Chemical Studies on Tuberactinomycin. IV.[1] The Chemical Structure of γ-Hydroxy-β-lysine, A New Amino Acid Isolated from Tuberactinomycin A and N[2]

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γ-Hydroxy-β-lysine is a new basic amino acid isolated from the hydrolyzates of antitubercular peptides, tuberactinomycin A and N as one of the composite amino acids. The chemical structure and stereochemistry of the amino acid were established on the basis of chemical reactions and physical measurements, *i.e.*, IR, NMR, and ORD. One stereoisomer obtained in hydrochloric acid hydrolysis was determined as *threo*-γ-hydroxy-L-β-lysine (Ia), while the other one obtained in concentrated sulfuric acid hydrolysis as an *erythro* isomer (Ib).

Tuberactinomycin is a family of peptide antibiotics effective against tubercular bacilli.[3] Tuberactinomycin A and B were isolated from the broth filtrate of *Streptomyces griseoverticillatus* var. *tuberacticus*[4] and a mutant of the original microorganism produced tuberactinomycin N and O.[5]

Tuberactinomycin A is composed of following five amino acids, *i. e.*, L-serine, L-α,β-diaminopropionic acid, 3-ureidodehydroalanine,[6] L-tuberactidine,[7] and a new basic amino acid (I) with the molecular ratio of 2 : 1 : 1 : 1 : 1, while tuberactinomycin N contains capreomycidine[8] instead of tuberactidine and the other amino acids are common to those of tuberactinomycin A.[9]

Acid hydrolyzate of tuberactinomycin A or N was separated by ion-exchange column chromatography, using pyridine–formic acid buffer as an eluent. Serine, α,β-diaminopropionic acid, tuberactidine[10] (capreomycidine, in the case of tuberactinomycin N) and finally a new amino acid Ia were eluted from the column in this order. However, 3-ureidodehydroalanine was not obtained because of ease of decomposition to NH_3 and CO_2 during acid hydrolysis.

From the final fraction, the new basic amino acid was secured as hydrochloride. It had a molecular formula of $C_6H_{14}O_2N_2Cl_2$ (IIa·2HCl) and showed γ-lactone absorption at 1780 cm^{-1} in IR spectrum. A spot of IIa on thin-layer chromatogram was not identical with the original one in the eluate. This indicated that Ia is a basic γ-hydroxy amino acid and was converted to the γ-lactone IIa in the course of isolation procedure after column chromatography.

Taking advantage of the easy formation of γ-lactone from Ia as mentioned above and of insolubility of its derivatives, we attempted the direct and sole isolation of this amino acid from the tuberactinomycin hydrolyzate as acyl derivatives of the lactone. Thus, the acid hydrolyzate of tuberactinomycin A was benzyloxycarbonylated. The oily product formed even in alkaline medium was extracted and then purified by silica gel column chromatography. In this way the expected benzyloxycarbonyl lactone derivative $C_{22}H_{24}O_6N_2$ (IIIa) was obtained. This compound was identical with the benzyloxycarbonyl derivative of IIa. The compound IIIa thus obtained was debenzyloxycarbonylated with 30% hydrogen bromide in glacial acetic acid and the lactone IIa was prepared as dihydrobromide without difficulty.

For the purpose of structural determination of the amino acid Ia, the lactone IIa was saponified to prepare a hydroxy acid which was then reduced with hydriodic acid and red phosphorus. When the product was 2,4-dinitrophenylated, a small amount of di-DNP-β-lysine[11] was obtained together with di-DNP-IIa. Secondly, when the saponified product was oxidized with periodic acid followed by potassium permanganate treatment, β-alanine was detected by thin-layer chromatography as a single product of positive ninhydrin reaction. From these results, the structure of the new basic amino acid Ia could be assigned to γ-hydroxy-β-lysine unequivocally as shown in Scheme 1.

