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Chemical Studies on Tuberactinomycin. V.[1] Structures of Guanidino Amino Acids in Tuberactinomycins[2]

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Tuberactidine (III) is a guanidino amino acid component of antitubercular peptides, tuberactinomycin A and B, while the known guanidino amino acid capreomycidine (V) is involved in tuberactinomycin N and O. The structure of tuberactidine was assigned to be α-(4-hydroxy-2-iminohexahydro-6-pyrimidinyl)glycine and its stereochemistry was also established. During isolation of tuberactidine from an eluate in the column chromatography of tuberactinomycin hydrolyzate, it was converted to N^{α}-formyltuberactidine dimer (I). In this dimer, structural alternation between a carbinolamine form of cyclol type and an amide lactone form was noticed depending to a pH of the solution. I was hydrolyzed to tuberactidine and viomycidine (IV) with hydrobromic acid. In addition, a plausible pathway for the formation of viocidic acid (VII), one of the artifacts formed during hydrolysis of tuberactinomycin, was proposed.

Tuberactinomycins are antitubercular peptides isolated from the broth filtrate of *Streptomyces griseoverticillatus* var. *tuberacticus*[3] and its mutant.[4] A family of this antibiotic comprises four congeners, A, B, N and O. Among them, tuberactinomycin B was found to be the same to the one known as viomycin.[4] Amino acid compositions of these four peptides were listed in Table 1.

Acid hydrolyzate of tuberactinomycin A was separated by ion-exchange column chromatography, using a buffer solution of pyridine and formic acid as an eluent. Serine, α,β-diaminopropionic acid, a mixture of guanidino amino acids, *i.e.*, viomycidine, tuberactidine and viocidic acid, and finally γ-hydroxy-β-lysine[1] were eluted in this order. In the case of viomycin, a similar elution pattern was obtained except a replacement of γ-hydroxy-β-lysine with β-lysine. As a guanidino amino acid in viomycin, viomycidine (IV) has already been isolated from its hydrolyzate by many workers,[5] and its structure was determined by Büchi and Raleigh as shown in Fig. 1.[6] From hydrolyzates of tuberactinomycin N and O, capreomycidine (V) was obtained as a guanidino amino acid component. First isolation of this amino acid from the known

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1) Part IV: T. Wakamiya, T. Shiba, and T. Kaneko, This Bulletin, **45**, 3668 (1972).

2) Presented at the 24th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1971; p. 1783. A part was reported: T. Wakamiya, T. Shiba, T. Kaneko, H. Sakakibara, T. Take, and J. Abe, *Tetrahedron Lett.*, **1970**, 3497.

3) A. Nagata, T. Ando, R. Izumi, H. Sakakibara, T. Take, K. Hayano, and J. Abe, *J. Antibiotics*, **21**, 681 (1968).

4) R. Izumi, T. Noda, T. Ando, T. Take, and A. Nagata, *ibid.*, **25**, 201 (1972).

5) a) T. H. Haskell, S. A. Fusari, R. P. Frohardt, and Q. Bartz, *J. Amer. Chem. Soc.*, **74**, 599 (1952). b) J. H. Bowie, A. W. Johnson and G. Thomas, *Tetrahedron Lett.*, **1964**, 863. c) J. R. Dyer, H. B. Hayes, E. G. Miller, Jr., and R. F. Nassar, *J. Amer. Chem. Soc.*, **86**, 5363 (1964). d) T. Takita and K. Maeda, *J. Antibiotics*, **21**, 512 (1968).

6) G. Büchi and J. A. Raleigh, *J. Org. Chem*, **36**, 873 (1971). J. A. Raleigh, Ph. D. Dissertation, Massachusetts Institute of Technology, Oct. 1966.

