Chemical Studies on Tuberactinomycin. VII.[1] Synthesis of γ-Hydroxy-β-lysine[2]

Tateaki WAKAMIYA, Tadashi TESHIMA, Ichiro KUBOTA,*
Tetsuo SHIBA, and Takeo KANEKO**
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560
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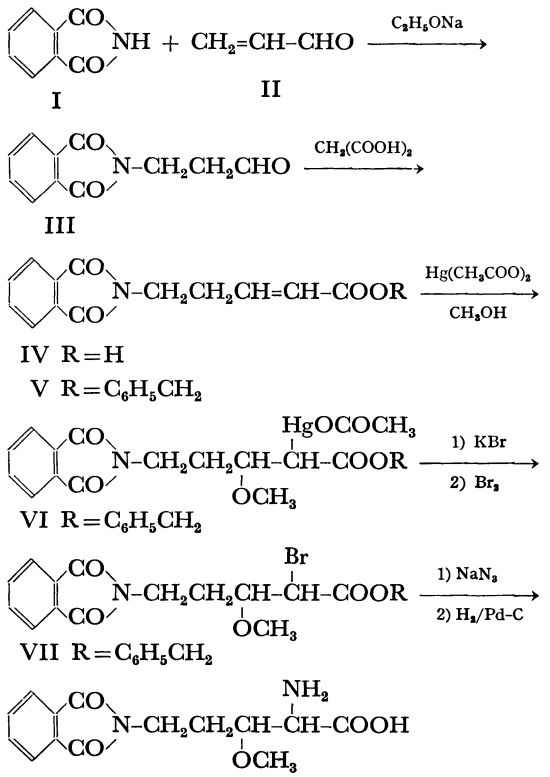
γ-Hydroxy-β-lysine is a new basic amino acid isolated from the hydrolyzates of antitubercular peptides, tuberactinomycin A and N. In order to confirm the structure and establish the stereochemistry of the amino acid, two diastereoisomers of γ-hydroxy-DL-β-lysine were synthesized by Arndt-Eistert synthesis *via* β-hydroxyornithine. Synthetic *erythro* and *threo* isomers were found to be identical spectroscopically and chromatographically with the corresponding forms obtained from the antibiotics respectively.

The isolation and the structural determination of γ-hydroxy-β-lysine in antitubercular peptides tuberactinomycin A and N[3] were reported in the previous paper.[4] Two different stereoisomers of the amino acid were obtained depending on acid reagent and reaction condition for the hydrolysis. The complete hydrolysis of the peptides by heating with hydrochloric acid gave *threo*-γ-hydroxy-β-lysine, while the partial hydrolysis with concentrated sulfuric acid at room temperature afforded the *erythro* isomer.[4] Our stereochemical investigation on *N,O*-acyl migration reaction of vicinal hydroxy amino acid revealed that the *threo* form of this amino acid was a genuine configuration in the intact molecules of tuberactinomycins, since the treatment with concentrated sulfuric acid caused an epimerization of the configuration at carbon atom carrying the hydroxyl group.[1,5] This paper presents a total synthesis of both isomers of the amino acid in order to confirm the conclusion of the structural and stereochemical studies.

In our synthetic plan, β-hydroxyornithine is required as an important intermediate which is subjected to the homologation to γ-hydroxy-β-lysine through Arndt-Eistert reaction. The synthesis of β-hydroxyornithine was carried out as follows (Scheme 1). Coupling of phthalimide with acrolein gave 3-phthaliminopropionaldehyde (III), which was then condensed with malonic acid to yield 5-phthalimino-2-pentenoic acid (IV).[6] Acid IV was esterified to benzyl ester V with benzyl alcohol and *p*-toluenesulfonic acid in benzene. From the coupling constant of 16 Hz for olefinic protons in its NMR spectrum, the benzyl ester V thus obtained was indicated to be a *trans* form. The ester V was allowed to react with mercuric acetate in methanol to give benzyl 2-acetoxymercuri-3-methoxy-5-phthaliminopentanoate (VI). A conversion of 2-acetoxymercuri compound VI to 2-bromomercuri derivative with potassium bromide followed by bromination gave benzyl 2-bromo-3-methoxy-5-phthaliminopentanoate (VII) in a good yield. A direct amination of VII in alkaline medium should be avoided in view of possible cleavage of phthalyl group to phthalamic acid derivative.[7] Since a substitution of phthalimino group into α-carbon

