*, 1963, 4260; B. W. Bycroft and T. J. King, *Chem. Comm.*, in the press.
[38] E. Suzuki, S. Inoue, and T. Goto, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 933.
[39] R. B. Woodward, *Pure Appl. Chem.*, 1964, **9**, 49.
[40] T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *Tetrahedron*, 1965, **21**, 2059.
[41] K. Tsuda, *Naturwiss.*, 1966, **53**, 171.

of an *O*-methyl derivative with methanolic hydrogen chloride, the formation of a 2-aminoquinazoline in a

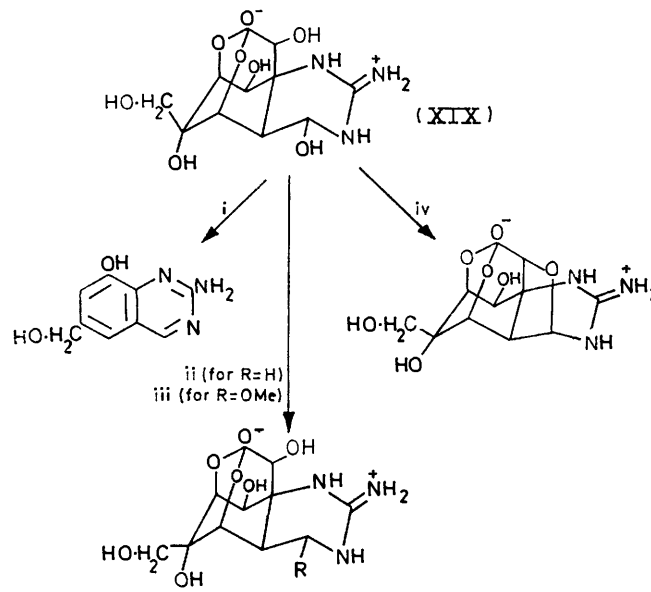


Reagents: i, MeOH–HCl; ii, H_2O; iii, NaOH; iv, HCl; v, Pt–H_2 then H^+.

base-catalysed elimination involving the production of the aromatic ring, and the intramolecular nucleophilic substitution of the hydroxy-group by another neighbouring hydroxy-group with the formation of an ether function and the hydrogenolysis of the carbinol function.[42]

The cyclic guanidino-carbinol structure in viomycin exists in equilibrium with the acyclic guanidine aldehyde tautomer, which accounts for the positive Sakaguchi and Fehling reactions shown by viomycin. In this connection, reference should be made to the work of Takita and Maeda,[43] who reduced viomycin with sodium borohydride and hydrolysed the product. From the hydrolysate they isolated a compound which they named dihydroviomycidine hydrochloride, $C_6H_{12}N_4O_2$,HCl,1·5-H_2O, which showed positive ninhydrin and Sakaguchi reactions. A comparison of the physical properties, especially n.m.r. spectra, showed that the new compound was not identical with the hydrochloride of capreomycidine (XIV), and they therefore inferred that dihydroviomycidine and capreomycidine were stereoisomers, and assigned structure (XX) to the former. However the n.m.r. spectrum of dihydroviomycidine hydrochloride also differed from that of the hydrochloride of a sample of racemic *epi*-capreomycidine (XX) which we had synthesised,[44] and we therefore concluded[45] that the product obtained by the Japanese workers was derived from the acyclic guanidine aldehyde structure, *i.e.* it was (XXI), which in fact agreed better with the microanalytical figures quoted. In a further note,[30] Takita and Maeda reported further work, including the preparation of an *O*-acetyl derivative, on the structure of their product which led them to accept our formulation (XXI), $C_6H_{12}N_4O_3$,HCl,0·5H_2O.

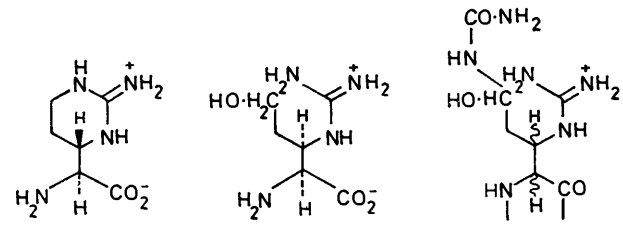
An attempt to assign the configuration of the viomycidine hydroxy-substituent from the n.m.r. spectrum did not give a definitive result, but the question may be resolved by further study of the mechanism of formation of viomycidine (VIII), or by the *X*-ray examination of viomycin dihydrobromide hydrochloride at present in progress. No evidence of mutarotation of viomycin has



Reagents: i, NaOH; ii, H_2–Pt; iii, MeOH–HCl; iv, H^+.

been observed even though the carbinol system is an anomeric centre.