Furthermore, this structure was confirmed by NMR spectrum of dibenzyloxycarbonyl derivative IIIa in $CDCl_3$ as follows; α-CH_2 (d,d at δ 2.36 and 2.82 ppm), β-CH and γ-CH (overlapped at 4.50 ppm), δ-CH_2 (q at 1.80 ppm), ε-CH_2 (q at 3.30 ppm, changed to a triplet in addition of D_2O), β-NH and ε-NH (d at 6.2 ppm and t at 5.4 ppm, both disappeared in addition of D_2O).[12]

When tuberactinomycin A or N was 2,4-dinitrophenylated and then hydrolyzed, no other DNP amino acids than di-DNP-γ-hydroxy-β-lysine lactone (IVa) were detected. Therefore, γ-hydroxy-β-lysine is a sole N-terminal amino acid in the molecules of tuberac-

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1) Part III: T. Noda, T. Take, A. Nagata, T. Wakamiya, and T. Shiba, *J. Antibiot.* (Tokyo), **25**, 427 (1972).

2) Presented at the 13th Symposium of The Chemistry of Natural Products, Sapporo, Japan, September, 1969; p. 17.

3) M. Toyohara, *Kekkaku*, **43**, 245 (1968).

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

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

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

7) T. Wakamiya, T. Shiba, T. Kaneko, H. Sakakibara, T. Take, and J. Abe, *ibid.*, **1970**, 3497.

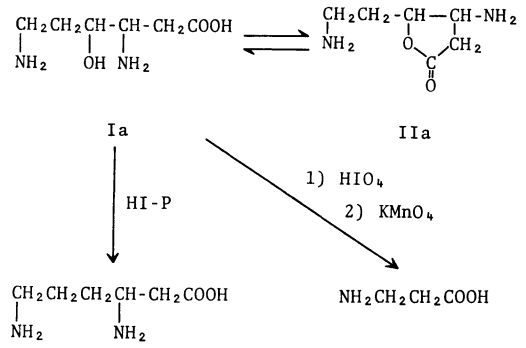
8) E. B. Herr, Jr., Antimicrobial Agents Chemotherapy 1962, 201 (1963).

9) 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.

10) It was obtained as a mixture with viomycidine.

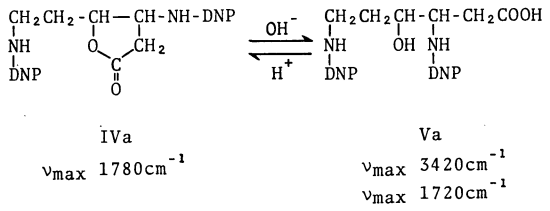
11) DNP stands for 2,4-dinitrophenyl.

12) The terms of d, t, and q show doublet, triplet, and quartet respectively.



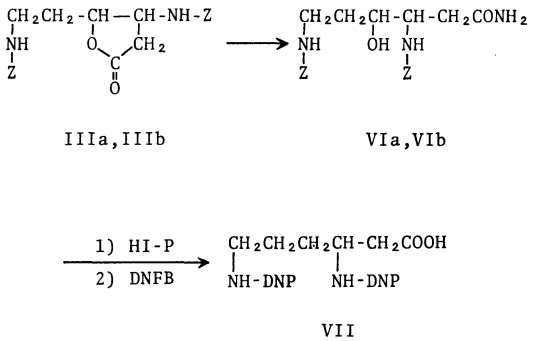
Scheme 1.

tinomycin A and N. The DNP derivative IVa showed the absorption band characteristic to γ-lactone at 1780 cm^{-1} likely as that of the lactone IIa. The lactone IVa was saponified with 1 N NaOH to prepare the corresponding hydroxy acid Va. Reversely, Va was easily converted to IVa by acid treatment.



