Communications to the Editors

THE STRUCTURE OF THE GUANIDO AMINO ACID MOIETY OF VIOMYCIN

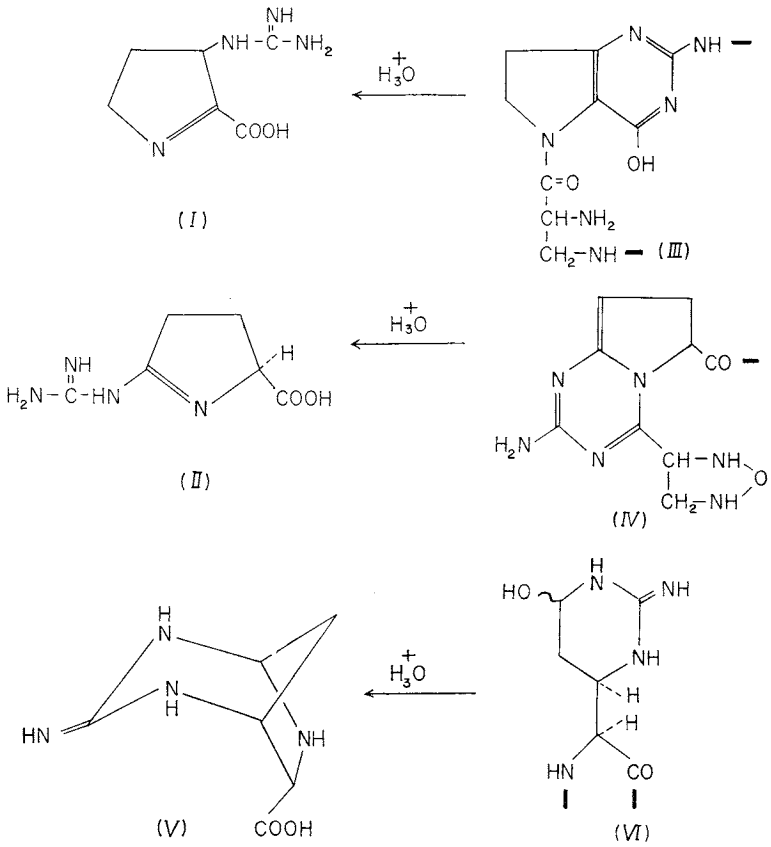
Sir:

A recent paper by BYCROFT *et al.*[1] on degradative studies of viomycin prompted us to describe our findings.*

For the structure of viomycidine, a guanido compound obtained by acid hydrolysis of viomycin, structures I[2] and II[3] have been suggested by BOWIE *et al.*[2] and DYER *et al.*[3] They pointed out further that these structures originated from partial structures III[4] and IV[5] of viomycin, respectively.

In 1966, RALEIGH proposed another structure V[6] for viomycidine. The present communication indicates that this structure is derived from partial structure VI of viomycin.

We agreed with the formulation of viomycidine as V for the following reasons. We isolated viomycidine by ion exchange chromatography (Dowex 50W×4 in pyridine acetate buffer) from the acid hydrolyzate of viomycin (hydrolysis: reflux in 6 N hydrochloric acid for 24 hours). Viomycidine was characterized as its crystalline monohydrochloride, m.p. 200~205°C (dec., uncorrected). Found: C 34.67, H 5.64, N 27.01. Calcd. for $C_6H_{10}N_4O_2 \cdot HCl$ (MW 206.7): C 34.87, H 5.37, N 27.11. $[\alpha]_D^{16}$ −74° (*c* 1, H_2O), −19° (*c* 1, 6 N HCl). It gave positive ninhydrin and negative SAKAGUCHI and DRAGENDORFF tests. Potentiometric titration showed the presence of one carboxyl group



* The content of this report was presented at the 159th meeting of Japan Antibiotics Research Association (January 26, 1968, in Tokyo). Abstract paper: *Journal of the Fermentation Association, Japan*, Vol. 26, No. 7, 1968.