Lechowski[46] has suggested (XXII) as a viomycin guanidine–urea chromophoric fragment, but, as shown in the following paper, the urea is not attached to the viomycidine precursor but rather to the dehydroserine chromophore.



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[45] B. W. Bycroft, L. R. Croft, A. W. Johnson, and T. Webb, *J. Antibiotics*, 1969, **22**, 133.

[46] L. Lechowski, *Tetrahedron Letters*, 1969, 479; *Roczniki Chem.*, 1971, **25**, 581.

Recently,[47] an *X*-ray structural study has been reported on tuberactinomycin O, which is closely related to viomycin and capreomycin. In an earlier note,[48] the authors reported the isolation from tuberactinomycin A[5] of a degradation product ($C_{14}H_{20}N_8O_6$) of unspecified structure; when subjected to further acid hydrolysis, this yielded viomycidine and the guanidinocarbinol (XVIII; R = H, R′ = OH) named tuberactidine. This structure was assigned largely on the basis of an interpretation of the n.m.r. spectrum and a comparison of the spectrum with that of the parent 2-amino-4-hydroxy-1,4,5,6-tetrahydropyrimidine.[38] The reduction of tuberactidine with sodium borohydride to give (XXI) was shown to follow the course we had postulated.[44] The same guanidino-carbinol was detected [48] in acid hydrolysates of viomycin (tuberactinomycin B)[47] but experimental details are not yet available.

The evidence detailed above finds a logical explanation in the existence of the unit (XVIII) in viomycin.

EXPERIMENTAL

M.p.s were measured on a Kofler hot-stage apparatus. U.v. spectra were recorded with a Perkin-Elmer 137UV instrument, for solutions in water unless otherwise stated, and i.r. spectra for potassium bromide discs with a Unicam SP 200 spectrophotometer. N.m.r. spectra were measured at 60 MHz with a Perkin-Elmer R10 spectrometer, for deuterium oxide solutions (unless otherwise stated), usually with sodium 3-(trimethylsilyl)propane-1-sulphonate as internal reference, but where it was deemed more important to recover the material, with the HOD absorption as reference (although the position of this band is not absolutely constant, the solutions were at approximately the same pH, and the variation was not large). pK_a Values were determined for solutions in water, by the method of Parke and Davies.[49] Paper chromatography (descending) was carried out on Whatman no. 3 paper in the following solvent systems: (A) t-butyl alcohol–acetic acid–water (2 : 1 : 1); (B) ethanol–water (7 : 3). High voltage electrophoresis was performed using a Pherograph apparatus, with Whatman no. 3 paper and acetic acid–formic acid buffer (pH 1·9). A BioCal 200 amino-acid analyser was used for the determination of amino-acids.

Viomycin.—The antibiotic was obtained in the form of the crystalline sulphate (Found: C, 35·9; H, 5·9; N, 21·5; S, 5·7. Calc. for $C_{25}H_{43}N_{13}O_{10},1{\cdot}5H_2SO_4$: C, 36·05; H, 5·55; N, 21·9; S, 5·7%), $\lambda_{max.}$ (0·1N-HCl) 269 nm (ε 23,910), $\lambda_{max.}$ (0·1N-NaOH) 285 nm (ε 15,300), $\nu_{max.}$ 3184, 3090, 3070, 2957, 1669, 1650, 1621, 1508, 1352, 1327, 1228, 1110, and 1057 cm^{-1}, pK_a (by titration) 8·2, 9·7, and >12·5, pK_a (spectrophotometric) 12·65, *M* (thermistor drop), 825, *M* (bromine titration), 860, *M* (permanganate oxidation), 888 (calc. for $C_{25}H_{43}N_{13}O_{10},1{\cdot}5H_2SO_4$: *M*, 836). For the counter-current purification of viomycin, n-butanol was equilibrated with aqueous 6·4% sodium chloride solution for 24 h, then loaded into the counter-current distribution machine (80 tubes), n-butanol only being added to the first four tubes. Viomycin free base (500 mg), dissolved in 100 ml of aqueous phase, was introduced equally into the first four tubes. The distribution procedure was as follows: volume of each phase, 25 ml; settling time, 15 min; mixing time, 5 min; transfer and decanting time, 30 s; number of transfers, 329. The distribution train was operated by the recycling procedure and when this was terminated 5 ml of the aqueous phase was removed from each tube and diluted with ethanol (10 ml), and the optical density was read at 268 nm. A graph of optical density against tube number indicated one symmetrical peak. An attempted mol. wt. determination on viomycin by mass spectrometry showed a strong peak at *m/e* 95, which by high resolution was shown to correspond to $C_4H_5N_3$ (2-aminopyrimidine).