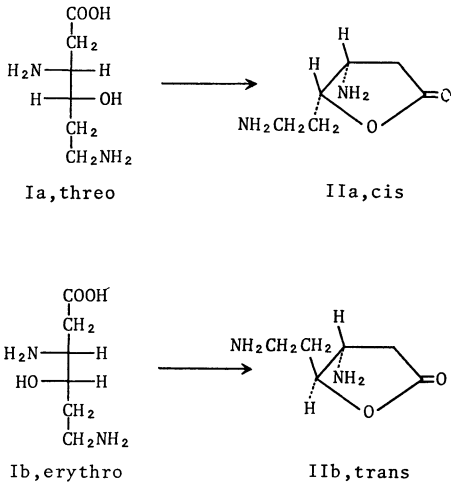
Interestingly, we have obtained a diastereoisomer of the amino acid Ia mentioned above as its lactone derivatives, when tuberactinomycin A was treated with concentrated sulfuric acid with the intention of carrying out N,O-migration reaction. It was benzyloxycarbonylated or 2,4-dinitrophenylated to prepare the corresponding derivatives, IIIb and IVb respectively, both of them showing the γ-lactone absorption in IR spectrum. Though these compounds IIIb and IVb were certainly the derivatives of γ-hydroxy-β-lysine lactone, they were not identical with IIIa and IVa obtained above in respects of IR spectrum, thin-layer chromatogram and melting point. Moreover, debenzyloxycarbonylated products IIa and IIb dihydrobromide prepared respectively from IIIa and IIIb evidently differed each other in thin-layer chromatogram, paper electrophoresis, $[\alpha]_D$, IR, NMR spectra and so on. Thus it was concluded that IIIb and IVb should be the diastereoisomers of IIIa and IVa respectively.

Concerning the stereochemistry of the amino acid I, it was first attempted to determine the configuration of β-carbon atom by the reduction of γ-hydroxyl group to drive to β-lysine. When γ-hydroxy-β-lysine I itself was used for the reduction, always the lactone was obtained as a main product indicating that the lactone formation proceeded much faster than the reduction. Therefore, dibenzyloxycarbonyl-γ-hydroxy-β-lysine amide VIa and VIb prepared by ammonolysis of IIIa and IIIb were used for this purpose. VIa and VIb were reduced with hydriodic acid and red phosphorus, and then 2,4-dinitrophenylated respectively. From the products of dinitrophenylation, di-DNP-β-lysine was separated and purified by preparative thin-layer chromatography and found to be completely identical with the authentic di-DNP-L-β-lysine (VII) in its ORD spectrum in both cases. In this respect, both isomers of γ-hydroxy-β-lysine must belong to L-series at β-carbon atom configurations.



On the other hand, the configurations of γ-carbon atoms of Ia and Ib were assigned by extended application of Hudson's lactone rule[13] as in the case of γ-hydroxylysine.[14] Thus, the lactone IIa and IIb were saponified with silver acetate to the corresponding hydroxy acids Ia and Ib respectively. Although these acids could not be isolated in pure states, mutarotations of them were measured in acidic solutions. From the facts that the rotation of Ia showed the positive shift in the course of time, while that of Ib indicated negative shift (Fig. 1), the configuration of γ-carbon atom was determined as D_g in Ia and L_g in Ib. In these cases, D_g should correspond to *R* and L_g to *S* configuration.

Consequently, it was concluded that Ia must be assigned to *threo*-γ-hydroxy-L-β-lysine and Ib to *erythro*-γ-hydroxy-L-β-lysine. This conclusion was supported by the following investigations. First, in the NMR spectra of IIa and IIb as shown in Fig. 2 and 3, coupl-



13) B. Witkop, *Experientia*, **12**, 372 (1956). Hudson's lactone rule says that γ- or δ-sugar lactones in which the carbon atom carrying the hydroxyl group contributed to the lactone linkage is related to D-glyceraldehyde show positive value of $[\alpha]_D$ (lactone) − $[\alpha]_D$ (acid) and those to L-glyceraldehyde give negative one.

14) N. Izumiya, Y. Fujita, F. Irreverre, and B. Witkop, *Biochemistry*, **4**, 2501 (1965).

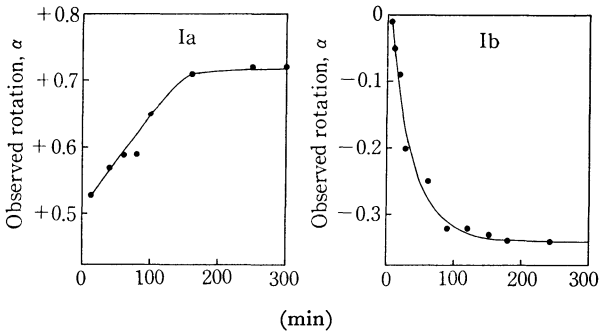


Fig. 1. Mutarotations of Ia and Ib.