of $pK_a'<2.0$ and two basic functions of pK_a' 5.5 and >12.0, with an epuivalent weight of 210. Formation of guanidine by oxidation with aqueous permanganate and the strongly basic function suggested the presence of a substituted gunaidine in viomycidine. The weakly basic function was characterized as a secondary amine adjacent to the strongly basic guanido group by the pK value (5.5), color reactions and no VAN SLYKE nitrogen. No hydrogen absorption was observed by catalytic hydrogenation of viomycidine with platinum oxide in 0.5 N hydrochloric acid solution, indicating two

Fig. 1. The n.m.r. spectrum of viomycidine free base in D_2O solution (60 Mc)

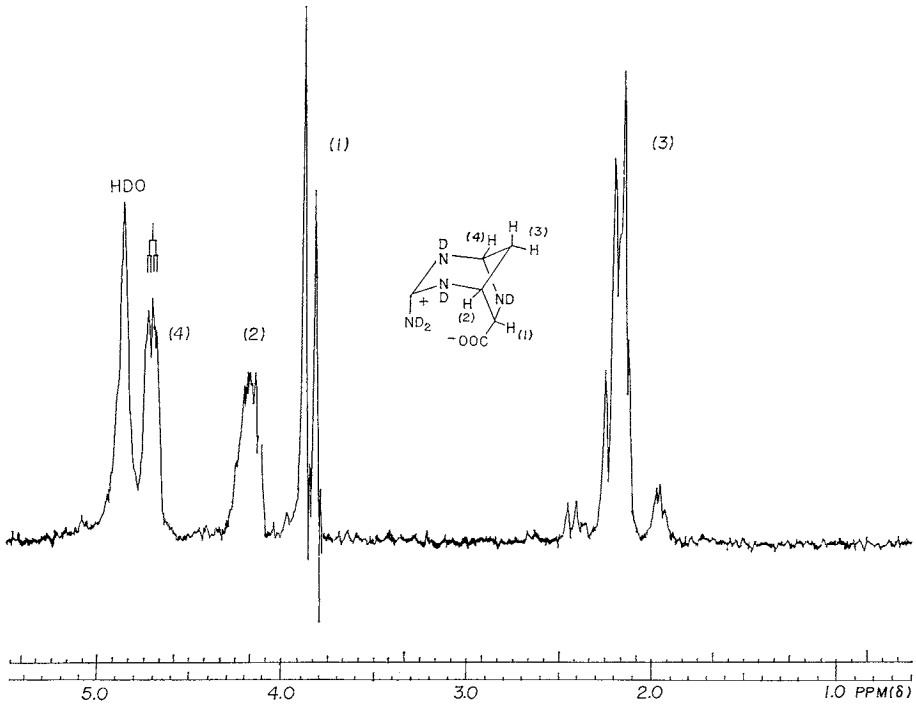
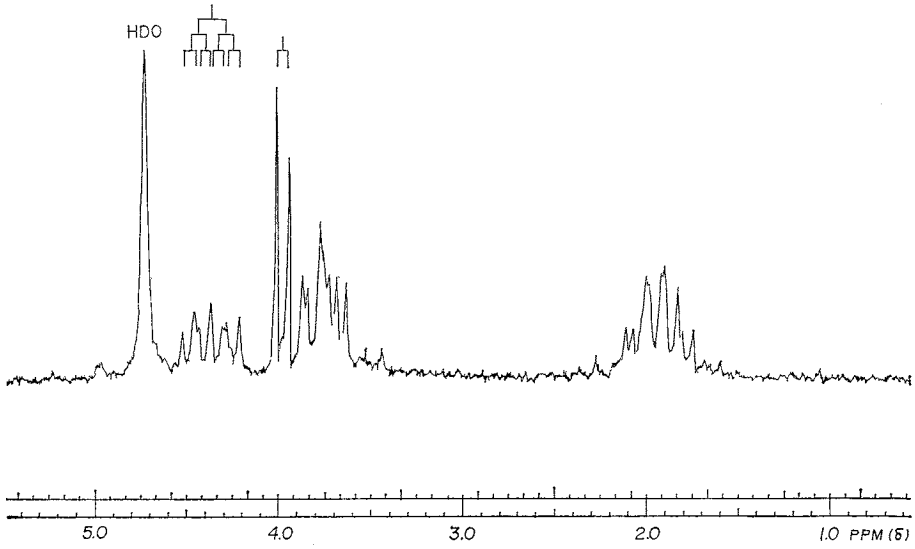


