# *Studies on Antibiotics and Related Substances. IV.* Synthesis of* DL-*threo-β-Cyclohexylserine and* DL-*threo-2-Dichloroacetamido-1-cyclohexylpropane-1, 3-diol*

By Sumio UMEZAWA and Yoshihisa OGUMA

(Received August 23, 1956)

**Synthesis of DL-*threo*-β-cyclohexylserine and its Derivatives.**— In earlier papers[1] the reduction of E. Erlenmeyer's phenylserine[2] with Raney nickel catalyst in ethanol was found to yield DL-*threo*-2-benzylideneamino-1-phenylpropane-1, 3-diol (a Schiff base) as the main product. The present paper records the result of the reduction of the N-acetyl ester of phenylserine.

When ethyl DL-*threo*-N-acetyl-β-phenylserinate (I) was reduced with hydrogen over Raney nickel catalyst in ethanol, the product was ethyl DL-*threo*-N-acetyl-β-cyclohexylserinate (II) melting at 94–95°C. Hydrolysis of the N-acetyl derivative (II) by refluxing in dilute hydrochloric acid was followed by concentration of the solution *in vacuo* and subsequent neutralization to pH 6.8 with sodium hydroxide yielding crystalline DL-β-cyclohexylserine (III) melting at 231°C (dec.).

Confirmation of the structure of β-cyclohexylserine (III) was obtained in the following ways. III was oxidized by sodium periodate to give 62% crude yield of cyclohexanealdehyde, which was identified as the 2, 4-dinitrophenylhydrazone and did not depress the melting point of an authentic sample of 2, 4-dinitrophenylhydrazone of cyclohexanealdehyde prepared by hydrolysis and decarboxylation of ethyl cyclohexylglycidate[3]. The N-acetylcyclohexylserinate(II) was further acetylated with acetic anhydride in the presence of pyridine to yield ethyl N, O-diacetyl-β-cyclohexylserinate, m.p. 115.5–116.5°C, which, on hydrolysis under Kunz's conditions[4] (for O-acyl group), liberated two moles of carboxylic acid. As anticipated, ethyl N-acetylcyclohexylserinate liberated one mole of carboxylic acid under the same conditions. Thus, the presence of the cyclohexyl group and that of contiguous hydroxyl and amino groups in β-cyclohexylserine (III) were ascertained.

Since it is well established that the configuration of Erlenmeyer's phenylserine, the starting material, is of the *threo* configuration, it is necessary for III to be DL-*threo*-β-cyclohexylserine.

---

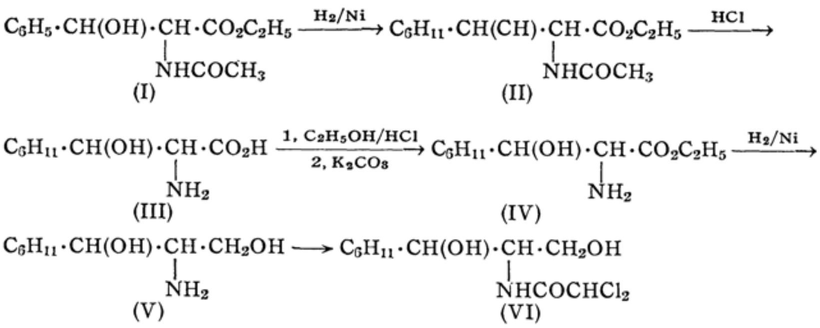
* Presented before the Division of Organic Chemistry at the Annual Meeting of the Chemical Society of Japan, at Kyoto University, April 3, 1956. Previous papers of this series: This Bulletin, 27, 477 (1954); 29, 417 (1956); 29, 979 (1956).

[1] S. Umezawa and T. Suami, This Bulletin, 27, 477 (1954); T. Suami, and S. Umezawa, ibid., 29, 979 (1956).

[2] E. Erlenmeyer, *Ber.*, 25, 3445 (1892).

[3] G. Darzens, *Compt. rend.*, 142, 214 (1906).