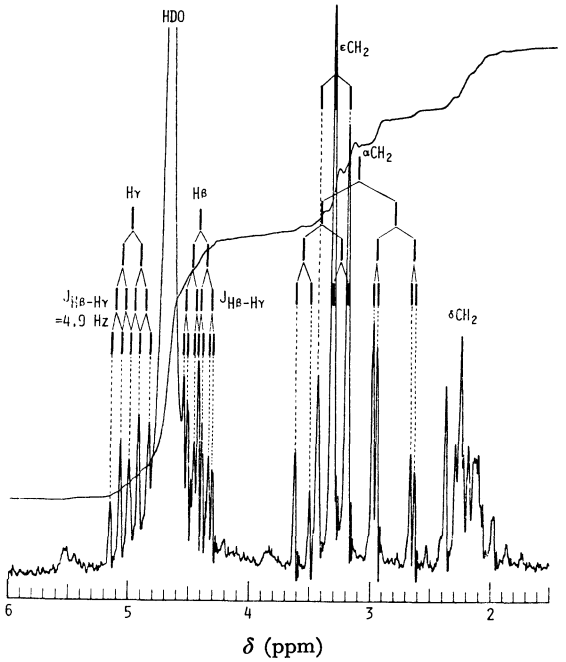


Fig. 2. NMR spectrum of IIa in D_2O.

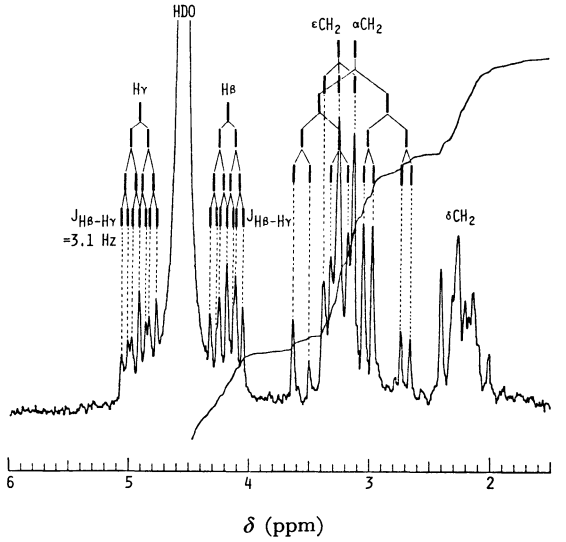


Fig. 3. NMR spectrum of IIb in D_2O.

ing constants between β and γ protons, of 4.9 Hz in the former and 3.1 Hz in the latter, indicated that vicinal protons in the lacton ring of IIa has a *cis* configuration and that of IIb a *trans* configuration, since dihedral angle consisting of vicinal protons in the five-membered lactone ring is near to 0° in *cis* form and to 120° in *trans* form, and therefore the coupling constant of *cis* configuration must be larger than that of of *trans* configuration. This assignment is consistent with the expectation that *threo*-γ-hydroxy-L-β-lysine forms *cis* γ-lactone ring and *erythro*-γ-hydroxy-L-β-lysine forms *trans* γ-lactone ring.

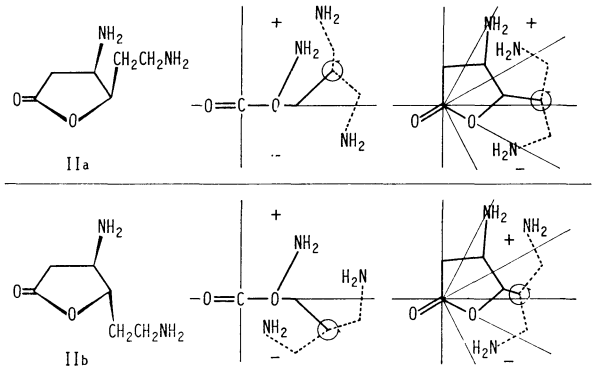


Fig. 4. Estimate of Cotton effects of IIa and IIb.