Table 1. Amino acid compositions of tuberactinomycins (TUM)

TUM	Molecular Formula	Ser	Dpr	Uda	Tbd	Cpd	γ-Hy-β-lys	β-Lys
A	$C_{25}H_{43}O_{11}N_{13}$	2	1	1	1	—	1	—
B	$C_{25}H_{43}O_{10}N_{13}$	2	1	1	1	—	—	1
N	$C_{25}H_{43}O_{10}N_{13}$	2	1	1	—	1	1	—
O	$C_{25}H_{43}O_9N_{13}$	2	1	1	—	1	—	1

Ser: L-serine, Dpr: L-α,β-diaminopropionic acid, Tbd: L-tuberactidine, Cpd: L-capreomycidine, γ-Hy-β-lys: γ-hydroxy-L-β-lysine, β-Lys: L-β-lysine, Uda: 3-ureidodehydroalanine

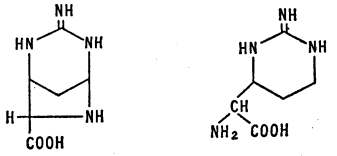


Fig. 1. Structures of viomycidine(IV) and capreomycidine(V).

antibiotic capreomycin had been reported by Herr and its structure was decided as above (Fig. 1).[7]

The existence of 3-ureidodehydroalanine as a composite amino acid in the antibiotics was confirmed not only by X-ray analysis in the case of tuberactinomycin O,[8] but also by the chemical evidences and NMR data[9] which were coincided with those reported in the studies of viomycin by Bycroft and his colleagues.[10] However, this moiety could not be isolated from the hydrolyzate because of ease of decomposition to NH_3 and CO_2 during acid hydrolysis.

For the isolation of tuberactidine (III), fractions of a positive Sakaguchi reaction in the eluates of column chromatography were combined and evaporated to give colorless prisms. Although the original eluate containing tuberactidine gave positive reactions both for ninhydrin and Sakaguchi reactions, the crystals obtained above were found to be negative for both reactions. Molecular formula of $C_{14}H_{20}O_6N_8$ (I) was deduced to the compound from the results of elemental analysis and molecular peak (396) in the mass spectrum. This molecular weight was supported by the results of mass spectrum of its diacetyl derivative $C_{18}H_{24}O_8N_8$ (480) (II).

When I was treated with 15% hydrobromic acid at 50°C, it was degradated to tuberactidine (III) and viomycidine (IV), in almost equal amounts, both of which were isolated separately by fractional crystallization from aqueous ethanol. As reported in the preliminary form, we decided the structure of tuberactidine on the basis of NMR (Fig. 2) and ORD spectrum (Fig. 3) to α-(4-hydroxy-2-iminohexahydro-6-pyrimidinyl)glycine (4S, 6R, 7S) (Fig. 4).[2] The signals of the NMR spectrum in D_2O could be assigned as follows: a triplet at δ 5.26 (1H, J=3.0 Hz) is ascribed to C-4 equatorial proton: a multiplet at δ 4.40 (1H, J=3.0, 3.45, 6.9 Hz) to C-6 axial proton; a doublet at δ 3.91 (1H, J=3.0 Hz) to C-7 proton; a multiplet at δ 2.45 (1H, J=3.45, 6.9, 14.7 Hz) to C-5 axial proton; a multiplet at δ 2.12 (1H, J=3.45, 3.45, 14.7 Hz) to C-5 equatorial proton.

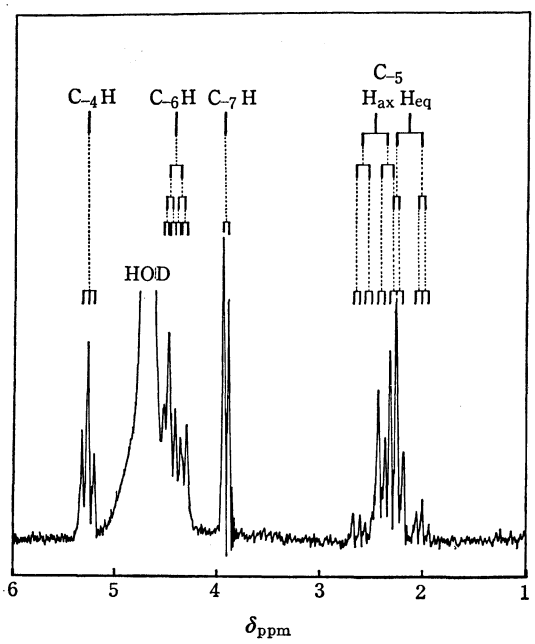


Fig. 2. NMR spectrum of tuberactidine in D_2O.