[4] A. Kunz and C. S. Hudson, *J. Am. Chem. Soc.*, 48, 1982 (1926); M. L. Wolfrom, M. Königsberg and S. Stolzberg, ibid., 58, 409 (1936).



$$C_6H_5\cdot CH(OH)\cdot \underset{\substack{|\\ NHCOCH_3\\ (I)}}{CH}\cdot CO_2C_2H_5 \xrightarrow{H_2/Ni} C_6H_{11}\cdot CH(CH)\cdot \underset{\substack{|\\ NHCOCH_3\\ (II)}}{CH}\cdot CO_2C_2H_5 \xrightarrow{HCl}$$

$$C_6H_{11}\cdot CH(OH)\cdot \underset{\substack{|\\ NH_2\\ (III)}}{CH}\cdot CO_2H \xrightarrow[2,\ K_2CO_3]{1,\ C_2H_5OH/HCl} C_6H_{11}\cdot CH(OH)\cdot \underset{\substack{|\\ NH_2\\ (IV)}}{CH}\cdot CO_2C_2H_5 \xrightarrow{H_2/Ni}$$

$$C_6H_{11}\cdot CH(OH)\cdot \underset{\substack{|\\ NH_2\\ (V)}}{CH}\cdot CH_2OH \longrightarrow C_6H_{11}\cdot CH(OH)\cdot \underset{\substack{|\\ NHCOCHCl_2\\ (VI)}}{CH}\cdot CH_2OH$$

**Synthesis of DL-*threo*-2-Dichloroacetamido-1-cyclohexylpropane-1, 3-diol.**—The derivatives of 2-aminopropane-1, 3-diol have received considerable attention in recent years as analogues of chloramphenicol, an important antibiotic found by scientists of Parke, Davis and Company[5]. Ashley and Davis[6] recently synthesized an isomer of 2-amino-1-cyclohexylpropane-1, 3-diol by reduction of 2-nitro-1-cyclohexylpropane-1, 3-diol which was obtained by condensation of cyclohexanealdehyde with $\beta$-nitroethanol in the presence of sodium methoxide. The dichloroacetyl derivative of this isomer of 2-amino-1-cyclohexylpropane-1, 3-diol had no antibacterial activity and it was likely that this isomer is of the *erythro* configuration.

It became of interest to us to convert the ethyl ester of the above-mentioned $\beta$-cyclohexylserine (III) into the corresponding aminoalcohol. Ethyl DL-*threo*-$\beta$-cyclohexylserinate (IV), m. p. 60–61°C, was readily prepared from the above-mentioned $\beta$-cyclohexylserine (III) by the Fischer-Speier method. The catalytic reduction* of IV with hydrogen over Raney nickel catalyst at 150 kg./cm$^2$ and 100°C for 4.5 hours gave DL-*threo*-2-amino-1-cyclohexylpropane-1, 3-diol(V), m.p. 112–113°C. Acylation of V by refluxing with methyl dichloroacetate gave DL-*threo*-2-dichloroacetamido-1-cyclohexylpropane-1, 3-diol (VI), m. p. 167–168°C. It has now been found that, though the chloramphenicol analogue possesses the *threo* configuration, it shows no significant antibacterial activity.

## Experimental

**Ethyl DL-*threo*-N-Acetyl-$\beta$-phenylserinate(I).**—Ethyl DL-*threo*-phenylserinate was prepared by treatment of Erlenmeyer's phenylserine in absolute ethanol with dry hydrogen chloride followed by liberating the aminoester by rendering alkaline with potassium carbonate, as reported by G. Carrara and G. Weitnauer[7]. The compound was very hygroscopic and decomposed upon prolonged standing.