Viomycin hydrochloride (Found: C, 37·35; H, 6·2; N, 23·0. $C_{25}H_{43}N_{13}O_{10},3HCl$ requires C, 37·6; H, 5·8; N, 22·8%) and the *sulphate picrate* (Found: C, 37·1; H, 5·0; N, 22·2. $C_{25}H_{43}N_{13}O_{10},H_2SO_4,C_6H_3N_3O_7$ requires C, 36·75; H, 4·75; N, 22·1%) were also prepared. A crystallographic molecular weight determination (carried out by Professor J. Carlisle, Birkbeck College, London) on the latter gave a value of 1025, corresponding to 686 for viomycin free base, in agreement with the calculated value. Viomycin orange II sulphate was also made (Found: C, 43·7; H, 5·0; N, 19·0. $C_{25}H_{43}N_{13}O_{10},H_2SO_4,C_{16}H_{12}N_2O_4S$ requires C, 44·15; H, 5·15; N, 18·85%).

Acidic Hydrolysis of Viomycin. Isolation of the Amino-acid Components.—Several previous workers have described the isolation of L-serine, L-αβ-diaminopropionic acid, L-β-lysine, and viomycidine from the hydrolysate of viomycin, but a check was made to ensure that they were also present in the viomycin used in this investigation. Following the method of Haskell *et al.*,[10] the amino-acids were isolated and characterised. Serine was isolated as the hydrochloride and crystallised from aqueous ethanol to give needles, identical in i.r. spectrum and chromatographic behaviour in solvents (A) and (B) with an authentic sample of L-serine hydrochloride. αβ-Diaminopropionic acid was isolated as the monohydrochloride, m.p. 245° (from aqueous ethanol) (lit.,[50] 245°), $[\alpha]_D^{23}$ +3·7° (*c* 0·76 in H_2O), pK_a (H_2O) 6·6 and 9·1 (lit.,[51] 6·6 and 9·1); paper chromatographic behaviour identical with that of an authentic sample in systems (A) and (B). The bisdinitrophenyl derivative was identical [t.l.c. in n-propanol–ammonium hydroxide (2 : 1)] with an authentic sample. The bisbenzyloxycarbonyl derivative was prepared, following the procedure of Poduška *et al.*[52] (Found: C, 60·95; H, 5·7; N, 7·3. Calc. for $C_{19}H_{20}N_2O_6$: C, 61·3; H, 5·4; N, 7·5%); τ ($CDCl_3$) 0·8 (1H, s), 2·8 (10H, s, aromatic protons), 5·0 (4H, s, 2 × PhCH_2), 5·6 (1H, m), and 6·4 (1H, m). β-Lysine was isolated as the dihydrochloride, m.p. 148°, identical [i.r. spectrum, paper chromatography in system (A), and electrophoresis] with the same amino-acid isolated from a sample of streptothricin, following the procedure of Johnson and Westley.[53] Viomycidine was isolated as the free base and crystallised from ethanol–ether; $\nu_{max.}$ 3410, 1660, 1600, 1417, 1375, 1370, 1280, 1172, 1150, 1100, 1030, 960, 937, 900, 850, 805, and 720 cm^{-1} identical with the i.r. spectrum of a sample supplied by

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[50] J. P. Greenstein and M. Winitz, ' Chemistry of the Amino Acids,' Wiley, London, 1961, p. 650.

[51] Ref. 50, p. 487.

[52] K. Poduška, J. Rudiger, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1955, **20**, 1174.

[53] A. W. Johnson and J. W. Westley, *J. Chem. Soc.*, 1962, 1642.

Professor Büchi;[25] τ 7·9 (2H, m, 5-H_2), 6·2 (1H, d, side-chain methine, J 4 Hz), 5·9 (1H, m, 4-H), with an additional one-proton signal coinciding with the HOD peak. The monohydrochloride, crystallised from aqueous ethanol, had m.p. 200—204° (lit.,[22] 200—208°), $[\alpha]_D^{22}$ −85° (*c* 0·11 in H_2O) {lit.,[22] $[\alpha]_D^{30}$ −78° (*c* 1·78 in H_2O)}, $\nu_{max.}$ 3500, 3200, 2900, 1700, 1660, 1620, 1590, 1550, 1475, 1420, 1350, 1280, 1240, 1160, 1060, 940, 900, 820, and 740 cm^{-1}, τ 4·45 (1H, t, J 2 Hz), 5·48 (2H, s), and 7·5 (2H, t, J 2 Hz). Viomycidine methyl ester dihydrochloride was prepared following the procedure of Büchi and Raleigh[25] (Found: C, 32·6; H, 5·5; N, 22·6. Calc. for $C_7H_{12}N_4O_2,2HCl$: C, 32·7; H, 5·45; N, 21·8%), $\nu_{max.}$ 3200, 1750, 1675, 1630, 1580, 1550, 1450, 1392, 1352, 1280, 1240, 1215, 1162, 1060, 1010, 912, 840, 795, and 700 cm^{-1}, τ 4·4 (2H, m), 6·05 (3H, s), 7·5 (2H, t, J 2·5 Hz), and a 1H signal under the HOD peak.