The ORD spectrum of tuberactidine showed a positive Cotton effect at 221 nm ([Φ]+807 pk, 0.1 M hydrochloric acid) which is characteristic of L-α-amino acid. In connection with this observation, ORD curves of a series of guanidino amino acids obtained from tuberactinomycins were measured (Fig. 3). Tuberactidine, capreomycidine and dihydroviomycidine showed a positive Cotton effect characteristic of L-α-amino acids whereas viomycidine a negative effect characteristic of L-α-imino acids as proline or hydroxy proline.

The structure of viomycidine was determined by X-ray analysis as shown in Fig. 4. The unit cell of crystalline hydrobromide was orthorhombic with a=9.37, b=12.49, c=15.41Å, Z=8, ρ_c=1.865, and

7) E. B. Herr, Jr., Antimicr. Agents and Chemoth. 1962, **1963**, 201.

8) H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T. Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba, and T. Kaneko, *Tetrahedron Lett.*, **1971**, 2043.

9) Tuberactinomycin A, N and viomycin show a olefin proton signal ascribed to -NH-C(H)=C(NHCONH$_2$)-CO- at δ 8.01, 8.01 and 8.10 in NMR respectively. When tuberactinomycin A, N and viomycin were hydrogenated with palladium on charcoal catalyst, urea was liberated and hydrogenated compounds were hydrolyzed to produce one mole of alanine newly.

10) B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Tetrahedron Lett.*, **1968**, 5901.

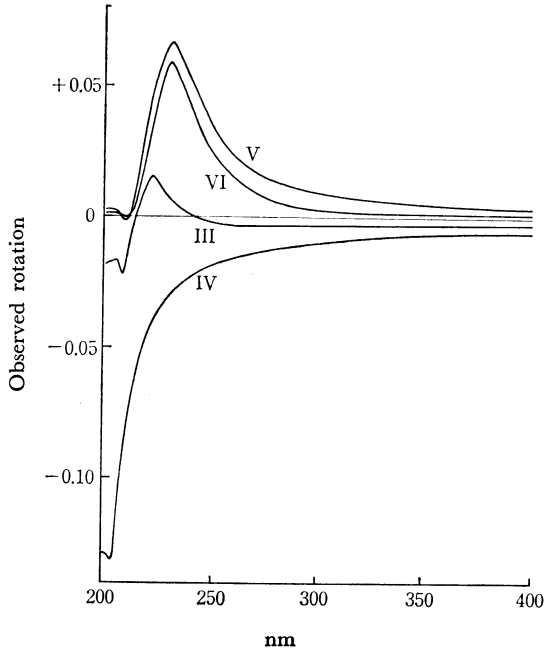


Fig. 3. Observed ORD curves of guanidino amino acids, *i.e.* tuberactidine (III), viomycidine (IV), capreomycidine (V) and dihydroviomycidine (VI). Molecular rotations [Φ] were calculated to +807° (III, 221 nm), −5373° (IV, 204 nm), +3992° (V, 229 nm), and +3926° (VI, 229 nm) respectively.

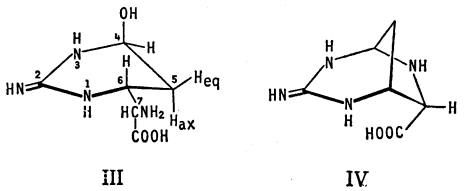


Fig. 4. The absolute structure of tuberactidine(III) and viomycidine(IV).

space group $C222_1$. The result was consistent with that reported by other investigators.[11]

The facts obtained above indicated that I could be a condensed product of two molecules of formyl derivative of tuberactidine or viomicidine. From the measurement of pK_a', it was shown that free amino group is not present in the molecule of I. In the NMR spectrum of I as shown in Fig. 7, two signals ascribed to formyl protons were recognized at the position centered at δ 8.5. However, no more useful data for the structural determination of I were obtained from I itself at the stage of the investigation. In the meantime, hydrobromide (II), $C_{14}H_{20}O_6N_8 \cdot 2HBr$, of I was readily prepared. It showed the absorption of carbonyl of either ester or lactone at 1740 cm^{-1} and 1760 cm^{-1} in IR spectrum, while no absorption was recognized in I between 1700 cm^{-1} to 1800 cm^{-1} (Fig. 5). Moreover, when an aqueous solution of II was neutralized with pyridine, it was reconverted to I.