With the aid of gentle warming, 6.0 g. of ethyl DL-*threo*-$\beta$-phenylserinate was dissolved in 6 cc. of glacial acetic acid and 2.8 cc. of acetic anhydride was added with stirring. The ethyl DL-*threo*-N-acetyl-$\beta$-phenylserinate separated as a crystalline mass and was filtered after keeping for thirty minutes, and then washed well with ether and water. The yield was 6.8 g. (91.8%) and m. p. 175–176°C. The compound was almost insoluble in ether and acetone, soluble in hot ethyl acetate and ethanol. Recrystallization from ethanol failed to raise the melting point.

*Anal.* Found: N, 5.52. Calcd. for $C_{13}H_{17}O_4N$: N, 5.57%.

**Ethyl DL-*threo*-N-Acetyl-$\beta$-cyclohexylserinate (II).**—A solution of 6.0 g. of ethyl DL-*threo*-N-acetyl-$\beta$-phenylserinate (I) in 150 cc. of absolute ethanol was shaken with 10 g. of Raney nickel catalyst[8] at 50–60°C and 130 kg./cm$^2$ for sixteen hours. The content was filtered to remove the catalyst and the solvent was removed *in vacuo*. The oily residue was dissolved in 25 cc. of ether and the solution was diluted with an equal volume of petroleumether, whereupon there separated a crystalline precipitate, which was collected after standing overnight; yield 5.3 g. (86.8%) and m. p. 83–88°C. Two recrystallizations from ether afforded 1.5 g. of pure ethyl DL-*threo*-N-acetyl-$\beta$-cyclohexylserinate (II) melting at 94–95°C. The compound liberated one mole of carboxylic acid on hydrolysis under Kunz's conditions[4].

*Anal.* Found: C, 60.96; H, 8.81; N, 5.65; saponification equivalent*, 275. Calcd. for $C_{13}H_{23}O_4N$: C, 60.68; H, 8.95; N, 5.44%; saponification equivalent*, 257.

**DL-*threo*-$\beta$-Cyclohexylserine (III).**—A mixture of 0.8 g. of ethyl DL-*threo*-N-acetyl-$\beta$-cyclohexylserinate in 16 cc. of 5% hydrochloric acid was refluxed for 2.5 hr. The solution was evaporated *in vacuo* to dryness and the resulting solid was dissolved in 10 cc. of water and the solution was neutralized to pH 6.8 by addition of 5 N sodium hydroxide. After standing overnight the cry-

---

[5] J. Ehrlich. Q. R. Bartz, R. M. Smith and A. A. Joslyn, *Science*, **106**, 417 (1947); J. Controulis. M. C. Rebstock and H. M. Crooks, Jr., *J. Am. Chem. Soc.*, **71**, 2463 (1949), and later papers.

[6] N. J. Ashley and M. Davis, *J. Chem. Soc.*, **1952**, 63.

* When this reduction was carried out at 50°C and 150 kg./cm$^2$ for seven hours, the original aminoester was recovered in a high yield.

[7] G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949); *C. A.* **44**, 7268 (1650).

[8] Organic Syntheses, **21**, 15 (1940).

* Saponified under Kunz's conditions.

stalline precipitate was separated from the solution; yield 0.42 g. (72.5%) and m. p. 223°C (dec.) Two recrystallizations from distilled water gave 0.22 g. of pure DL-*threo*-β-cyclohexylserine, m. p. 231°C (dec.).

*Anal*. Found: C, 57.50; H, 9.38; N, 7.18. Calcd. for $C_9H_{17}O_3N$: C, 57.73; H, 9.15; N, 7.48%.

**Ethyl DL-*threo*-N, O-Diacetyl-β-cyclohexylserinate.**—A mixture of 0.52 g. of ethyl DL-*threo*-N-acetyl-β-cyclohexylserinate (II), 3.0 cc. of acetic anhydride and 2 cc. of pyridine was allowed to stand overnight at room temperature. The solution was poured into about 20 cc. of ice water with stirring. After standing for 2.5 hr., a yield of 0.40 g. of crude ethyl DL-*threo*-N, O-diacetyl-β-cyclohexylserinate was collected, m. p. 113.5–114.5°C. Purification for analysis was accomplished by dissolving the product in ether with subsequent addition of petroleum ether; yield 0.16 g. and m. p. 115.5–116.5°C. The N, O-diacetyl ester liberated two moles of carboxylic acid on hydrolysis under Kunz's conditions[4], indicating the presence of an O-acetyl and a carbethoxyl group.

