Tetrahedron Letters No.10, pp. 585-592, 1965. Pergamon Press Ltd.
Printed in Great Britain.

VIOMYCIN: II. THE STRUCTURE OF VIOMYCIN

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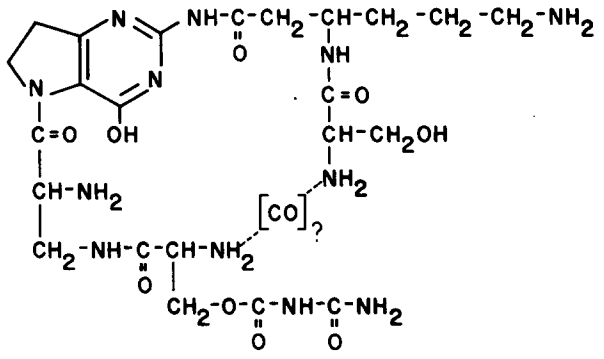
(Received 3 December 1964; in revised form 18 January 1965)

The tuberculostatic streptomyces antibiotic viomycin (1,2) is degraded on acid hydrolysis, and yields urea, carbon dioxide, ammonia, L-serine, L-α,β-diaminopropionic acid (2,3), L-β-lysine (4,5), and a basic guanido amino acid (2,3), which we have named viomycidine (6,7). The recent publication of Bowie, Cox, Johnson, and Thomas (8), which suggested I as the structure of viomycin, prompts us to report our findings, which we

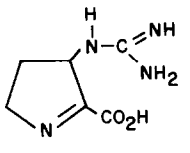


I

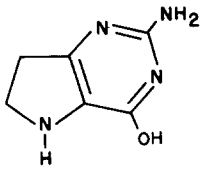
believe more logically suggest a different structure for this antibiotic. Structure I does not satisfy much of the physical data and chemical evidence obtained for viomycin. There is no evidence that viomycin contains an acidic group, as would be expected of the hydroxypyrimidine nucleus of I, but instead, viomycin has pK_a values of 8.2, 10.3, and 12 (in water), attributed to two primary amino groups (van Slyke analysis) and a guanidine function. The acylaminopyrimidine unit of I would not be strongly basic nor would it be expected to give a positive Sakaguchi reaction, which viomycin does. The n.m.r. spectra of viomycin and its simple degradation products (see below) exhibit absorption caused by one proton at ca. 2 τ (deuterium oxide solution), which cannot be accounted for by structure I. Full acid hydrolysis of viomycin releases only one equivalent of carbon dioxide, whereas two would be required from the suggested structure I.

Bowie, Johnson, and Thomas (9) reported the isolation (experimental details not yet available) of a dipeptide component ("peptide A") of viomycin that gave, on full acid hydrolysis, viomycidine and α,β-diaminopropionic acid. The amino groups of α,β-diaminopropionic acid were shown to be free in the dipeptide by the preparation of the N-2,4-dinitrophenyl derivative, which gave, on acid hydrolysis, α,β-bis(2,4-dinitrophenylamino)propionic acid. Because the ultraviolet spectrum of the dipeptide (λ_{max} 275 mμ, ε 5100 at pH 1 and λ_{max} 295 mμ, ε 3600 at pH 10) was similar to that of viomycin (λ_{max} 268 mμ, ε 24,600 at pH 1 and λ_{max} 290 mμ, ε 16,100 at pH 10), the chromophoric unit of the dipeptide was proposed to be the same as that present in viomycin. Based on a provisional structure (II) that we proposed (6) for viomycidine, Bowie, Johnson, and Thomas (9) proposed that viomycidine exists in viomycin as the cyclized structure III, and that the dipeptide

isolated from viomycin has structure IV.