The peculiar interconversion between I and II reminded us of lactone-lactal equilibrium in tetrodotoxin,[12] cyclol-amide lactone carbonyl interaction in ergotamine[13] or depsipeptides,[14] and amide-ortho-amide transannular interaction in cyclic peptides,[15] all of which were realized rather recently in the field of chemistry of natural compounds. By an application of the above concept to our observation, the reversible structural alternation between I and its hydrobromide II was concluded to be interconversion of cyclic carbinolamine form of cyclol type and amide lactone form (Fig. 6).

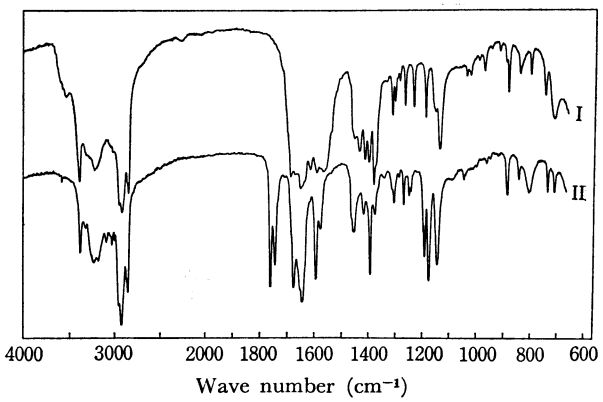


Fig. 5. IR Spectra of I and its hydrobromide II.

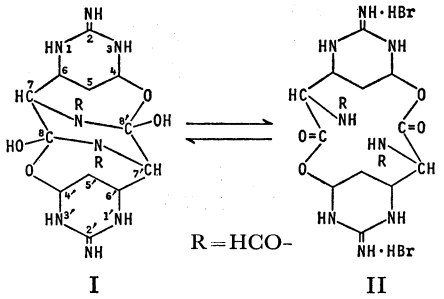


Fig. 6. Structures and interconversion of I and II.

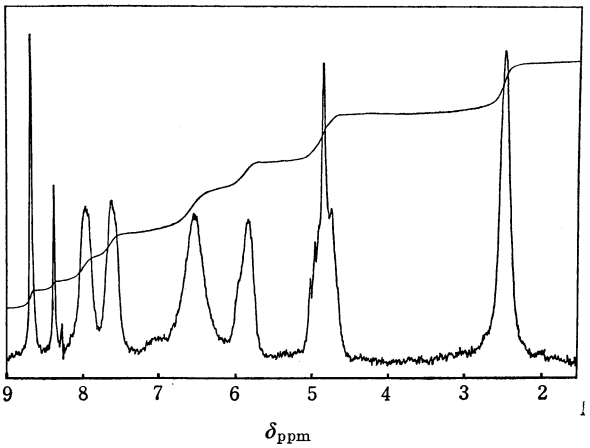


Fig. 7. NMR spectrum of I in TFA.

11) a) J. C. Floyd, J. A. Bertrand, and J. R. Dyer, *Chem. Commun.*, **1968**, 998. b) G. Koyama, H. Nakamura, S. Omoto, T. Takita, K. Maeda, and Y. Iitaka, *J. Antibiotics*, **22**, 34 (1969).

12) T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *Tetrahedron*, **21**, 2059 (1965).

13) A. Hofmann, H. Ott, G. Griot, P. A. Stadler, and A. J. Frey, *Helv. Chim. Acta*, **46**, 2306 (1963).

14) M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and Z. E. Agadzhanyan, *Tetrahedron*, **21**, 3537 (1965).

15) A. Prox, J. Schmid, and H. Ottenheym, *Ann. Chem.*, **722**, 179 (1969).

On the basis of the proposed structure, the signals of the NMR spectrum of I in TFA (Fig. 7) could be now assigned as follows: 4H protons at δ 2.5 were ascribed to C-5 and C-5′ methylene; 4H at δ 4.85 to C-6, C-6′, C-7 and C-7′ methine; 2H at δ 5.8 to C-4 and C-4′ methine; 4H at δ 6.5 to =NH bearing on C-2 and C-2′, and –OH on C-8 and C-8′; 2H at δ 7.6 to N-1 and N-1′ protons; 2H at δ 7.95 to N-3 and N-3′ protons; 2H centered at δ 8.5 to two formyl protons.

