Chemical Studies on Tuberactinomycin. XVII.[1] Synthesis of *threo*-γ-Hydroxy-L-β-lysine, Constituent Amino Acid of Tuberactinomycin A and N

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Synopsis. *threo*-γ-Hydroxy-L-β-lysine which is a constituent amino acid of antibiotics tuberactinomycin A and N, was synthesized from *threo*-β-hydroxy-L-ornithine derivative by the Arndt-Eistert reaction. The synthetic amino acid was identical with the natural sample in all respects. Furthermore, a route was established to prepare a protected γ-hydroxy-β-lysine derivative useful for the peptide synthesis.

γ-Hydroxy-β-lysine is a constituent amino acid of peptide antibiotics, tuberactinomycin A and N.[2] Either *threo* or *erythro* isomer of this amino acid was obtained from both tuberactinomycin A and N depending on the hydrolysis conditions. Thus, *threo* isomer was obtained from a hydrolyzate with 6 M hydrochloric acid, whereas *erythro* isomer from that with concentrated sulfuric acid.[2] An intact configuration of the amino acid in the original antibiotics was supposed to be of *threo* form from an assumption of the reaction mechanisms in the acidolysis.[3-5]

Racemic *threo* and *erythro* forms of γ-hydroxy-β-lysine were synthesized from a key intermediate, β-methoxyornithine derivative, resulting in a confirmation of the structure of this amino acid.[6] In the present study, a genuine stereoisomer of the natural amino acid, *i.e.*, *threo*-γ-hydroxy-L-β-lysine, was synthesized via *threo*-β-hydroxy-L-ornithine according to a scheme shown in Fig. 1. An intermediate in the synthetic route, N^{β},N^{ε}-bis(benzyloxycarbonyl)-*O*-*t*-butyl-γ-hydroxy-L-β-lysine, was successfully used to the total synthesis of tuberactinomycin N, which will be reported elsewhere.[1]

In the Arndt-Eistert reaction for an elongation of the carbon chain, hydroxyl group must be blocked anyhow. Moreover, it was found in our previous study that γ-hydroxy-β-lysine was very liable to be lactonized.[2] Therefore, a protection of the hydroxyl group was necessary particularly for the purpose of the peptide synthesis. *t*-Butyl group was chosen as the protecting group because of a mildness in the introducing reaction. N^{δ}-Benzyloxycarbonyl-*threo*-β-hydroxy-L-ornithine (**1**) was obtained by a coupling of β-(benzyloxycarbonylamino)propionaldehyde with aqua[*N*-(1-carboxylatoethylidene)glycinato]copper(II) followed by a diastereomeric separation and then an optical resolution enzymatically according to the method reported previously.[7] The compound **1** was converted to bis(benzyloxycarbonyl) derivative **2**, and then to its methyl ester **3**. It was treated with isobutylene in the presence of an acid catalyst to give a fully protected derivative **4**. Saponification of **4** produced a free carboxylic acid **5**, which was treated with ethyl chloroformate and then diazomethane to afford a diazo ketone. Wolff rearrangement for the diazo ketone was carried out in the presence of silver benzoate in methanol. Hydrolysis of the product with 6 M hydrochloric acid gave *threo*-γ-hydroxy-L-β-lysine lactone dihydrochloride (**7**). The lactone dihydrochloride **7** thus synthesized was completely identical with the natural specimen obtained from the hydrochloric acid hydrolyzate of tuberactinomycin N[2] in all respects (Table 1). This is the first synthesis of the natural form of γ-hydroxy-β-lysine, and gave a confirmative conclusion for the supposed configurations of this natural amino acid.

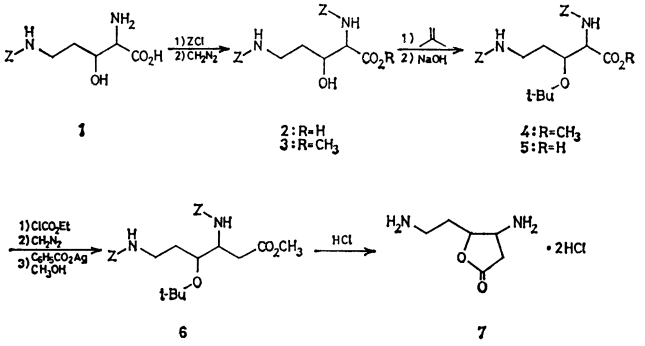

Fig. 1.

Table 1. Comparisons of the natural and synthetic γ-hydroxy-L-β-lysine lactone dihydrochlorides

	Synthetic	Natural
mp (°C, dec)	252—255	254—257
$[\alpha]_D^{25}$ (*c* 0.5, H_2O)	+63°	+65°
Thin-layer chromatography[a]	0.48	0.48
Paper electrophoresis[b]	13 cm	13 cm

a) R_f value on developing solvent: phenol-water-28% ammonia (30:10:1). b) Migration distance toward cathode. Buffer solution: pyridine-acetic acid-water (30:4:966).

Experimental

All melting points are uncorrected. NMR spectra were obtained with a Varian XL-100-15 spectrometer using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. The optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were obtained with a Hitachi EPI-G2 Grating Infrared Spectrophotometer. Thin-layer chromatographies were carried out by the ascending method on silica gel 60 F_{254} using developing solvent, phenol-water-28% ammonia (30:10:1). Paper electrophoreses were carried out at 750 V and 1 mA/cm 1 h on Toyo Roshi No. 51 paper using a buffer solution of pyridine–acetic acid–water (30:4:966).