The above conclusion concerning the configurations of asymmetric carbon atoms in γ-hydroxy-β-lysine was also supported by ORD curve on the basis of Klyne's lactone sector rule.[15] According to this rule, it is expected that the ORD curves of *threo*- and *erythro*-γ-hydroxy-β-lysine lactone will show the positive Cotton effects as illustrated in Fig. 4, if they have β-L-configurations. There may exist negative contribution due to free rotations at δ-carbon atoms, which is, however, overcome by positive contribution as a whole. In fact, both ORD curves of IIa and IIb, in the range of 350 nm to 210 nm, showed positive plain curves indicating presences of positive Cotton effects at lower wavelength than 210 nm.

Recently, we carried out the synthetic work for this amino acid. Synthetic *erythro*-γ-hydroxy-DL-β-lysine in a form of lactone dihydrobromide was identical with that of *erythro* form of the natural amino acid.[16] Therefore, the conclusion of our study on chemical structure and stereochemistry of the new basic amino acid isolated from the antibiotics tuberactinomycin A and N was confirmed unequivocally.

Finally a problem remains, whether *threo* or *erythro* form of this amino acid is original one in the molecule of tuberactinomycin A or N and which is the artificial one converted during the acid treatment. At present, this subject is not yet clarified and must be left to a future investigation.

Experimental

All melting points are uncorrected. The infrared spectrum was obtained in nujol mull with a Nihon Bunko IR–S spectro-

15) W. Klyne, P. M. Scopes and A. Williams, *J. Chem. Soc.*, **1965**, 7211, 7299.

16) T. Wakamiya, T. Teshima, and T. Shiba, Presented at the 26th Annual Meeting of the Chemical Society of Japan, Hiratsuka, April, 1972; p. 1275.

photometer. The NMR spectrum was obtained with a Hitachi NMR R-20 spectrometer and Nihon Denshi C-60 spectrometer. Tetramethylsilane (TMS) was used as an internal reference in deuterochloroform solution, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external reference in the case of deuterium oxide solution. The chemical shifts are expressed as ppm from TMS or DSS. The ORD curve was obtained with a Yanagimoto ORD-185 spectropolarimeter in 1N hydrochloric acid and in methanol. The specific rotation was obtained with a Perkin-Elmer 141 polarimeter in water and in dimethylformamide. Thin-layer chromatography was carried out by the ascending method on silica gel G and preparative thin-layer chromatography on silica gel H.

Isolation of threo-*γ-Hydroxy*-L-*β-lysine Lactone (IIa) Dihydrochloride.* A solution of 20 g of tuberactinomycin A hydrochloride in 180 m*l* of 6N hydrochloric acid was heated at 100°C in a sealed tube or under reflux for 24 hr. Hydrochloric acid was evaporated *in vacuo* and the residue was dissolved in water. The evaporation was repeated three times. The solution of the residue thus 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 equilibriated with a buffer solution of 0.2 M pyridine and formic acid (pH 3.1). In the elution using 5.5 *l* of the same buffer solution (pH 3.1), serine, α, β-diaminopropionic acid and a mixture of tuberactidine and viomycidine were eluted successively. When the buffer solution was changed to a mixture of 2 M pyridine and formic acid (pH 5.2), γ-hydroxy-β-lysine was eluted. Fractions containing γ-hydroxy-β-lysine were collected and evaporated *in vacuo*. The residue was dissolved in hydrochloric acid and evaporated again. Crystalline residue was dissolved in ethanol and allowed to stand in refrigerator. Colorless prisms obtained were recrystallized from water–ethanol, mp 250—252°C (decomp.), $[\alpha]_D^{25}+65°$ (*c* 0.5, H_2O).

Found: C, 33.04; H, 6.54; N, 12.85; Cl, 32.65%. Calcd for $C_6H_{14}O_2N_2Cl_2$: C, 33.19; H, 6.50; N, 12.90; Cl, 32.66%.