II



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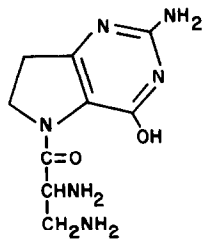
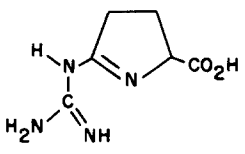


IV

Since viomycidine, as obtained from viomycin hydrolysate, is optically active, structures III and IV are rejected as integral structural units of the viomycin molecule. We have recently determined that viomycidine is R-2-guanido-Δ^1-pyrroline-5-carboxylic acid (V) (7), rather than II. We propose that the dipeptide isolated (9) from viomycin hydrolysate has structure VI, 2-amino-4-(1,2-diaminoethyl-6,7-dihydropyrrolo[1,2,a]-s-triazine-6-carboxylic acid (10), and is responsible for the ultraviolet absorption of viomycin.



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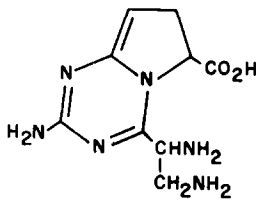
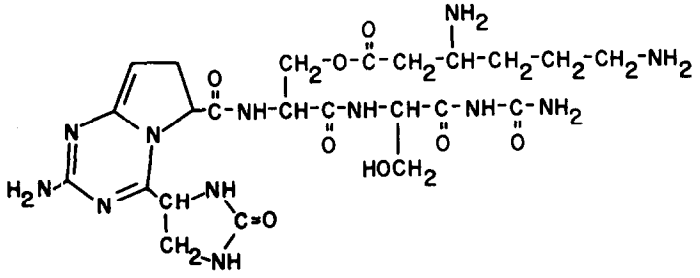


VI

Structure VI is the first example of a derivative of the pyrrolo-[1,2,a]-s-triazine ring system reported. A large number of dihydro-s-triazines have been synthesized and many possess a broad spectrum of biological activity (11), including activity against many microorganisms. It is possible that the antibacterial activity exhibited by viomycin results from the presence in the molecule of the structural unit VI.

Acid hydrolysis of viomycin ($C_{23}H_{36}N_{12}O_8$) releases two equivalents of L-serine and one equivalent each of the previously mentioned hydrolysis products. Ammonia, reported by others (2) as a primary hydrolysis product, has been shown to arise from too vigorous hydrolytic conditions. Based on our findings, described below, we suggest structure VII for viomycin.



VII

Hydrazinolysis (12) of viomycin indicated no free carboxyl group, a conclusion in agreement with the potentiometric titration data. Acid hydrolysis of bis-2,4-dinitrophenylviomycin gave all of the hydrolysis products

described previously except β-lysine; bis-2,4-dinitrophenyl-β-lysine[1] was obtained from the hydrolysate in 70% yield. Thus the three basic centers of viomycin are present as the amino groups of β-lysine[2] and the guanidine function.

When viomycin was treated with 0.1 N hydrochloric acid at 95° for six hours, one equivalent of urea was released;[3] no other amino acid was found in significant amount. The resulting product, desureaviomycin (3), upon vigorous acid hydrolysis gave all of the usual hydrolysis products of viomycin except urea. Hydrazinolysis of desureaviomycin ($C_{22}H_{36}N_{10}O_9$) revealed a free serine carboxyl group, and hydrolysis of bis-2,4-dinitro-

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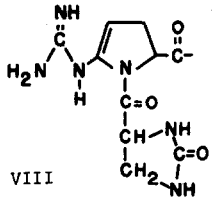
[1] Bowie et al. (8) report the isolation of mono-ε-, rather than bis-2,4-dinitrophenyl-β-lysine. We find that when bis-2,4-dinitrophenylviomycin is hydrolyzed by boiling 6 N hydrochloric acid, little 2,4-dinitrophenyl-β-lysine results, but considerable decomposition occurs, and 2,4-dinitrophenol and a mono-2,4-dinitrophenyl-β-lysine result. On the other hand, hydrolysis of bis-2,4-dinitrophenylviomycin by 12 N hydrochloric acid at 95° (pressure bottle) results in little decomposition. Less decomposition results, leading to superior yields of 2,4-dinitrophenylamino acids in the hydrolysis of 2,4-dinitrophenylpeptides, if concentrated acid is used (13).