Fig. 2. The n.m.r. spectrum of dihydroviomycidine·HCl in D_2O solution (60 Mc)



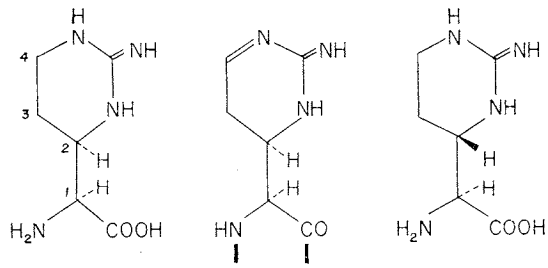
rings. The structure of viomycidine as V was deduced finally by interpretation of the n. m. r. spectrum of the free base in deuterium oxide solution[6] (Fig. 1).

The fact that viomycidine gives a negative SAKAGUCHI test[7], whereas viomycin itself give a positive reaction, suggests that viomycidine is an artefact formed by secondary reactions involving the guanido group during acid hydrolysis of viomycin.

Viomycin was reacted with excess sodium borohydride in aqueous solution. The reaction product, which we named deoxyviomycin, could not be differentiated from viomycin by paper chromatography and paper electrophoresis. The UV spectrum of deoxyviomycin was the same as viomycin. The n. m. r. spectrum of deoxyviomycin was very similar to viomycin with the exception of two additional protons at δ 3.7 ppm (multiplet). The total acid hydrolysis of deoxyviomycin yielded β-lysine, α,β-diamino propionic acid, serine, urea, carbon dioxide, ammonia and a SAKAGUCHI-positive substance, instead of viomycidine.

These facts indicate that a moiety of viomycin which yields viomycidine on acid hydrolysis is reduced by sodium borohydride. However, the chromophoric moiety is not reduced by sodium borohydride and exists independently in the molecule from the SAKAGUCHI-positive moiety.

We named the SAKAGUCHI-positive substance obtained from deoxyviomycin dihydroviomycidine. It was isolated by ion exchange chromatography and was crystallized as the monohydrochloride from aqueous ethanol, m. p. 182°C (dec., uncorrected). Found: C 30.57, H 6.89, N 23.26, O 24.00, Cl 15.10. Calcd. for $C_6H_{12}N_4O_2 \cdot {}^3/_2H_2O \cdot HCl$ (MW 235.7): C 30.58, H 6.84, N 23.77, O 23.76, Cl 15.04. $[\alpha]_D^{25}$ +25° (*c* 0.71, 6 N HCl). Potentiometric titration showed the presence of one carboxyl group of $pK_a' <$ 2.0 and two basic functions of pK_a' 7.4 and >12.0, with an equivalent weight of 241. It gave positive ninhydrin and SAKAGUCHI tests. Determination of primary amino group by the VAN SLYKE method showed the presence of one primary amino group. The structure VII was finally deduced by inter-



(VII) (VIII) (IX)

Fig. 3. The n.m.r. spectrum of capreomycidine·HCl in D_2O solution (60 Mc)