Viocidic Acid (XI).—Viomycin sulphate (10 g) was heated under reflux for 24 h with hydrochloric acid (10N; 60 ml). The resulting solution was evaporated to dryness, and small quantities of water were then added and distilled out to remove traces of volatile acid. The hydrolysate was taken up in distilled water (50 ml) and added to a column (3 × 80 cm) of Dowex 50 W × 8 (H^+) cation-exchange resin. The column was eluted with hydrochloric acid of increasing strength (1—6N). Fractions (15 ml) were taken every 30 min; by fraction 500 (4N-HCl) all the amino-acids had been removed and the strength of the acid was increased to 6N. Fractions 700—750 contained viocidic acid [paper chromatography in system (A) was used to monitor each fraction]; these fractions were evaporated to dryness, to give viocidic acid hydrochloride as a glass (800 mg). This was dissolved in distilled water and the pH was adjusted to 7 with Amberlite CG (400) (OH^-) anion-exchange resin. After removal of the resin a saturated solution of picric acid was added, which caused the precipitation of viocidic acid picrate. After five crystallisations from water, pure viocidic acid picrate (100 mg) was obtained as *needles*, m.p. 173—175° [Found: C, 35·2; H, 2·7; N, 22·8. $C_8H_{13}N_5O_2,(C_6H_3N_3O_7)_2$ requires C, 35·8; H, 2·7; N, 22·7%], $\nu_{max.}$ 3400, 3100, 2940, 2600, 2520, 1682, 1640, 1555, 1495, 1435, 1364, 1340, 1270, 1198, 1160, 1080, 990, 925, 918, 792, 742, and 710 cm^{-1}. The *dihydrochloride* was prepared from viocidic acid picrate (90 mg) by dissolution in 2N-hydrochloric acid (10 ml) and extraction with diethyl ether (50 ml). The resulting solution was evaporated to dryness *in vacuo* and the crystalline residue, after several crystallisations from ethanol–water, gave needles (20 mg), m.p. 210—212° (decomp.) (Found: C, 31·7; H, 5·5; Cl, 19·9; N, 23·4. $C_8H_{13}N_5O_2,2HCl,H_2O$ requires C, 31·6; H, 5·6; Cl, 23·5; N, 23·1%), $\nu_{max.}$ 3399, 3247, 3184, 3077, 3006, 2914, 2615, 2532, 1692, 1633, 1598, 1582, 1482, 1419, 1383, 1356, 1287, 1259, 1228, 1192, 1169, 1118, 1099, 1062, 998, 96 26, 798, and 732 cm^{-1}, τ 4·25 (1H, d, J 8·5 Hz), 5·4 (1H, s), and 6·0 (5H, m), $[\alpha]_D^{22}$ −51° (*c* 0·08 in H_2O), pK_a <2, 5·8, 6·8, and >12·5. Viocidic acid did not give a positive Sakaguchi reaction. The *dihydrobromide* was prepared from the dihydrochloride (17 mg) by dissolution in a small amount of distilled water and addition of 48% hydrobromic acid (4 ml). The excess of acid was removed under reduced pressure and the residue was repeatedly evaporated with water. It crystallised from aqueous ethanol to give *viocidic acid dihydrobromide trihydrate* (20 mg), m.p. 222—225° (Found: C, 22·4; H, 4·45; Br, 36·7; N, 16·5. $C_8H_{13}N_5O_2,2HBr,3H_2O$ requires C, 22·5; H, 4·45; Br, 38·0; N, 16·4%), $\nu_{max.}$ 3400, 1680, 1620, 1390, 1320, 1215, 1185, 1100, 1080, 1010, 800, and 720 cm^{-1}.