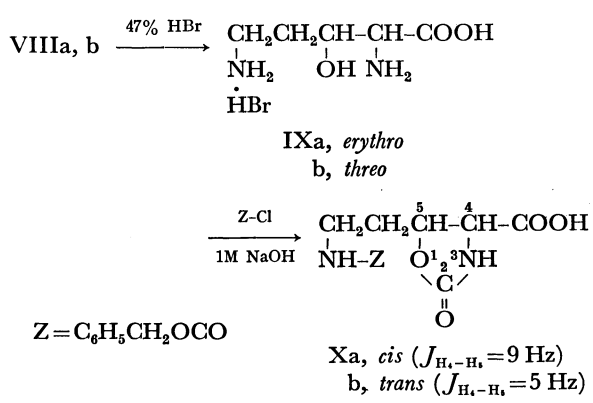


Scheme 1.

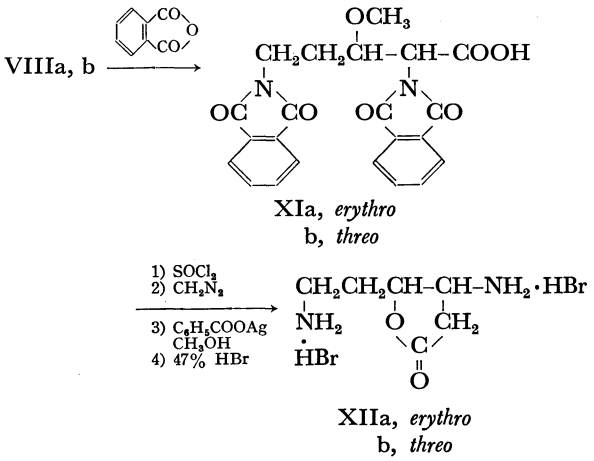
atom of VII with potassium phthalimide failed, the compound VII was converted to an azide derivative with sodium azide in aqueous dimethylformamide and then hydrogenated to N^{δ}-phthalyl-β-methoxyornithine (VIII) using palladium on charcoal as a catalyst. The amino acid VIII thus obtained was a mixture of two diastereoisomers. One of them was easily crystallized as hydrochloride from ethanolic solution, while another isomer was crystallized as free base from the mother liquor on addition of triethylamine.

In order to determine the configuration of these products, the NMR study for oxazolidone derivative of β-hydroxy-α-amino acid was applied.[8] Thus VIII was hydrolyzed to β-hydroxyornithine (IX) with hydrobromic acid in the presence of anisole. The amino acid IX was then benzyloxycarbonylated in aqueous sodium hydroxide solution to afford the oxazolidone

* Present address: Department of Agricultural Chemistry, Nagoya University, Chikusa-ku, Nagoya 464.
** Present address: Shiseido Laboratory, Nippa-cho, Kohoku-ku, Yokohama 223.



Scheme 2.



Scheme 3.

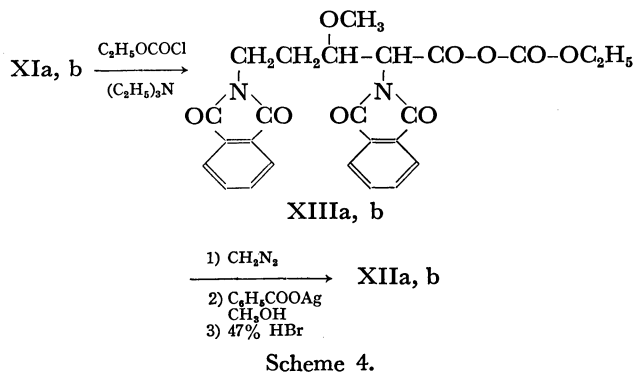
derivative X (Scheme 2). Oxazolidone compounds derived from β-hydroxy-α-amino acids may possess a fixed structure of nearly planar five-membered ring. Therefore, the coupling constants of *cis* and *trans* isomers will be distinguishable. In fact, the coupling constants of vicinal protons on the ring of several oxazolidone derivatives are converged at around 9 Hz in *cis* configurations and at 5 Hz in *trans* ones as far as tested.[8] Actually, the coupling constant between C-4 and C-5 protons was measured to be 9 Hz in Xa and 5 Hz in Xb. Consequently, the configurations of Xa and Xb were assigned to *cis* and *trans* forms which should have been derived from *erythro* and *threo* compounds respectively. Therefore, the configurations of VIIIa and VIIIb must be *erythro* and *threo* forms respectively. In general, a so-called hydroxymercuration, an addition reaction of mercuric acetate to an olefin in methanol, is known to proceed by *trans* addition preferentially.[9] However, since the following bromination induced racemization at α-carbon atom more or less,[9,10] the fact is not unreasonable that the product VII and hence VIII were mixtures of the diastereoisomers.