N^{β},N^{ε}-*Dibenzyloxycarbonyl*-threo-*γ-hydroxy*-L-*β-lysine Lactone (IIIa).* Hydrolysis of each 10 g of tuberactinomycin A or N hydrochloride was carried out under the similar condition to that mentioned above and hydrolyzate was evaporated *in vacuo*. Residue was dissolved in 100 m*l* of water and neutralized with sodium carbonate on cooling. The neutralized hydrolyzate was benzyloxycarbonylated by the usual method using 50 g of benzyloxycarbonylchloride and 100 m*l* of 2N sodium hydroxide. Oily substance formed before acidification was extracted with ethyl acetate and the extract was washed with aqueous sodium carbonate solution and then water thoroughly. The organic layer was dried over anhydrous sodium sulfate and then evaporated *in vacuo*. Oily residue obtained was purified by silica gel (Merck, 0.05—0.20 mm mesh) column chromatography. Chloroform (500 m*l*) and then chloroform–methanol (100 : 1, 1500 m*l*; 100 : 1.5, 200 m*l*) were passed through the column. Benzyl carbamate was eluted first, followed by the desired N^{β},N^{ε}-dibenzyloxycarbonyl-*threo*-γ-hydroxy-L-β-lysine lactone (IIIa). Recrystallization of 2.5 g of crude IIIa from ethyl acetate gave colorless needles, yield 2.1 g, mp 149.5°C, $[\alpha]_D^{20}+48.0°$ (*c* 0.4, DMF).

Found: C, 64.02; H, 5.73; N, 6.84%. Calcd for $C_{22}H_{24}O_6N_2$: C, 64.06; H, 5.87; N, 6.79%.

Finally, when a mixture of chloroform–methanol (100 : 4, 1200 m*l*) was used as an elution solvent, N^{α},N^{β}-dibenzyloxycarbonyl-L-α,β-diaminopropionamide was eluted.

Reduction of γ-Hydroxy-β-lysine to β-Lysine. In 2 m*l* of 1N sodium hydroxide, 50 mg of IIa dihydrochloride was dissolved, and allowed to stand at room temperature. After 2 hr, the reaction mixture was neutralized with 1N hydrochloric acid and concentrated *in vacuo*. The residue was dissolved in 5 m*l* of hydriodic acid, and 50 mg of red phosphorus was added. After heating for 3 hr under reflux at 135°C, the solution was evaporated *in vacuo*. The residue was dissolved in water and evaporation was repeated several times. To the solution of the residue in water was added silver oxide and the suspension was stirred for a half hour. Insoluble materials were filtered off and the filtrate was acidified with 1N hydrochloric acid. After filtration of silver chloride, the filtrate was neutralized with aqueous sodium hydrogencarbonate. To this solution was added 100 mg of 2,4-dinitrofluorobenzene in 5 m*l* of acetone and the pH of the solution was controlled always in alkaline side by further additions of sodium carbonate. After stirring at room temperature for 5 hr, it was acidified with diluted hydrochloric acid and the reaction mixture was extracted with ethyl acetate. Di-DNP-β-lysine was detected in the extract on thin-layer chromatogram using developing solvents of chloroform–methanol–acetic acid (95 : 5 : 1) and benzene–pyridine–acetic acid (40 : 10 : 1).

Periodic Acid Oxidation of γ-Hydroxy-β-lysine. To a solution of 50 mg of IIa dihydrochloride in 5 m*l* of 0.5N sodium hydroxide, 50 mg of crystalline periodic acid were added. After allowing to stand at room temperature for 2 hr, diluted potassium permanganate solution was added dropwise to the reaction mixture until the color of the solution remained. In the reaction mixture, β-alanine was detected by thin-layer chromatography. The reaction mixture was subjected to 2,4-dinitrophenylation. Thus, formation of DNP-β-alanine was confirmed also by thin-layer chromatography.