2-Aminopyrimidine from Viomycin.—Viomycin sulphate (3·0 g) was dissolved in N-sodium hydroxide (150 ml) and heated under reflux for 1 h. The solution was cooled and extracted exhaustively with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated, leaving a crystalline residue (250 mg) which was purified by sublimation to give 2-aminopyrimidine, m.p. 127°, identical (mixed m.p., u.v., and i.r. spectra) with an authentic specimen,[54] $\lambda_{max.}$ (EtOH) 227 and 297 nm (ε 14,600 and 3160).

Isolation of Capreomycidine [α-(2-*Iminohexahydropyrimidin-4-yl*)*glycine*] (XIV) *from Viomycin.*—Viomycin hydrochloride (2 g) was hydrogenated in 3N-hydrochloric acid (50 ml) over platinum (2 g) at room temperature and 3 atm pressure; after 60 h the reaction was terminated. After filtration and careful washing of the catalyst, the solvent was removed under reduced pressure to yield a glass, which was hydrolysed with 6N-hydrochloric acid overnight. The hydrolysate, which was slightly coloured was evaporated to dryness and dissolved in water. This solution was adjusted to pH 6 by passing it through an Amberlite 4B (OH^-) anion-exchange column (3 × 42 cm). The column was eluted with distilled water to remove all ninhydrin-positive material and the combined washings were introduced on to an Amberlite CG 400 (OH^-) anion-exchange column (3 × 60 cm). The column was eluted with distilled water and fractions (15 ml) were taken every 6 min. Electrophoresis indicated that fractions 17—20 contained α-(2-iminohexahydropyrimidin-4-yl)glycine. These fractions were combined and concentrated to dryness, and the residue was crystallised from aqueous ethanol to give small crystals (77 mg), identical (i.r. and n.m.r. spectra) with the same amino-acid isolated from capreomycin; t.l.c. (silica gel G; potassium dihydrogen phosphate buffer at pH 6·0) R_F 0·6 (R_F of viomycidine 0·5 under similar conditions).

Basic Hydrolysis of Viomycidine.—Viomycidine hydrochloride (1·06 g) in N-sodium hydroxide (50 ml) was heated under reflux for 20 h; the solution was then cooled and extracted with ether at pH 10, 7, and 2 (the pH was altered by careful addition of 50% sulphuric acid). The extracts were then dried and evaporated. The extract from the basic solution gave crystalline material (4 mg) which was purified by sublimation and identified as 2-aminopyrimidine (m.p. and i.r. absorption). The extract of the acidic solution yielded pyrrole-2-carboxylic acid (100 mg), m.p. 190—192° (from methanol), identical (mixed m.p. and i.r. spectrum) with authentic material.

2-Guanidino-Δ¹-pyrroline Toluene-p-sulphonate (with D. Cameron).—Guanidine hydrochloride (40·25 g) was added to a solution of sodium (9·25 g) in dry ethanol (500 ml) and the mixture was heated under reflux for 45 min, and cooled. The precipitated sodium chloride was separated and washed with ethanol (2 × 70 ml) and to the combined filtrates and washings was added 2-ethoxy-Δ¹-pyrroline[55] (22·5 g). The solution was heated under reflux for 18 h, cooled, filtered, and evaporated at 40° *in vacuo* until the solid product precipitated. The remaining solvent was removed by filtration and the solid was washed with ether and crystallised from a small volume of ethanol. The crystalline product was washed with a little ethanol at −50° and then with ether; it then had m.p. 140—143°. It gave a strong Sakaguchi reaction and showed $\lambda_{max.}$ 241 nm (ε 9670) in

[54] E. Büttner, *Ber.*, 1903, **36**, 2227.
[55] A. Etienne and Y. Correia, *Compt. rend.*, 1964, **259**, 2660.

water, and 242·5 nm (ε 10,580) as the hydrochloride, m.p. 138—138·5°. Satisfactory analytical figures for the free base and the hydrochloride were not obtained because of their hygroscopic properties, but the *toluene*-p-*sulphonate*, m.p. 134—136° (from ethanol), formed crystals (Found: C, 48·1; H, 5·7; N, 18·65. $C_{12}H_{18}N_4O_3S$ requires C, 48·3; H, 6·05; N, 18·8%), $\lambda_{max.}$ 223·5, 228infl, and 240 nm (ε 16,610, 14,500, and 11,700). It gave a strong positive Sakaguchi reaction. Biguanide [56] (for comparison) showed $\lambda_{max.}$ 230 nm (ε 8900) at pH >14 and 230 nm (ε 12,360) at pH 9.

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[1/1872 ...*eived, 13th October,* 1971]

[56] R. C. Hirt and R. G. Schmitt, *Spectrochim. Acta,* 1958, **12** 127.