For Arndt-Eistert synthesis, each isomer of VIII was then phthalylated respectively to N^{α},N^{δ}-diphthalyl-β-methoxyornithine (XIa) (XIb) with phthalic anhydride either by fusion method or by heating in dioxane. Use of ethoxycarbonylphthalimide as phthalylation reagent[11] did not give satisfactory result, because of a possible cleavage of N^{δ}-phthalyl group to phthalamic acid derivative during the reaction under a basic condition.

According to the usual manner of Arndt-Eistert reaction, diphthalyl acid XI was converted to an acid chloride and then to diazoketone which was rearranged to N^{β},N^{ϵ}-diphthalyl-γ-methoxy-β-lysine methyl ester in methanol using silver benzoate. Without isolation of the ester, the product was immediately hydrolyzed with hydrobromic acid in the presence of anisole. Eventually, both desired isomers of γ-hydroxy-DL-β-lysine were obtained starting from VIIIa and VIIIb respectively as their lactone (XIIa) (XIIb) dihydrobromide (Scheme 3).

Since the configurations of the asymmetric carbon atoms are known to be maintained through Arndt-Eistert synthesis involving Wolff rearrangement reaction, the final product XIIa prepared from *erythro* compound XIa can be assigned to *erythro* form and XIIb from XIb to *threo* form. The lactone XIIa dihydrobromide synthesized here was completely identical with *erythro*-γ-hydroxy-L-β-lysine lactone dihydrobromide obtained by *N,O*-acyl migration with concentrated sulfuric acid from tuberactinomycin A and N, in IR, NMR spectra, thin-layer chromatogram, and paper electrophoresis. Another isomer XIIb was also identified with *threo*-γ-hydroxy-L-β-lysine lactone obtained from the hydrochloric acid hydrolyzates of tuberactinomycin A and N in all respects.

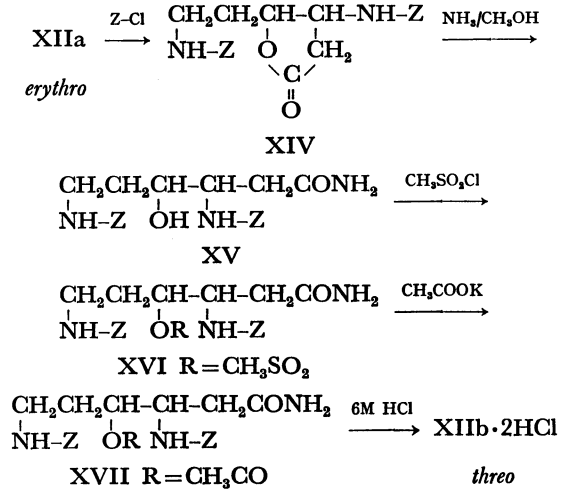
The synthesis of γ-hydroxy-β-lysine by the usual Arndt-Eistert reaction *via* acid chloride could not always give good yields. This defect seems to be due to an undesirable formation of chloroketone which may be produced from diazoketone and hydrogen chloride in the reaction course. Actually, mixed crystals composing of the diazoketone and the chloroketone was readily precipitated in the case of *erythro* compound and purification of the diazoketone by recrystallization of the mixed crystals was made extremely difficult. By this reason, an improved method for preparation of the diazoketone was attempted by application of mixed carbonic-carboxylic acid anhydride method.[12] This method afforded a satisfactory result in yield of the product compared with the acid chloride method (Scheme 4). However, partial racemization occurred through the reaction particularly in *threo* isomer. Therefore, for the preparation of *erythro*-γ-hydroxy-β-lysine, Arndt-Eistert synthesis *via* the mixed anhydride procedure serves as a fairly good synthetic process, though



Scheme 4.

the purification of the *threo*-isomer was shown to be difficult even after repetition of recrystallization. However, the mixed anhydride method could be improved for diazoketone synthesis, if a prevention of the partial racemization becomes feasible for instance by choice of protection of α-amino group or by use of an adequate amine like *N*-methylmorpholine in place of triethylamine.