N^{β},N^{ε}-*Di-2,4-dinitrophenyl*-threo-*γ-hydroxy*-L-*β-lysine Lactone (IVa).* To a solution of 15 g of tuberactinomycin A hydrochloride in 400 m*l* of water, 15 g of 2,4-dinitrofluorobenzene in 400 m*l* of acetone was added. A mixture was stirred for 20 hr magnetically. It was evaporated *in vacuo* to remove acetone. Precipitate was filtered and washed with water, ethanol, and ether successively. DNP-Tuberactinomycin A obtained was reprecipitated from dimethylformamide–ether, yield 10.2 g. When 7.0 g of DNP-peptide prepared as above was suspended in 350 m*l* of 6N hydrochloric acid and heated under reflux for 24 hr, dark green insoluble material formed. It was filtered and recrystallized from dimethylformamide–ether to give 2.2 g of yellowish needles of N^{β},N^{ε}-di-DNP-*threo*-γ-hydroxy-L-β-lysine lactone (IVa), mp 245—246°C (decomp.), $[\alpha]_D^{22}-63.9°$ (*c* 0.5, DMF).

Found: C, 45.47; H, 3.50; N, 17.66%. Calcd for $C_{18}H_{16}O_{10}N_6$: C, 45.38; H, 3.39; N, 17.64%.

N^{β},N^{ε}-*Di-2,4-dinitrophenyl*-threo-*γ-hydroxy*-L-*β-lysine (Va).* A suspension of 600 mg of IVa in 6 m*l* of 1N sodium hydroxide was warmed at 90°C. After IVa was dissolved, the reaction mixture was immediately cooled and neutralized with 6 m*l* of 1N hydrochloric acid. Oily product formed was extracted with ethyl acetate and washed with water. Organic layer was dried over anhydrous sodium sulfate. It was concentrated *in vacuo* to give a crystalline residue which was then recrystallized from methanol, yield 0.4 g, mp 161—162°C (sint.) and 245°C (decomp.).

Found: C, 43.76; H, 3.86; N, 16.93%. Calcd for $C_{18}H_{18}O_{11}N_6$: C, 43.73; H, 3.67; N, 17.00%.

N^{β},N^{ε}-*Dibenzyloxycarbonyl*-erythro-*γ-hydroxy*-L-*β-lysine Lactone (IIIb).* A solution of 5.0 g of tuberactinomycin A hydrochloride in 140 m*l* of concentrated sulfuric acid was allowed to stand for five weeks in a sealed tube. The solution was poured into acetone on cooling with a freezing mixture.

A gel precipitated was filtered off, washed with acetone and ether, and then dried *in vacuo*. Dried precipitate was dissolved in 6N hydrochloric acid and allowed to stand at room temperature. After 18 hr, the acidic solution was neutralized with sodium hydrogencarbonate on cooling and benzyloxycarbonylation was carried out by the usual way. Oily product was extracted with ethyl acetate and washed with saturated aqueous sodium chloride solution. Organic layer was dried and concentrated *in vacuo*. Oily residue obtained was washed with petroleum ether and then made to powder by trituration with ether. The powder was extracted with ether using Soxhlet's extractor. Combined filtrate and extract were evaporated and residual oil was dissolved in ether. Crystals formed on addition of petroleum ether were filtered off and recrystallized from ethyl acetate and petroleum ether to afford 0.5 g of colorless needles, mp 96.5—97.5°C, $[\alpha]_D^{20}$ −9.0°C (*c* 0.4, DMF).

Found: C, 64.07; H, 5.87; N, 6.81%. Calcd for $C_{22}H_{24}O_6N_2$: C, 64.06; H, 5.87; N, 6.79%.

*N^{β},N^{ε}-Di-2,4-dinitrophenyl-*erythro-*γ-hydroxy-*L-*β-lysine Lactone (IVb).* Tuberactinomycin A was treated with concentrated sulfuric acid as described above and 500 mg of the product obtained was treated with 35 ml of 6N hydrochloric acid at room temperature for 20 hr. After the acid solution had been neutralized with sodium hydrogencarbonate, it was 2,4-dinitrophenylated by the usual manner. The reaction mixture was evaporated *in vacuo* to remove acetone and then insoluble materials were filtered off. The filtrate was extracted with ethyl acetate and washed with aqueous sodium hydrogencarbonate and water. Organic layer was dried and concentrated *in vacuo*. Crystalline residue was recrystallized from acetone and ether, yield 70 mg, mp 145°C (decomp. after sint.), $[\alpha]_D^{22}$ −177.8° (*c* 0.5, DMF).