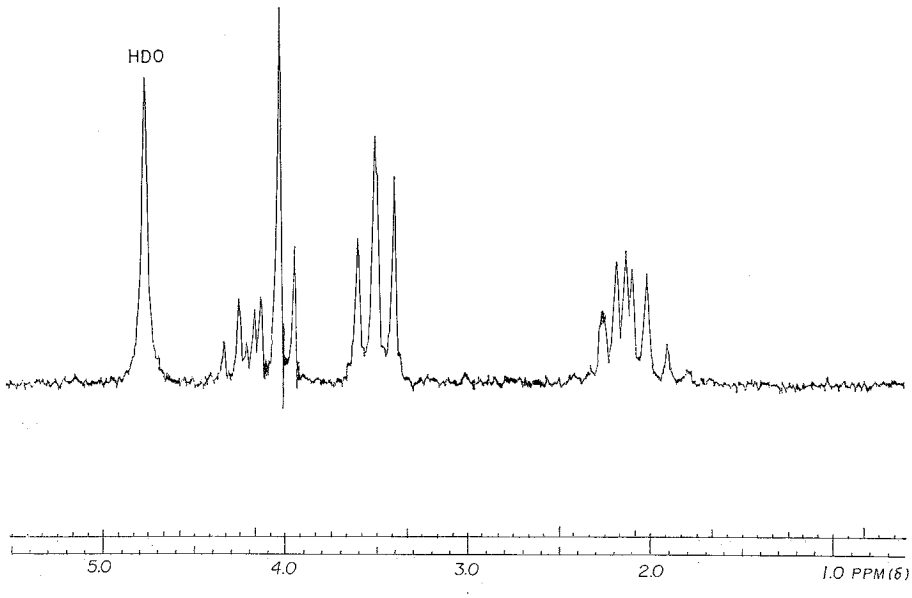
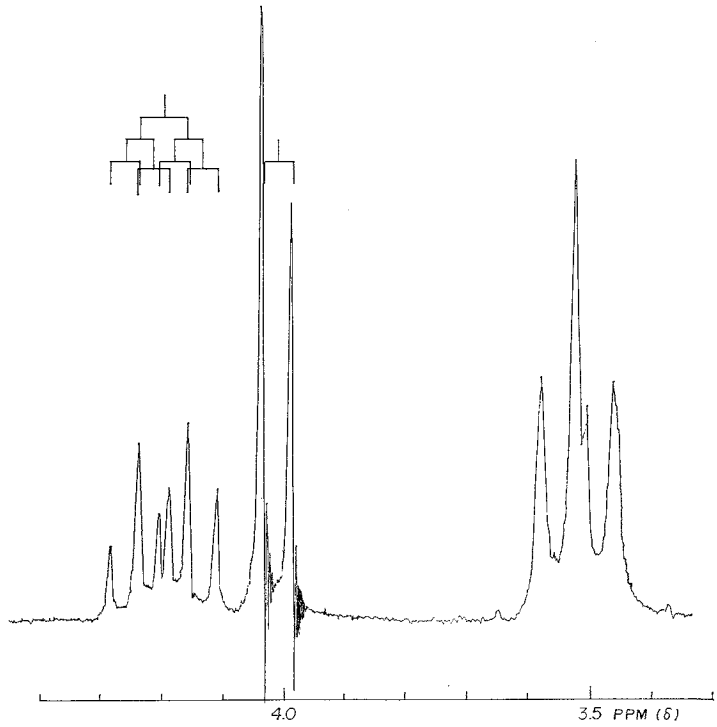


Fig. 4. The n.m.r. spectrum of capreomycidine·HCl in D_2O solution (100 Mc, δ 3.3~4.4 ppm)



pretation of the n. m. r. spectrum of dihydroviomycidine monohydrochloride in deuterium oxide solution (Fig. 2).

The assignment of the peaks is as follows: C_1 δ 3.96 (1 H, doublet J=4 Hz), C_2 4.35 (1 H, triple doublets J=4, 5 and 9 Hz), C_3 1.92 (2 H, multiplet), C_4 3.76 (2 H, multiplet).

BYCROFT *et al.*[1] reported that they obtained a compound of the same structure VII from total acid hydrolysate of hydrogenated viomycin and they also named it dihydroviomycidine. However, they did not describe the properties of VII and we could not obtain it from the acid hydrolyzate of hydrogenated viomycin (hydrogenation : with platinum oxide, under atmospheric pressure at room temperature overnight in 10 % aqueous acetic acid solution).