In attempt of an interconversion of diastereoisomers of γ-hydroxy-β-lysine, an epimerization of γ-carbon atom of the amino acid was investigated. As shown in



Scheme 5.

Scheme 5, synthetic *erythro*-γ-hydroxy-DL-β-lysine lactone XIIa was benzyloxycarbonylated to N^{β},N^{ϵ}-dibenzyloxycarbonyl-*erythro*-γ-hydroxy-DL-β-lysine lactone (XIV) which was then converted to its amide XV. Otherwise, the reverse lactonization proceeded very easily when free hydroxy acid was attempted to be obtained. Hydroxyl group of the amide XV was mesylated with methanesulfonyl chloride in pyridine. When this *O*-mesyl compound XVI was treated with potassium acetate in absolute ethanol, substitution of acetoxyl group was accompanied by an inversion of the configuration on γ-carbon atom. Through hydrolysis of γ-acetoxy derivative XVII thus prepared with 6 M hydrochloric acid, *threo*-γ-hydroxy-DL-β-lysine lactone (XIIb) dihydrochloride was obtained. This conversion was also confirmed by identity of XIIb with natural *threo*-γ-hydroxy-L-β-lysine from the antibiotics on thin-layer chromatogram and IR spectra.

From the result of this synthetic study, not only the assignment of the chemical structure of the new natural amino acid in tuberactinomycins to γ-hydroxy-β-lysine, was assured, but also the relationship between the stereoisomers of the amino acid was clearly established.

Experimental

All melting points are uncorrected. The IR spectra were obtained in nujol mull with a Nihon Bunko IR-S spectrophotometer. The NMR spectra were obtained with a Varian T-60 spectrometer (60 MHz). Tetramethylsilane (TMS) was used as an internal reference in deuterochloroform and deuteromethanol solution, and as an external reference in the case of deuterium oxide solution. Thin-layer chromatography was carried out by the ascending method on silica gel G using a developing solvent of phenol–water–28% ammonium hydroxide (30: 10: 0.6). Paper electrophoresis was carried out at 750 volt and 10 mA for 1.5 hr on Toyo Roshi No. 51 filter paper using a buffer solution of pyridine–acetic acid–water (30: 4: 966).

Benzyl 5-Phthalimino-2-pentenoate (V). To a suspension of 5-phthalimino-2-pentenoic acid[6] (36.9 g, 0.150 mol) in 220 ml of benzene, there were added benzyl alcohol (26.0 g, 0.241 mol) and *p*-toluenesulfonic acid monohydrate (2.16 g, 11.4 mmol). The mixture was heated under reflux and the liberated water was removed azeotropically by means of Dean and Stark distilling receiver. After water had been no longer distilled off, the reaction mixture was permitted to cool to room temperature. Clear benzene solution was washed with sodium hydrogencarbonate solution and then water. Organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo*. Oily residue was triturated in petroleum ether under cooling to deposit crystals, yield 38.3 g (76%). Recrystallization from methanol gave prisms, mp 57—58 °C.

Found: C, 71.58; H, 5.20; N, 4.15%. Calcd for $C_{20}H_{17}O_4N$: C, 71.63; H, 5.11; N, 4.18%.

Benzyl 2-Bromo-3-methoxy-5-phthaliminopentanoate (VII). To a solution of V (63.0 g, 0.188 mol) in 500 ml of methanol, mercuric acetate (89.9 g, 0.282 mol) was added. The clear reaction mixture was stirred at room temperature for four days. The colorless crystals of 2-acetoxymercuri-3-methoxy derivative (VI) precipitated were filtered, yield 86.0 g. To the suspension of the above crystals VI in 500 ml of acetone, a solution of potassium bromide (41.8 g, 0.351 mol) in 75 ml of water was added on stirring. A clear solution thus obtained was concentrated *in vacuo*. A residue obtained was dissolved in water and then extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate and concentrated to 200 ml *in vacuo*. To the chloroform solution containing 2-bromomercuri derivative a solution of bromine (28.0 g, 0.175 mol) in 50 ml of chloroform was added dropwise at 50 °C. The precipitate of mercuric bromide was filtered off and the filtrate was washed with aqueous sodium hydrogencarbonate and then water. It was dried over anhydrous sodium sulfate and concentrated *in vacuo*. Residual oil was triturated with petroleum ether to make powder, yield 66.1 g (78.7%). Recrystallization from benzene gave needles, mp 112—114 °C.