Found: C, 45.39; H, 3.58; N, 17.77%. Calcd for $C_{18}H_{16}O_{10}N_6$: C, 45.38; H, 3.39; N, 17.64%.

*N^{β},N^{ε}-Dibenzyloxycarbonyl-*threo-*γ-hydroxy-*L-*β-lysine Amide (VIa).* A solution of 500 mg of IIIa in methanol was saturated with ammonia. After allowing to stand at room temperature for a few days in a pressure bottle, methanol was evaporated under reduced pressure. Residual crystals were recrystallized from methanol to give colorless needles, yield 410 mg, mp 209°C, $[\alpha]_D^{20}$ +13.0° (*c* 0.4, DMF).

Found: C, 61.28; H, 6.34; N, 9.79%. Calcd for $C_{22}H_{27}O_6N_3$: C, 61.52; H, 6.34; N, 9.79%.

*N^{β},N^{ε}-Dibenzyloxycarbonyl-*erythro-*γ-hydroxy-*L-*β-lysine Amide (VIb).* Colorless needles of VIb were prepared from 170 mg of IIIb as shown in VIa, yield 140 mg, mp 209—210°C, $[\alpha]_D^{20}$ −3.5° (*c* 0.4, DMF).

Found: C, 61.18; H, 6.24; N, 9.78%. Calcd for $C_{22}H_{27}O_6N_3$: C, 61.52; H, 6.34; N, 9.79%.

Reduction of VIa and VIb with Hydriodic Acid and Red Phosphorus. To a solution of 50 mg of VIa or VIb in 2 ml of 57% hydriodic acid, 80 mg of red phosphorus was added. After heating at 100°C for 20 hr in a sealed tube, excess red phosphorus was filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in water. Hydriodic acid was decomposed with hydrogen peroxide. Iodine was filtered off and the filtrate was extracted with ethyl acetate. An aqueous layer was treated with 5% Pd–C to decompose excess hydrogen peroxide. After concentration, it was subjected to 2,4-dinitrophenylation. The product was purified by preparative thin-layer chromatography using developing solvent of chloroform–methanol–acetic acid (95 : 5 : 3). Di-DNP-β-lysine obtained was identified with an authentic sample by thin-layer chromatography. It was applied to the measurement of ORD curve.

Debenzyloxycarbonylation of IIIa to IIa dihydrobromide. A solution of 1 g of IIIa in 5 g of 30% hydrogen bromide in glacial acetic acid was kept at room temperature for 1 hr. When ether was added, IIa was precipitated as hydrobromide, yield 740 mg. Recrystallization from 94% ethanol gave prisms, mp 235—6°C (decomp.), $[\alpha]_D^{20}$ +48° (*c* 0.5, H_2O).

Found: C, 23.88; H, 4.53; N, 8.84; Br, 51.80%. Calcd for $C_6H_{12}O_2N_2 \cdot 2HBr$: C, 23.54; H, 4.61; N, 9.16; Br, 52.23%.

Debenzyloxycarbonylation of IIIb to IIb Dihydrobromide. Removal of benzyloxycarbonyl group from 280 mg of IIIb was carried out as mentioned in the reaction of IIIa to IIa, to obtain IIb hydrobromide, yield 100 mg, mp 222—223°C (decomp.), $[\alpha]_D^{20}$ −18.0° (*c* 0.5, H_2O).

Found: C, 23.92; H, 4.62; N, 8.95; Br, 51.98%. Calcd for $C_6H_{12}O_2N_2 \cdot 2HBr$: C, 23.54; H, 4.61; N, 9.16; Br, 52.23%.

Preparation of Hydroxy Acid Ia and Ib. To a solution of 200 mg of the lactone IIa or IIb hydrobromide in 5 ml of water, 1.0 g of silver acetate was added. Suspended solution was heated at 90°C with stirring for 2 hr. Silver bromide formed and excess silver acetate were filtered off. Filtrate was acidified to pH 3.5 with 1N hydrochloric acid. Precipitated silver chloride was filtered off and the filtrate was concentrated below the temperature of 30°C *in vacuo*. The hydroxy acids, Ia and Ib were obtained as oily products. These were applied to the measurement of mutarotation directly.

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