[2] That the two free amino groups of β-lysine are free in viomycin is more in agreement with the pK_a values of the amino groups of viomycin (8.2 and 10.3) than if the α-amino group of α,β-diaminopropionic acid and the ε-amino group of β-lysine were free, as suggested by Bowie, et al. (8). β-Lysine has pK_a values of 8.7 and 10.2 in water and α,β-diaminopropionic acid has pK_a values of 6.6 and 9.1 in water.

[3] Desureaviomycin shows no λ_{max} in the ultraviolet region, so the structural unit in viomycin responsible for ultraviolet absorption is altered, without fragmentation, during this hydrolysis. Because desureaviomycin and viomycinic acid show n.m.r. absorption caused by a single proton at 2 τ (deuterium oxide solution), we believe the 6,7-dihydropyrrolo[1,2,a]-s-triazine unit of viomycin is changed to part structure VIII in these two compounds.

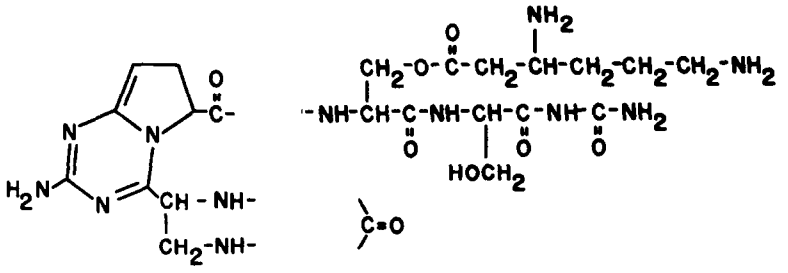


VIII

phenyldesureaviomycin gave bis-2,4-dinitrophenyl-β-lysine and the hydrolysis products of viomycin except urea and β-lysine. Carbon dioxide is not produced during the preparation of desureaviomycin, as would be required by formula I. Indeed, that the source of carbon dioxide was not from the hydrolysis of a base sensitive urethane is indicated by the fact that carbon dioxide is not released from viomycin, desureaviomycin, or viomycinic acid on treatment with mild alkali.

When desureaviomycin was treated with carboxypeptidase, one equivalent of serine was released. Hydrazinolysis of the product, which we have named viomycinic acid ($C_{19}H_{31}N_9O_7$), revealed a free seryl carboxyl group, and hydrolysis of bis-2,4-dinitrophenylviomycinic acid gave bis-2,4-dinitrophenyl-β-lysine and all of the usual hydrolysis products of viomycin except urea and β-lysine. Viomycinic acid was found to be inert to further action by carboxypeptidase. It is concluded that viomycin contains a seryl-serylurea unit.

When a solution of viomycin sulfate was boiled in water for three weeks, there was isolated a dipeptide that gave, on hydrolysis, serine and β-lysine. As this dipeptide cannot occur in viomycin as an N-β-lysylseryl unit, it is formulated as arising from an O-β-lysylseryl unit and is probably identical with "peptide B" obtained by Bowie *et al.* (8). Further evidence for the presence of a β-lysyl ester linkage in viomycin is derived from the fact that β-lysine was released very much more rapidly than serine on partial hydrolysis of viomycinic acid. Ordinarily, peptides containing seryl units are hydrolytically cleaved most rapidly at the N-seryl bond, owing to N → O acyl migration. The observations summarized above lead to a partial structure of viomycin as shown in IX.



IX

Bowie et al. (8) reported the isolation from viomycin partial hydrolysates of a number of peptides only one of which, in addition to "peptide A," was obtained in pure form and characterized as a crystalline derivative. This compound, "peptide D," was assigned the formula $C_{15}H_{27}N_7O_3$ and was reported to yield serine, β-lysine, and viomycidine on further hydrolysis. VII is the only rational formula that can be derived for viomycin that satisfies this observation. Structure VII cannot explain all of the peptides obtained by Bowie et al. (8) by partial hydrolysis; however, some of those peptides reported may have been mixtures.

Acknowledgment. -- This work was supported in part by Research Grant E-2007 from the Institute of Allergy and Infectious Disease of the National Institutes of Health, Public Health Service.

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