Found: C, 56.73; H, 4.55; N, 3.15; Br, 17.64%. Calcd for $C_{21}H_{20}O_5NBr$: C, 56.51; H, 4.52; N, 3.14; Br, 17.91%.

N^{δ}-Phthalyl-β-methoxy-DL-ornithine (VIII). To a suspension of sodium azide (24.5 g, 0.377 mol) in 60 ml of dimethylformamide and 20 ml of water, a solution of VII (11.2 g, 0.025 mol) in 20 ml of dimethylformamide was added dropwise at 50 °C for 30 min. The solution was stirred at the same temperature overnight. The reaction mixture was diluted with water, saturated with sodium chloride, and then extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate and evaporated *in vacuo*. Residual oil was dissolved in a mixture of 30 ml of methanol and 2 ml of concentrated hydrochloric acid. Catalytic hydrogenation was carried out by passing a slow stream of hydrogen gas through the above solution added with 5% palladium on charcoal as a catalyst until the IR absorption band of the azide group disappeared in the reaction mixture. After filtration of catalyst, the filtrate was concentrated *in vacuo*. The residue was dissolved in water and extracted with ether several times. Aqueous layer was again concentrated *in vacuo*, and the residue was dissolved in ethanol. An addition

of ether precipitated *erythro* isomer VIIIa as hydrochloride. Recrystallization from aqueous ethanol and ether gave needles, yield 1.30 g (15.8%), mp 221 °C (decomp.).

Found: C, 50.92; H, 5.37; N, 8.55; Cl, 11.12%. Calcd for $C_{14}H_{17}O_5N_2Cl$: C, 51.15; H, 5.21; N, 8.52; Cl, 10.79%.

The filtrate of VIIIa was concentrated *in vacuo* and the residue was dissolved in a small amount of ethanol. On addition of triethylamine and then ether to the solution, precipitate of a free amino acid was formed. It was filtered and washed with ethanol. Recrystallization from aqueous ethanol gave needles of *threo* isomer VIIIb, yield 1.10 g (15.1%), mp 214—215 °C (decomp.).

Found: C, 57.43; H, 5.53; N, 9.65%. Calcd for $C_{14}H_{16}O_5N_2$: C, 57.53; H, 5.52; N, 9.59%.

erythro-*β-Hydroxy*-DL-*ornithine Hydrobromide (IXa).* A solution of VIIIa (1.00 g, 3.04 mmol) in 30 ml of 47% hydrobromic acid was heated under reflux in the presence of 15 ml of anisole for 20 hr. Hydrolyzate was extracted with benzene and aqueous layer was concentrated *in vacuo*. Crystalline residue was recrystallized from aqueous ethanol and ether, yield 0.46 g (66%). Recrystallization was repeated from aqueous ethanol for analysis, mp 230 °C (decomp.).

Found: C, 26.58; H, 5.85; N, 12.15; Br, 34.70%. Calcd for $C_5H_{13}O_3N_2Br$: C, 26.21; H, 5.72; N, 12.23; Br, 34.89%.

threo-*β-Hydroxy*-DL-*ornithine Hydrobromide (IXb).* Hydrolysis of VIIIb (1.00 g, 3.42 mmol) was similarly carried out as for the *erythro* isomer to obtain IXb, yield 0.59 g (75%). Recrystallization from aqueous ethanol gave needles, mp 110—111 °C.

