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CHEMICAL STUDIES ON TUBERACTINOMYCIN. I.
THE STRUCTURE OF TUBERACTIDINE, GUANIDINO AMINO ACID COMPONENT.

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A new antibiotic, tuberactinomycin (TUM) has been isolated from the broth filtrate of Streptomyces griseoverticillatus var. tuberacticus obtained from a soil sample at Ohito-cho, Shizuoka prefecture (1). TUM is a peptide antibiotic effective against tubercular bacilli (2).

The structural study of this antibiotic started with isolation and characterization of amino acid components. Acid hydrolyzate of TUM was chromatographed on Dowex-50W×2 (200-400 mesh, H^+ form) column, and eluted with a buffer solution of 0.2M pyridine and formic acid (pH 3.1). From the column, L-serine, L-α,β-diaminopropionic acid, guanidino amino acid and γ-hydroxy-L-β-lysine (3) were eluted successively.

Although an original eluate containing the guanidino amino acid gave positive reactions both for ninhydrin and Sakaguchi reagents, the crystals of fine prisms obtained through concentration in vacuo were negative for both reactions, m.p. 245°(decomp.). Molecular formula of $C_{14}H_{20}N_8O_6$(I) was deduced to this compound from the results of elementary analysis of I and molecular peak(480) in the mass spectrum of its diacetyl derivative (4).

When 1.1g of the compound I was treated with 4.5ml of 15% hydrobromic acid at 50°, it was degradated to two compounds (II and III) which were isolated separately by fractional crystallization with aqueous ethanol in a yield of 400mg and 320mg respectively.

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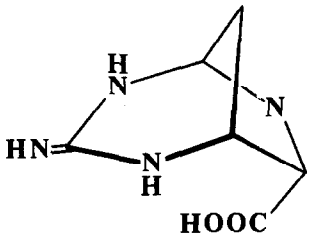
The compound II gave a positive ninhydrin but a negative Sakaguchi reaction. Elementary analysis of the hydrobromide indicated the molecular formula $C_6H_{10}N_4O_2 \cdot HBr$, m.p.210°(decomp.)(5). The hydrochloride, m.p.219°(decomp.) (6,7), $[\alpha]_D^{13}$-89°(c 0.5, H_2O), $[\alpha]_D^{16}$-32°(c 0.5, N HCl) (6). The structure and absolute configuration of the compound II were determined by X-ray analysis of crystalline hydrobromide. The unit cell of this crystal was orthorhombic with a=9.37, b=12.49, c=15.41 Å, z=8, ρ_c=1.865, and space group $C222_1$. From these facts, II was identified with viomycidine obtained from analogous antibiotic viomycin (VM). Furthermore, IR, NMR, and ORD spectra of II hydrochloride were identical with those of viomycidine hydrochloride.

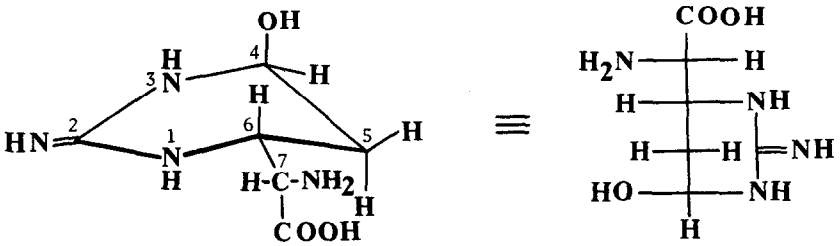


II = Viomycidine

2,4,6-triaza-3-iminobicyclo[3,2,1]

octane-7-carboxylic acid

The another degradation product III obtained from I gave positive ninhydrin as well as positive Sakaguchi reaction. Elementary analysis of III hydrobromide indicated the molecular formula $C_6H_{12}N_4O_3 \cdot HBr$, m.p.182°(decomp.), $[\alpha]_D^{15}$-25.8°(c 0.5, H_2O). The ORD spectrum of III shows positive Cotton effect at 221mμ ([Φ]+807pk, 0.1N hydrochloric acid) which is characteristic to L-α-amino acid. From the results obtained above and the NMR spectrum of III mentioned below, the absolute structure of III was determined as α-(2-imino-4-hydroxyhexahydro-6-pyrimidinyl)glycine (4S,6R,7S).