N^{α}, N^{δ}-*Bis(benzyloxycarbonyl)*-threo-β-*hydroxy*-L-*ornithine* (**2**). To a suspension of N^{δ}-benzyloxycarbonyl-*threo*-β-hydroxy-L-ornithine (**1**)[7] (1.00 g, 3.55 mmol) and triethylamine (1.15 ml, 8.24 mmol) in 300 ml of water–tetrahydrofuran (2:1), was added benzyloxycarbonyl chloride (800 mg, 4.69 mmol) in 4 ml of tetrahydrofuran portionwise with stirring under ice cooling for 2.5 h. After the usual work up for

benzyloxycarbonylation, crystals of the product were obtained: yield 1.32 g (89.2%). It was recrystallized from ethyl acetate–hexane: yield 1.23 g (83.1%), mp 141.5—143 °C, $[\alpha]_D^{20}$ +13.8° (c 1.04, CH_3OH).

Found: C, 60.80; H, 5.83: N, 6.62%. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.81; N, 6.73%.

N^{α},N^{δ} - *Bis (benzyloxycarbonyl)* - threo - *β* - *hydroxy* - L - *ornithine Methyl Ester* (**3**). To a solution of N^{α},N^{δ}-bis(benzyloxycarbonyl)-*threo*-*β*-hydroxy-L-ornithine (**2**) (1.20 g, 2.88 mmol) in 20 ml of methanol, a solution of diazomethane in ether was added until its yellow color remained. After excess diazomethane was decomposed with acetic acid, the reaction mixture was concentrated *in vacuo*. The oily residue was triturated with hexane to give crystals: yield 1.22 g (98.5%). It was recrystallized from ethyl acetate–hexane: yield 1.19 g (95.9%), mp 85—88 °C, $[\alpha]_D^{18}$ +5.42° (*c* 1.02, CH_3OH).

Found: C, 61.40; H, 6.31; N, 6.28%. Calcd for $C_{22}H_{26}N_2O_7$: C, 61.38; H, 6.09; N, 6.51%.

N^{α},N^{δ}-*Bis(benzyloxycarbonyl)*-O - t - *butyl* - threo - *β*-*hydroxy*-L-*ornithine* (**5**). To a solution of N^{α},N^{δ}-bis(benzyloxycarbonyl)-*threo*-*β*-hydroxy-L-ornithine methyl ester (**3**) (1.00 g, 2.33 mmol) in 100 ml of dichloromethane and 300 ml of liquid isobutylene in a pressure bottle, 0.2 ml of concentrated sulfuric acid was added under cooling. The mixture was shaken at 37 °C for 11 d and then neutralized with triethylamine. The solution was concentrated *in vacuo*, and the oily residue was purified by silica gel column chromatography using a developing solvent of benzene–ethyl acetate (9:1); yield 910 mg (80.5%), a pale yellow oil. *O-t*-Butyl methyl ester (**4**) thus obtained was saponified with 2.81 ml of 1 M sodium hydroxide in 5 ml of *N,N*-dimethylformamide at 0 °C. After the usual work up, crystals of the carboxylic acid was obtained: yield 633 mg (71.6%). It was recrystallized from ethyl acetate–ether–hexane; yield 567 mg (64.1%), mp 92—95 °C, $[\alpha]_D^{21}$ −2.07° (*c* 5.07, CH_3OH).

Found: C, 63.65; H, 6.83; N, 5.92%. Calcd for $C_{25}H_{32}N_2O_7$: C, 63.55; H, 6.83; N, 5.93%.

threo-*γ*-*Hydroxy*-L-*β*-*lysine Lactone Dihydrochloride* (**7**). To a solution of N^{α},N^{δ}-bis(benzyloxycarbonyl)-*O-t*-butyl-*threo*-*β*-hydroxy-L-ornithine (**5**) (420 mg, 0.890 mmol) in 4 ml of ethyl acetate, *N*-methylmorpholine (0.100 ml, 0.890 mmol) and ethyl chloroformate (0.085 ml, 0.89 mmol) were added at −20 °C with stirring. The reaction mixture was stirred for 1 h, and insoluble material was filtered off. To the filtrate, excess diazomethane in ether was added at −20 °C with stirring. The reaction mixture was stirred at this temperature for 1 h and then at room temperature overnight. Insoluble material was filtered off and the filtrate was concentrated *in vacuo*. A diazo ketone was obtained as a yellow oil.

To a solution of this oily residue in 4 ml of methanol, silver benzoate (70 mg, 0.306 mmol) in 0.7 ml of triethylamine was added and the mixture was stirred at room temperature in the dark for 4 h and then concentrated *in vacuo*. After the residue was dissolved in ethyl acetate, insoluble inorganic material was filtered off. The filtrate was washed with 10% citric aicd, saturated sodium hydrogencarbonate, and water. Organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give a methyl ester **6**.

A suspension of **6** in 25 ml of hydrochloric acid was heated under reflux for 3 h, and concentrated *in vacuo*. The residue was triturated with ethanol and ether to give crystals: yield 58.1 mg (30.1%). It was recrystallized from ethanol–ether: yield 45 mg (23%), mp 252—255 °C (dec), $[\alpha]_D^{21}$ +63° (*c* 0.5, H_2O).

Found: C, 32.97; H, 6.59; N, 12.79; Cl, 32.33%. Cacld for $C_6H_{14}N_2O_2Cl_2$: C, 33.20; H, 6.50; N, 12.90; Cl, 32.66%.

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