Found: C, 25.47; H, 6.02; N, 11.77; Br, 33.49%. Calcd for $C_5H_{13}O_3N_2Br \cdot 1/2H_2O$: C, 25.22; H, 5.92; N, 11.77; Br, 33.56%.

cis-*5-(2-Benzyloxycarbonylaminoethyl)-2-oxazolidone-4-carboxylic Acid (Xa).* To a solution of IXa (0.28 g, 1.22 mmol) in 5 ml of 1 M sodium hydroxide, benzyloxycarbonyl chloride (0.50 g, 2.93 mmol) was added at 0 °C and the mixture was stirred for 30 min at the same temperature. After stirring at room temperature for 3 hr, the reaction mixture was extracted with ether and an aqueous layer was acidified with hydrochloric acid. Oily product formed was extracted with ethyl acetate and an organic layer was dried over anhydrous sodium sulfate. It was concentrated *in vacuo* to obtain the oily residue which was triturated with petroleum ether and then ether to yield crystals. Recrystallization from ethyl acetate and petroleum ether gave needles, yield 0.15 g (43.0%), mp 139—140 °C (decomp.). NMR: δ 4.33 (d, H-4, $J_{4-5}=9$ Hz).

Found: C, 54.63; H, 5.33; N, 8.98%. Calcd for $C_{14}H_{16}O_6N_2$: C, 54.54; H, 5.23; N, 9.09%.

trans-*5-(2-Benzyloxycarbonylaminoethyl)-2-oxazolidone-4-carboxylic Acid (Xb).* *trans*-Oxazolidone derivative Xb was obtained from IXb (1.00 g, 4.37 mmol) as in the preparation of Xa, yield 0.80 g (59.0%). It was recrystallized from ethyl acetate and petroleum ether, mp 103—105 °C. NMR: δ 4.08 (d, H-4, $J_{4-5}=5$ Hz).

Found: C, 54.24; H, 5.30; N, 9.06%. Calcd for $C_{14}H_{16}O_6N_2$: C, 54.54; H, 5.23; N, 9.09%.

N^{α},N^{δ}-*Diphthalyl*-erythro-*β-methoxy*-DL-*ornithine (XIa).* To a solution of VIIIa (1.00 g, 3.04 mmol) in methanol, triethylamine was added in excess and the mixture was concentrated *in vacuo*. Crystalline residue was suspended in anhydrous dioxane and phthalic anhydride (0.50 g, 3.38 mmol) was added. The suspension was refluxed at 130—135 °C for 30 hr, and then the clear solution was concentrated *in vacuo*. Residual oil was dissolved in sodium hydrogencarbonate solution and extracted with ethyl acetate. Aqueous layer was acidified with 6 M hydrochloric acid and extracted with ethyl acetate for several times. Ethyl acetate layer was washed with water and dried over anhydrous sodium sulfate. It was concentrated *in vacuo* and the oily residue was dissolved in a small amount of methanol. Crystals formed were filtered and recrystallized from methanol, yield 1.00 g (72.1%), mp 188—189 °C.

Found: C, 58.07; H, 4.82; N, 6.09%. Calcd for $C_{22}H_{18}O_7N_2 \cdot 2H_2O$: C, 57.64; H, 4.84; N, 6.11%.

After drying over phosphorus pentoxide at 80 °C for 36 hr under reduced pressure, elemental analysis was repeated.

Found: C, 62.13; H, 4.40; N, 6.67%. Calcd for $C_{22}H_{18}O_7N_2$: C, 62.56; H, 4.30; N, 6.63%.

N^{α},N^{δ}-*Diphthalyl*-threo-*β-methoxy*-DL-*ornithine (XIb).* The mixture of VIIIb (1.00 g, 3.42 mmol) and phthalic anhydride (0.76 g, 5.13 mmol) was heated at 150—160 °C for 1 hr. The melting product was cooled to room temperature and the residue was treated with ether. Insoluble material was recrystallized from methanol, yield 1.03 g (71.3%), mp 214—215 °C.

Found: C, 62.43; H, 4.36; N, 6.58%.

Mixed Anhydride of N^{α},N^{δ}-*Diphthalyl*-erythro-*β-methoxy*-DL-*ornithine with Ethoxycarbonic Acid (XIIIa).* To a solution of XIa (0.43 g, 1.02 mmol) in 5 ml of anhydrous dioxane and 10 ml of anhydrous ether, triethylamine (0.13 g, 1.29 mmol) and then ethoxycarbonyl chloride (0.12 g, 1.12 mmol) were added on ice cooling. After stirring for 30 min at 0 °C and then at room temperature for several hours, petroleum ether was added to the reaction mixture. Precipitate was filtered and washed rapidly with water. It was dried over phosphorus pentoxide in vacuum desiccator, yield 0.42 g (84.9%). Recrystallization from dioxane and petroleum ether gave needles, mp 160—161 °C.