*Anal*. Found: C, 60.13; H, 8.19; N, 4,96; O-acetyl, 13.8%. Calcd. for $C_{15}H_{25}O_5N$: C, 60.18; H, 8.35; N, 4.68; O-acetyl, 14.4%.

**Periodate Oxidation of DL-*threo*-β-Cyclohexylserine.**—A solution of 0.65 g. of sodium periodate hydrate in 25 cc. of distilled water containing ten drops of 6 N sulfuric acid was added with stirring to a solution of 0.5 g. of DL-*threo*-β-cyclohexylserine (III) in 15 cc. of distilled water containing five drops of 6 N sulfuric acid and warmed to about 40°C. After about thirty minutes the solution gave off the characteristic smell of an aldehyde. After standing overnight the reaction mixture was extracted four times with 15 cc. of ether. The pale-yellow ethereal extract was washed in turn with 5 cc. of 1% sodium carbonate solution and 5 cc. of distilled water and dried with anhydrous sodium sulfate. Removal of ether afforded 0.18 g. (62%) of crude hexahydrobenzaldehyde.

The crude hexahydrobenzaldehyde was dissolved in 1 cc. of ethanol and distilled water was added until the solution became just turbid. Again, ethanol was added until the mixture just clarified. To the solution were added 0.2 g. of semicarbazide hydrochloride and 0.3 g. of sodium acetate. The mixture was warmed for several minutes and allowed to stand overnight at room temperature. Concentration *in vacuo* gave 0.12 g. (44%) of crude semicarbazone, m. p. 168.5–170°C. After two recrystallizations from distilled water pure hexahydrobenzaldehyde semicarbazone, m. p. 172–173°C, was obtained, undepressed on admixture with the authentic sample prepared by decarboxylation of cyclohexylglycidic acid, as described below.

To a solution of 4.0 g. of sodium hydroxide in 11.5 cc. of distilled water was added 9.5 g. of ethyl cyclohexylglycidate[3] and the mixture was stirred for 6.5 hr. at 45–50°C. After standing overnight the reaction mixture was acidified with 6 N hydrochloric acid using Congo Red as indicator and then steamdistilled for about 4.5 hr. Extraction with benzene followed by vacuum distillation gave 10 g. of hexahydrobenzaldehyde, b. p. 56°C/24 mm. The semicarbazone melted at 171–172°C after recrystallization from distilled water.

**Ethyl DL-*threo*-β-Cyclohexylserinate (IV).**—A solution of 6.3 g. of DL-*threo*-β-cyclohexylserine (III) in 65 cc. of absolute ethanol was saturated with dry hydrogen chloride and allowed to stand overnight. Concentration *in vacuo* left an oily product, which was dissolved in 35 cc. of distilled water, washed twice with 10 cc. of ethyl acetate and rendered alkaline by addition of solid potassium carbonate. Extraction with ethyl acetate followed by removal of the solvent *in vacuo* gave 6.37 g. (86.5%) of crude ethyl DL-*threo*-β-cyclohexylserinate which could not be crystallized on prolonged standing. The crude ester was used in subsequent reduction without further purification.

However, it was found that the aminoester forms a crystalline oxalate and this furnishes a method of purifing and crystallizing the aminoester.

A sample of 0.4 g. of the crystalline oxalate prepared as described below was dissolved in 8 cc. of distilled water and rendered alkaline to pH 10 with potassium carbonate and extracted five times with 8 cc. of ethyl acetate, which were combined and dried with anhydrous sodium sulfate. Removal of the solvent *in vacuo* left a syrup which crystallized after standing overnight; yield 0.31 g. and m. p. 47–52°C. Four recrystallizations from petroleumether gave 0.09 g. of pure ethyl DL-*threo*-β-cyclohexylserinate, m. p. 60–61°C.