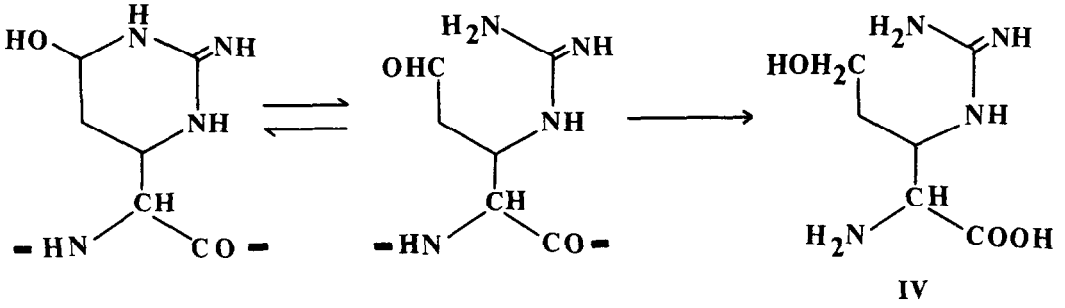


III , Tuberactidine

The signals of the NMR spectrum of III in D_2O could be assigned as follows: a triplet at 5.26 ppm (1H, J=3.45 cps) which is ascribed to the proton on C-4 atom bearing the hydroxyl and

guanidino groups; a doublet at 3.91 ppm (1H, J=3.0 cps) to C-7 proton; a multiplet at 4.40 ppm (1H, J=3.0, 3.45, 6.9 cps) to C-6 axial proton; a multiplet at 2.12 ppm (1H, J=3.45, 3.45, 14.7 cps) to C-5 equatorial proton; a multiplet at 2.45 ppm (1H, J=3.45, 6.9, 14.7 cps) to C-5 axial proton. The NMR study of 2-amino-4-hydroxy-1,4,5,6-tetrahydropyrimidine by Goto et al. supported our assignments to the structure of III, especially by coincidence of the chemical shifts and coupling constants of C-4 proton (8).

This newly isolated amino acid is now named tuberactidine, because this compound should be distinguished from viomycidine as a possible constituent of VM type antibiotics. A question arises whether viomycidine or tuberactidine is an original component in the intact molecule of tuberactinomycin. In order to clarify this problem, TUM was reduced with sodium borohydride in alkaline medium followed by acid hydrolysis to give a hydroxyl compound (IV) as monohydrochloride $C_6H_{14}N_4O_3 \cdot HCl$, m.p.180°(decomp.), $[\alpha]_D^{15}$+33°(c 0.5, N HCl). Physical properties, e.g., m.p., $[\alpha]_D$, NMR spectrum (in D_2O) of IV, are virtually identical with those of dihydroviomycidine which was obtained by reduction of viomycin in similar manner by Maeda et al. (6). Tuberactidine moiety may be considered to be interconvertible between a cyclol form and an amino-aldehyde form as shown below. The formation of IV can be understood by reduction of its aldehyde form.



IV

From the results obtained above, it is concluded that tuberactidine (III) is a true constituent of TUM and consequently viomycidine (II) is possibly an artifact derived from III during the isolation process. In fact, tuberactidine trends to convert to viomycidine, for instance, in trifluoroacetic acid, but not in reverse direction. Furthermore, the fact that TUM and tuberactidine showed a positive Sakaguchi reaction while viomycidine gave a negative reaction, seems to support the above interpretation of the constituent of the antibiotic.

Although Johnson et al.(7,9) as well as Maeda et al.(6) suggested that the intact molecule of VM may consist of the cyclol form of the guanidino amino acid, i.e., tuberactidine, this com-

pound has never been isolated from the hydrolyzate of VM so far. We not only isolated tuberactidine from TUM hydrolyzate for the first time, but also detected the same compound from the acid hydrolyzate of VM (10). Therefore, we can now conclude that tuberactidine is a common constituent as guanidino amino acid in TUM and VM.

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2. M. Toyohara, A. Nagata, K. Hayano and J. Abe, Amer. Rev. Respiratory Disease, 100,228(1969).
3. Structure determination and stereochemical study of this amino acid were presented in a preliminary form at 13th Symposium on the Chemistry of Natural Products, Sapporo, Sep. 25, 1969. Details will be reported soon elsewhere.
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10. Concerning the identification of the compounds I and III from VM hydrolyzate, details will be reported later.