Splitting of the formyl protons at δ 8.36 and 8.66 could be presumably accounted for by the equilibrium of two types of hydrogen bonding between two formyl groups as shown in Fig. 8. Thus the upfield signal may be due to non-hydrogen bonded proton and the lowfield to hydrogen bonded one. In fact, the latter is relatively stronger than the former.

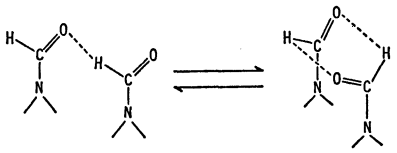


Fig. 8. Hydrogen bonding in formyl groups.

A mechanism for the formation of I was assumed from its structure as follows. Tuberactidine was first formylated with formic acid and pyridine in the eluate during concentration and then two molecules of them were condensed each other by dehydration followed by cyclization. However, a possibility of the formation of I through N^{α}-formylviomycidine could not be excluded, because I was actually obtained when the solution of viomycidine alone in the above buffer solution was evaporated. In addition to this, tuberactidine was easily converted to viomycidine with dehydration in the aqueous solution. Therefore, it is not clear at this stage whether N^{α}-formyltuberactidine or N^{α}-formylviomycidine is the real intermediate in the formation of I.

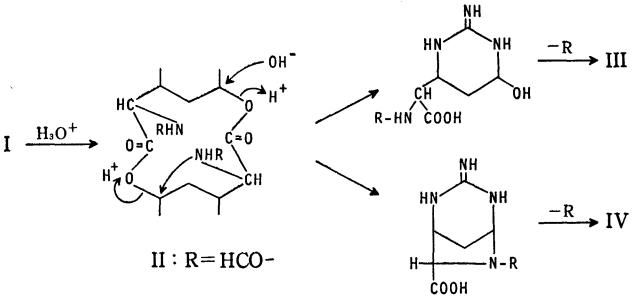
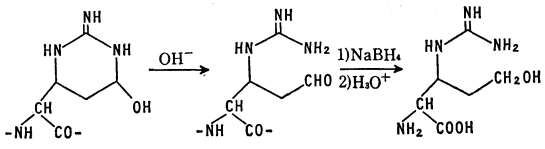


Fig. 9. Degradation of I to tuberactidine(III) and viomycidine(IV).

On the other hand, degradation of I to tuberactidine and viomycidine could be explained by concerted hydrolysis mechanism through II as shown in Fig. 9.

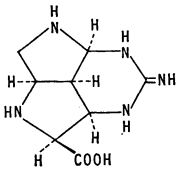
As mentioned previously,[2] it was contended that tuberactidine is a true constituent of tuberactinomycin A and viomycin, and viomycidine is possibly an artifact derived from tuberactidine during the isolation process. This assumption was supported by the following facts. First, tuberactidine as well as tuberactinomycin A and viomycin showed a positive Sakaguchi reaction whereas viomycidine gave a negative one. Secondly, when tuberactinomycin A was reduced with sodium borohydride in alkaline medium followed by acid hydrolysis, a hydroxy compound VI was obtained (Fig. 10). Physical properties such as mp, $[\alpha]_D$ and NMR spectrum in D_2O of monohydrochloride of VI were virtually identical with those of dihydroviomycidine hydrochloride which was obtained by reduction of viomycin in similar manner by Maeda *et al.*[16] The formation of VI can be elucidated by the interconversion between tuberactidine and its amino-aldehyde form in alkaline solution.



VI

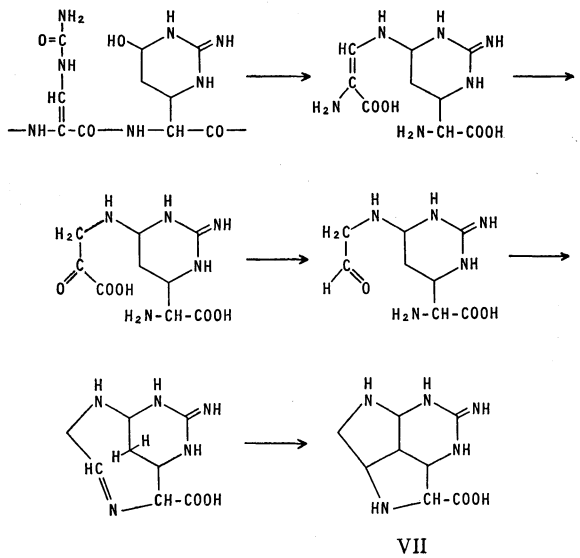
Fig. 10. Isolation of dihydroviomycidine(VI) from tuberactinomycin A.