*Anal*. Found: N, 6.55. Calcd. for $C_{11}H_{21}O_3N$: N, 6.51%.

**Oxalate of IV.**—A solution of 0.11 g. of oxalic acid hydrate in 1 cc. of absolute ethanol was added to a solution of 0.15 g. of the crude syrup of ethyl DL-*threo*-β-cyclohexylserinate (IV) in 0.5 cc. of absolute ethanol. After addition of ether followed by stirring, the mixture was allowed to stand for two hours at room temperature. Colorless minute crystals which separated, were collected and washed with ether; yield 0.12 g. and m. p. 182–185°C (dec.). Recrystallizations from ethanol-water mixture (7:3) gave the oxalate of ethyl DL-*threo*-β-cyclohexylserinate (IV), $C_6H_1{\cdot}_1CH(OH){\cdot}CHNH_2{\cdot}CO_2C_2H_5{\cdot}1/2(COOH)_2$, m. p. 181–181.5°C (dec.).

*Anal*. Found: N, 5.57. Calcd. for $C_{12}H_{22}O_5N$: N, 5.38%.

**DL-*threo*-2-Amino-1-cyclohexylpropane-1, 3-diol (V).**—A solution of 2.83 g. of ethyl DL-*threo*-β-cyclohexylserinate (a crude syrup) in 50 cc. of absolute ethanol was hydrogenated over 4 g. of Raney nickel catalyst[8] at 95–100°C for 4.5 hr. under an initial pressure of 130 kg./cm². The catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to about a fifth of its bulk, filtered from a small amount of solid impurity and the filtrate was again concentrated to give 2.97 g. of an oily product, which deposited a crystalline solid on standing overnight. Three recrystallizations from ethyl acetate gave colorless needles of DL-*threo*-2-amino-1-cyclohexylpropane-1, 3-diol; yield 0.32 g. (12.3%) and m. p. 112–113°C.

*Anal*. Found: C, 62.40; H, 10.89; N, 7.87. Calcd. for $C_9H_{19}O_2N$: C, 62.39; H, 11.05; N, 8.09%.

**DL-*threo*-2-Dichloroacetamido-1-cyclohexylpropane-1,3-diol (VI).**—A mixture of 0.13 g. of DL-

*threo*-2-amino-1-cyclohexylpropane-1, 3-diol (V) and 1.5 cc. of methyl dichloroacetate was refluxed for two hours. Removal of excess of the ester left 0.08 g. of a pale-green syrup. Washing with petroleum-ether and addition of ethyl acetate gave 0.03 g. (14%) of DL-*threo*-2-dichloroacetamido-1-cyclohexylpropane-1, 3-diol, m. p. 167–168°.

*Anal.* Found: N, 4.54. Calcd. for $C_{11}H_{19}O_3NCl_2$: N, 4.92%C.

The chloramphenicol analogue showed no significant antibacterial activity (test organism E. coli).

## Summary

(1) When the N-acetyl ethyl ester of Erlenmeyer's phenylserine was reduced with hydrogen over Raney nickel catalyst, ethyl DL-*threo*-N-acetyl-β-cyclohexylserinate was obtained in crude yields as high as 86.8 %. Subsequent hydrolysis with dilute hydrochloric acid afforded DL-*threo*-β-cyclohexylserine, which gave hexahydrobenzaldehyde by periodate oxidation.

(2) Raney nickel reduction of ethyl DL-*threo*-β-cyclohexyl-serinate afforded DL-*threo*-2-amino-1-cyclohexylpropane-1, 3-diol. The aminoalcohol was converted into DL-*threo*-2-dichloroacetamido-1-cyclohexylpropane-1, 3-diol, a chloramphenicol analogue.

The authors are indebted to Mr. S. Nakada of this Laboratory for the microanalytical data.

*Department of Applied Chemistry*
*Engineering Faculty of Keio*
*University, Tokyo*