Found: C, 60.50; H, 4.62; N, 5.57%. Calcd for $C_{25}H_{22}O_9N_2$: C, 60.73; H, 4.49; N, 5.67%.

Mixed Anhydride of N^{α},N^{δ}-*Diphthalyl*-threo-*β-methoxy-ornithine With Ethoxycarbonic Acid (XIIIb).* To a solution of XIb (0.84 g, 1.99 mmol) in 4 ml of dimethylformamide, triethylamine (0.23 g, 2.28 mmol) and ethoxycarbonyl chloride (0.24 g, 2.20 mmol) were added under ice cooling. After stirring at 0 °C for 1 hr, 20 ml of anhydrous ether and 100 ml of petroleum ether were added. Precipitate formed was filtered and washed rapidly with water. It was dried over phosphorus pentoxide in vacuum desiccator, yield 0.76 g (78%). Recrystallization from ethyl acetate and petroleum ether gave prisms, mp 143—144 °C.

Found: C, 60.67; H, 4.56; N, 5.74%. Calcd for $C_{25}H_{22}O_9N_2$: C, 60.73; H, 4.49; N, 5.67%.

erythro-*γ-Hydroxy*-DL-*β-lysine Lactone Dihydrobromide (XIIa).* *a)* via *Acid Chloride*: A solution of XIa (1.50 g, 3.55 mmol) in 15 ml of thionyl chloride was refluxed for 30 min. Thionyl chloride was thoroughly removed by evaporation *in vacuo* with anhydrous benzene. Residual oil was dried over sodium hydroxide in vacuum desiccator. Acid chloride thus obtained was dissolved in 70 ml of anhydrous benzene and added to a solution of diazomethane in ether on ice cooling for 2.5 hr. After stirring at room temperature for 3 hr, the reaction mixture was kept in a refrigerator. Pale yellow precipitate (0.90 g, 56%) obtained was suspended in methanol and a solution of silver benzoate (0.20 g, 0.87 mmol) in 1 ml of triethylamine was added. After stirring in the dark overnight, insoluble material was filtered off. Filtrate was concentrated *in vacuo* and oily residue was dissolved in ethyl acetate. Insoluble material formed again was filtered off and filtrate was washed with dilute hydrochloric acid and then water. Organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. Oily residue obtained was dissolved in 20 ml of 47% hydrobromic acid containing 7 ml

of anisole. It was heated under reflux for 20 hr. Aqueous layer was separated from organic layer and washed with benzene several times. Aqueous solution was concentrated *in vacuo* and the residue was crystallized from a small amount of ethanol. It was recrystallized from 94% ethanol and ether, yield 60 mg (9.7%), mp 217—219 °C (decomp.).

Found: C, 23.56; H, 4.67; N, 9.14; Br, 52.02%. Calcd for $C_6H_{14}O_2N_2Br_2$: C, 23.54; H, 4.61; N, 9.16; Br, 52.23%.

b) via *Mixed Anhydride*: A solution of XIIIa (1.07 g, 2.53 mmol) in 50 ml of anhydrous dioxane was added to a 0.1 mol solution of diazomethane in ether on ice cooling. After stirring at 0 °C for 6 hr and allowing to stand overnight at room temperature, the reaction mixture was concentrated *in vacuo* to obtain pale yellow oily residue of diazoketone in a quantitative yield. To a solution of the diazoketone in methanol, a solution of silver benzoate (0.10 g, 0.44 mmol) in triethylamine was added. Subsequent treatments were carried out as mentioned in a), yield 0.25 g (37.8%), mp 217—219 °C (decomp.).

threo-*γ-Hydroxy*-DL-*β-lysine Lactone Dihydrobromide (XIIb)*. *a)* via *Acid Chloride*: When the reaction of acid chloride of XIb and diazomethane was carried out at room temperature in a similar procedure to that in preparation of XIIa, crystalline diazoketone could not be isolated. Then the reaction mixture was warmed at 40 °C for removal of excess diazomethane and evaporated *in vacuo*. The oily residue thus obtained was immediately used for subsequent rearrangement and hydrolysis. From XIb (0.47 g, 1.11 mmol) 80 mg of *threo* isomer XIIb (23.5%) was obtained, mp 220—229 °C (decomp.).