The presence of the amino-aldehyde form in alkaline solution of tuberactidine can also explain a positive Sakaguchi reaction of tuberactidine, since this reaction should work on mono substituted guanidino compound like amino-aldehyde form derived from tuberactidine but not on disubstituted derivative like tuberactidine itself. Thus, it may be reasonable that VI gave a positive Sakaguchi reaction while viomycidine and capreomycidine were negative.



VII

Fig. 11. Structure of viocidic acid(VII)



VII

Fig. 12. Presumed formation pathway of viocidic acid(VII)

16) T. Takita and K. Maeda, *J. Antibiotics*, **22**, 39 (1969).

In several runs of column chromatography of the hydrolyzate of tuberactinomycin A, viocidic acid (VII), the other artifact formed during the hydrolysis, was eluted after viomycidine and tuberactidine. Viocidic acid had been also isolated from the hydrolyzate of viomycin, and the structure was determined using X-ray analysis by Bycroft *et al.* (Fig. 11).[17]

Pathway for the formation of viocidic acid remains unexplained as yet. However, it may be possibly interpreted by assuming the participation of 3-ureidodehydroalanine moiety as shown in Fig. 12, although the clarification of this problem must be left to a future investigation.

Experimental

All melting points are uncorrected. The infrared spectra were obtained in Nujol mull with a Hitachi EPI-G3 spectrophotometer. The NMR spectra were obtained with a Hitachi NMR R-20 spectrometer at 60 MHz in trifluoroacetic acid and deuterium oxide. Tetramethylsilane (TMS) was used as an internal reference in trifluoroacetic acid solution, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in the case of deuterium oxide solution. The ORD curves were obtained with a Yanagimoto ORD-185 spectropolarimeter in 0.1 M hydrochloric acid. The specific rotations were obtained with a Perkin-Elmer 141 polarimeter in water and 1 M hydrochloric acid. Amino acids were analyzed with a JEOL-JLC-5AH amino acid analyzer. Thin-layer chromatography was carried out by the ascending method on silica gel G using developing solvents of phenol–water–28% ammonium hydroxide (30 : 10 : 0.6) and *n*-butanol–acetic acid–water (4 : 1 : 2). Paper electrophoresis was carried out at 750 volt and 10 mA for 1.5 hr on Toyo Roshi No. 51 paper using a buffer solution of pyridine–acetic acid–water (30:4:966).

Isolation of Viomycidine (IV). A solution of 20 g of tuberactinomycin A hydrochloride or viomycin hydrochloride in 180 ml of 6 M hydrochloric acid was heated at 135—140°C under reflux for 20 hr. Hydrochloric acid was evaporated *in vacuo* and the residue was dissolved in water. The evaporation was repeated three times after addition of water. The solution of the residue obtained in a small amount of water was applied on a column (2.5×65 cm) of Dowex 50W×2 ion exchange resin which was previously equilibrated with buffer solution of 0.2 M pyridine and formic acid (pH 3.1). Fractions of a positive Sakaguchi reaction were collected and evaporated *in vacuo*. The residue was dissolved in diluted hydrochloric acid and evaporated again. Ethanol and ether were added and a powder thus obtained was recrystallized from water and ethanol two times. Colorless prisms of viomycidine (IV) hydrochloride were obtained, yield 500 mg, mp 215—219°C (decomp.), Lit, mp 200—208°C (decomp.)[5c], $[\alpha]_D^{13}$ −89° (*c* 0.5, H_2O), Lit, $[\alpha]_D^{22}$ −85° (*c* 0.11, H_2O).[18]

Found: C, 34.89; H, 5.61; N, 26.84; Cl, 16.97%. Calcd for $C_6H_{10}O_2N_4 \cdot HCl$: C, 34.87; H, 5.37; N, 27.12; Cl, 17.16%.

Isolation of N^{α}*-Formyltuberactidine Dimer (I).* The same fraction of a positive Sakaguchi reaction in the eluate at an another run of the above column chromatography was concentrated without acidification. Ethanol was added to the residue to make it powder. It was filtered off and crystallized from water. Colorless prisms of N^{α}-formyltuberactidine dimer (I) were obtained, yield 540 mg, mp 230°C (darkened) −245°C (decomp.). For analysis sample was dried *in vacuo* at 100°C.