All the above-mentioned results explain the presence of VI in viomycin. The alternative dihydro-2-amino-pyrimidine ring structure VIII is excluded by the results of the reduction studies and the absence of a signal in the n. m. r. spectrum of viomycin corresponding to a methine proton at C_4.

The two additional protons at δ 3.7 ppm (multiplet) in the n. m. r. spectrum of deoxyviomycin compared to viomycin correspond to the methylene protons formed by dehydroxylation of the guanidine-carbinol of viomycin with sodium borohydride. The signal of the methine proton of guanidine-carbinol of viomycin could not be exactly assigned because of overlapping of signals at δ 4.5~5.2.

It is evident that methylviomycin[8], obtained by heating viomycin with methanol, is an O-methyl derivative of the guanidine-carbinol, because deoxyviomycin did not yield the O-methyl derivative under the same conditions.

HERR[9] isolated α-(2-iminohexahydro-4-pyrimidyl) glycine from an acid hydrolyzate of capreomycin which is closely related to viomycin. We name this amino acid "capreomycidine". The structure of capreomycidine reported by HERR[9] is the same structure as dihydroviomycidine. However, the properties of capreomycidine were not available. We isolated it from an acid hy-

drolyzate of capreomycin. Chromatographic and electrophoretic behavior of capreomycidine was different from that of dihydroviomycidine. Capreomycidine was crystallized as the monohydrochloride, m. p. 241° C (dec., uncorrected). Found: C 33.20, H 6.57, N 25.29, O 18.17, Cl 16.76. Calcd. for $C_6H_{12}N_4O_2 \cdot \frac{1}{2}H_2O \cdot HCl$ (MW 217.7): C 33.10, H 6.48, N 25.74, O 18.38, Cl 16.29. $[\alpha]_D^{20}$ +35° (*c* 0.88, 6 N HCl). It had one carboxyl ($pK_a' < 2.0$) and two basic (pK_a' 7.5, >12.0) groups. The titration equivalent was 228. It gave positive ninhydrin and SAKAGUCHI tests and had one primary amino group (VAN SLYKE). The n.m.r. spectra are shown in Figs. 3 and 4. It can be concluded from the above and from comparison of the n.m.r. spectra that capreomycidine IX is a diastereoisomer of dihydroviomycidine.

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References

1) BYCROFT, B. W.; D. CAMERON, L. R. CROFT, A. W. JOHNSON, T. WEBB & P. COGGON: Viomycin, further degradative studies. Tetrahedron Letters No. 25: 2925~2930, 1968
2) BOWIE, J. H.; A. W. JOHNSON & G. THOMAS: The chromophore of viomycin. Tetrahedron Letters No. 15: 863~866, 1964
3) DYER, J. R.; H. B. HAYES, E. G. MILLER, Jr. & R. F. NASSAR: Viomycin. I. The structure of viomycidine. J. Am. Chem. Soc. 86: 5363~5364, 1964
4) BOWIE, J. H.; D. A. COX, A. W. JOHNSON & G. THOMAS: Viomycin. Tetrahedron Letters No. 45: 3305~3308, 1964
5) DYER, J. R.; C. K. KELLOGG, R. F. NASSAR & W. E. STREETMAN: Viomycin. II. The structure of viomycin. Tetrahedron Letters No. 10: 585~592, 1965
6) RALEIGH, J. A.: Dissertation (Supervisor, G. BÜCHI). Massachusetts Institute of Technology. Oct. 1966
7) JEPSON, J. B. & I. SMITH: Multiple dipping procedures in paper chromatography. A specific test for hydroxy-proline. Nature 172: 1100~1101, 1953
8) HOCHSTEIN, F. A. & R. L. MILLER: Viomycin. U. S. Pat. 3,920,998. Jan. 12, 1960
9) HERR, E. B., Jr.: Chemical and biological properties of capreomycin and other peptide antibiotics. Antimicr. Agents & Chemoth. 1962: 201~212, 1963