Found: C, 23.64; H, 4.61; N, 9.19; Br, 52.12%. Calcd for $C_6H_{14}O_2N_2Br_2$: C, 23.54; H, 4.61; N, 9.16; Br, 52.23%.

b) via *Mixed Anhydride*: By mixed anhydride method as mentioned in the preparation of XIIa, *threo* isomer XIIb was obtained from XIIIb (0.65 g, 1.54 mmol), yield 0.14 g (34%). It was recrystallized from 94% ethanol and ether, mp 205—212 °C (decomp.).

N^{β},N^{ε}-*Dibenzyloxycarbonyl*-erythro-*γ-hydroxy*-DL-*β-lysine Lactone (XIV)*. To an aqueous solution of XIIa (0.11 g, 0.36 mmol), sodium carbonate (0.27 g, 2.55 mmol) and benzyloxycarbonyl chloride (0.15 g, 0.87 mmol) were added at 0 °C. After stirring at 0 °C for 30 min and then at room temperature for 4 hr, the reaction mixture was acidified with dilute hydrochloric acid. Oily product formed was extracted with ethyl acetate and organic layer was dried over anhydrous sodium sulfate. It was concentrated *in vacuo* and the residual oil was treated with petroleum ether. Finally, the oil was dissolved in ethyl acetate. Addition of petroleum ether yielded crystals, yield 95 mg (64%). Recrystallization from ethyl acetate and petroleum ether gave needles, mp 79—81 °C.

Found: C, 63.78; H, 5.89; N, 6.71%. Calcd for $C_{22}H_{24}O_6N_2$: C, 64.06; H, 5.87; N, 6.79%.

N^{β},N^{ε}-*Dibenzyloxycarbonyl*-erythro-*γ-hydroxy*-DL-*β-lysine Amide (XV)*. A solution of XIV (80 mg, 0.19 mmol) in methanol was saturated with ammonia. After keeping at room temperature in a pressure bottle for 3 days, methanol was evaporated *in vacuo* to obtain crystals, yield 80 mg (96%). Recrystallization from methanol gave needles, mp 197—198 °C.

Found: C, 61.69; H, 6.44; N, 9.69%. Calcd for $C_{22}H_{27}O_6N_3$: C, 61.52; H, 6.34; N, 9.79%.

N^{β},N^{ε}-*Dibenzyloxycarbonyl*-O-*methanesulfonyl*-erythro-*γ-hydroxy*-DL-*β-lysine Amide (XVI)*. To a suspension of XV (0.10 g, 0.23 mmol) in 2 ml of pyridine, methanesulfonyl chloride (0.10 g, 0.87 mmol) was added. After stirring overnight, the reaction mixture was poured into ice-cold water. The aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. Organic layer was washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. It was concentrated *in vacuo* and the residual oil was purified by silica gel column chromatography. Elution with chloroform gave a pure mesylate XVI, yield 60 mg (51%). It was recrystallized from ethyl acetate and petroleum ether, mp 106—107 °C.

Found: C, 54.37; H, 5.72; N, 8.49; S, 6.65%. Calcd for $C_{23}H_{29}O_8N_3S$: C, 54.42; H, 5.76; N, 8.28; S, 6.32%.

Conversion of erythro *Amide (XVI) to* threo *Lactone (XIIb)*. To a solution of XVI (30 mg, 0.06 mmol) in anhydrous ethanol, potassium acetate (0.20 g, 2.04 mmol) was added. The reaction mixture was heated under reflux for 10 hr, and then concentrated *in vacuo*. The residue was dissolved in water and extracted with ethyl acetate. Organic layer was concentrated *in vacuo* after drying over anhydrous sodium sulfate. The oily residue obtained was hydrolyzed with 6 M hydrochloric acid. Hydrolyzate was extracted with ethyl acetate and aqueous layer was evaporated *in vacuo* to obtain crystals of XIIb, yield 5 mg (39%).

References and Notes

1) Part VI: T. Wakamiya, and T. Shiba, *J. Antibiotics*, in press.

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