Found: C, 42.19; H, 5.41; N, 28.09%. Calcd for $C_{14}H_{20}O_6N_8$: C, 42.42; H, 5.09; N, 28.27%.

Isolation of Viocidic Acid (VII). Mother liquor of I was evaporated *in vacuo* and the residue was dissolved in diluted hydrobromic acid. The acidic solution was concentrated and the residue was treated with ethanol to make it powder. It was recrystallized from aqueous ethanol several times to give colorless needles of viocidic acid (VII) dihydrobromide in a low yield, mp 208—210°C (decomp.), Lit. mp of trihydrate 222—225°C,[18] $[\alpha]_D^{25}$ −27° (*c* 0.5, H_2O).

Found: C, 25.70; H, 4.16; N, 18.64; Br, 42.71%. Calcd for $C_8H_{13}O_2N_5 \cdot 2HBr$: C, 25.70; H, 4.05; N, 18.77; Br, 42.84%.

N^{α}*-Formyltuberactidine Dimer from Viomycidine.* In a buffer solution of 0.2 M pyridine and formic acid (pH 3.1), 90 mg of viomycidine hydrochloride was dissolved. The solution was evaporated *in vacuo* at 50—60°C. The oily residue was treated with ethanol to make it powder. It was recrystallized from water to give colorless prisms. Melting point and infrared spectrum were virtualy identical with I obtained above.

Capreomycidine (V) from Tuberactinomycin N and O. A solution of 7 g of tuberactinomycin N hydrochloride in 21 ml of 6 M hydrochloric acid was heated at 100—110°C in a sealed tube for 24 hr. Hydrolyzate treated as mentioned above was column chromatographed on Dowex 50W×2 ion exchange resin (200—400 mesh, 2.5×25 cm) with a buffer solution of 0.2 M pyridine and acetic acid (pH 3.6). Fractions containing capreomycidine were evaporated and the residue was purified by column chromatography again with the same buffer solution. Crude capreomycidine hydrochloride was recrystallized from water and ethanol, yield 666 mg, mp 242—248°C (decomp.), Lit. mp 241°C (decomp.),[5] $[\alpha]_D^{25}$ +21° (*c* 1, H_2O), Lit, $[\alpha]_D$ +16.1° (*c* 1, H_2O).[7]

Found: C, 34.21; H, 6.17; N, 26.40; Cl, 17.17%. Calcd for $C_6H_{12}O_2N_4 \cdot HCl$: C, 34.54; H, 6.28; N, 26.85; Cl, 16.99%.

Detection of capreomycidine in a hydrolyzate of tuberactinomycin O was carried out by comparison with authentic sample chromatographically. Amino acid analysis, thinlayer chromatography and paper electrophoresis also characterized this guanidino amino acid in the hydrolyzate of tuberactinomycin O.

Hydrobromide of N^{α}*-Formyltuberactidine Dimer.* Crystals of 210 mg of I were dissolved in 1 ml of 25% hydrobromic acid, and then immediately 10 ml of ethanol and 20 ml of ether were added. After allowing to stand at room temperature until some crystals precipitated, it was stored in refrigerator overnight. Colorless needles were filtered off and washed with ethanol and ether thoroughly, yield 190 mg, mp 227°C (decomp.).

Found: C, 30.10; H, 4.04; N, 19.92; Br, 28.49%. Calcd for $C_{14}H_{20}O_6N_8 \cdot 2HBr$: C, 30.12; H, 3.97; N, 20.08; Br, 28.63%.

To the solution of the hydrobromide in a small amount of water, enough pyridine was added. Excess of pyridine was neutralized with formic acid. When it was allowed to stand for some time, I was recovered as a precipitate

17) a) B. W. Bycroft, D. Cameron, L. R. Croft, A. W. Johnson, T. Webb, and P. Coggon, *Tetrahedron Lett.*, **1968**, 2925. b) P. Coggon, *J. Chem. Soc., B*, **1970**, 838.

18) B. W. Bycroft, L. R. Croft, A. W. Johnson, and T. Webb, *J. Chem. Soc., Perkin I*, **1972**, 820.

Acetyl Derivative of N^{α}*-Formyltuberactidine Dimer.* In 25 ml of pyridine, 470 mg of I was suspended and 13 ml of acetic anhydride was added. After stirring overnight at room temperature, a clear solution obtained was poured on ice and concentrated *in vacuo*. The residue was dissolved in water and evaporation was repeated. Residual oil was dissolved in ethanol and allowed to stand, yield of crystals 390 mg. Analytical sample was obtained by recrystallization from water, mp 217—220°C (decomp.).

Found: 42.07; H, 5.26; N, 22.06%. Calcd for $C_{18}H_{24}O_8N_8 \cdot 2H_2O$: C, 41.86; H, 5.46; N, 21.70%.

Recrystallization from water and ethanol gave fine plates, mp 194—196°C (decomp.).

Found: C, 44.00; H, 5.68; N, 20.84%. Calcd for $C_{18}H_{24}O_8N_8 \cdot H_2O \cdot C_2H_5OH$: C, 44.11; H, 5.92; N, 20.58%

Degradation of I to Viomycidine and Tuberactidine. Crystals of 1.1 g of I were dissolved in 4.5 ml of 15% hydrobromic acid and evaporated *in vacuo* at about 50°C. The residue was dissolved in water and evaporation was repeated several times. The oily residue was dissolved in ethanol and allowed to stand in refrigerator overnight. Precipitate was filtered off and crystallized from water and ethanol to give colorless prisms of viomicidine hydrobromide, yield 350 mg, mp about 210°C (decomp.), Lit, mp 202—204°C (decomp.),[11b] $[\alpha]_D^{25}$ −63° (*c* 0.5, H_2O).

Found: C, 28.63; H, 4.61; N, 22.24; Br, 31.77%. Calcd for $C_6H_{10}O_2N_4 \cdot HBr$: C, 28.70; H, 4.42; N, 22.31; Br, 31.83%.

Mother liquor from viomycidine was evaporated. The residue was dissolved in ethanol and stored in refrigerator for a long time. Tuberactidine hydrobromide was obtained and recrystallized from aqueous ethanol repeatedly, if necessary, with addition of ether, yield 100 mg, mp about 182°C (decomp.), $[\alpha]_D^{13}$ −25.8° (*c* 0.5, H_2O).

Found: C, 26.98; H, 4.81; N, 20.94; Br, 29.65%. Calcd for $C_6H_{12}O_3N_4 \cdot HBr$: C, 26.78; H, 4.87; N, 20.82; Br, 29.70%.

Dihydroviomycidine (VI). In 100 ml of 0.3 M sodium hydroxide, 20 g of tuberactinomycin A hydrochloride was dissolved and 5 g of sodium borohydride in 70 ml of 0.3 M sodium hydroxide was added dropwise with stirring magnetically at 45°C for 8 hr. After stirring for 24 hr, 5 g of sodium borohydride was added by portions for 10 hr. Reaction mixture was acidified with hydrochloric acid and concentrated *in vacuo*. The residue was dissolved in 100 ml of concentrated hydrochloric acid and insoluble inorganic substance was filtered off. Filtrate was diluted with 100 ml of water and heated under reflux at 135°C for 8 hr. Hydrolyzate was column chromatographed as mentioned above. Next fraction to that of diaminopropionic acid showed a positive Sakaguchi reaction but was differentiated from those of viomycidine or tuberactidine. It was concentrated *in vacuo*, and the residue was dissolved in water. Evaporation was repeated and residual oil obtained was allowed to stand overnight. Crystalline product thus obtained was dissolved in a small amount of diluted hydrochloric acid, and then ethanol and ether were added. After allowing to stand overnight in a refrigerator, colorless needles were obtained. Recrystallization from water and ethanol gave 93 mg of hydroxy compound as hydrochloride, mp 180°C (decomp.), Lit, mp 182°C (decomp.),[5d] $[\alpha]_D^{15}$ +33° (*c* 0.5, 1 M HCl), Lit. $[\alpha]_D^{25}$ +25° (*c* 0.7, 6 M HCl).

Found: C, 30.90; H, 7.06; N, 23.59%. Calcd for $C_6H_{14}O_3N_4 \cdot HCl \cdot 1/2H_2O$: C, 30.58; H, 6.84; N, 23.77%.