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Chemical Synthesis of *O*-Ethyl-L-homoserine, a New Amino Acid Produced by *Corynebacterium Ethanolaminophilum*

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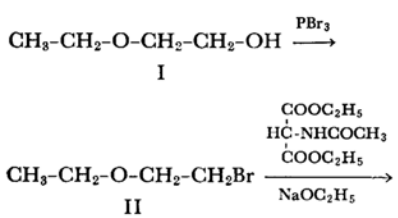
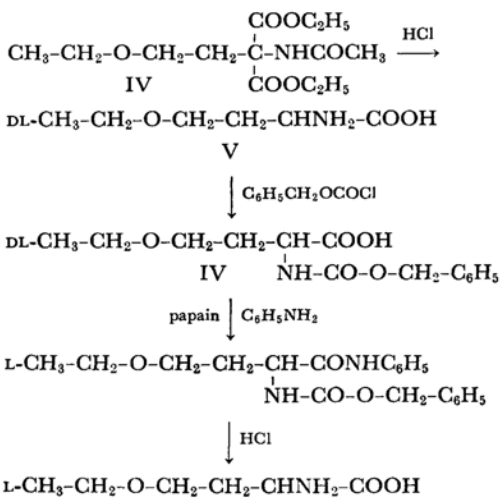
The synthesis of *O*-ethyl-DL-homoserine (V) was achieved by the condensation of β-ethoxyethylbromide (II) with diethyl acetoaminomalonate (III) and by the successive hydrolysis of diethyl ethoxyethylacetoaminomalonate (IV) with hydrochloric acid. The optical resolution of *O*-ethylhomoserine was performed enzymatically using papain. The *O*-ethyl-L-homoserine (VIII) obtained by the resolution was shown to be identical with the amino acid formed ethanol by *Corynebacterium ethanolaminophilum*, sp.nov.

In a previous paper,[1] *Corynebacterium ethanolaminophilum*, strain E17, was reported to form new amino acids when grown in a well-aerated medium containing ethanol, *n*-propanol, or *n*-butanol. The chemical properties and structures of these compounds were investigated by Murooka and Harada; the amino acids formed from ethanol, *n*-propanol, and *n*-butanol were assumed to be, respectively, *O*-ethylhomoserine (Compound E), *O*-propylhomoserine and *O*-butylhomoserine on the basis of a study of the infrared spectrum and the nuclear magnetic resonance spectrum and on the basis of an elemental analysis.

In the present paper, in an attempt to identify the structures of these compounds, the DL- and L-forms of *O*-ethylhomoserine were prepared by the process described in the following scheme (a preliminary account of part of this work has already appeared):[2]





The optical rotatory value of the L-compound (VIII) obtained was $[\alpha]_D^{30}$ −14° (*c* 2.5, water); this value was accordant with that[1] of the compound formed from ethanol by the bacterium. The infrared spectrum of the *O*-ethyl-L-homoserine (VIII) obtained by the optical resolution was identical with that of the bacterial product, while that of the racemate was rather different from that of the L-compound (Fig. 1). Thus, it was confirmed that Compound E was *O*-ethyl-L-homoserine. The syntheses of *O*-propylhomoserine and *O*-butylhomoserine are in progress.

Experimental

β-Ethoxyethylbromide (II). β-Ethoxyethanol (I) was brominated with phosphorus tribromide by the

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1) Y. Murooka and T. Harada, *Agr. Biol. Chem.*, **31**, 1035 (1967).

2) T. Harada, Y. Murooka and Y. Izumi, *Biochem. Biophys. Res. Commun.*, **28**, 485 (1967).

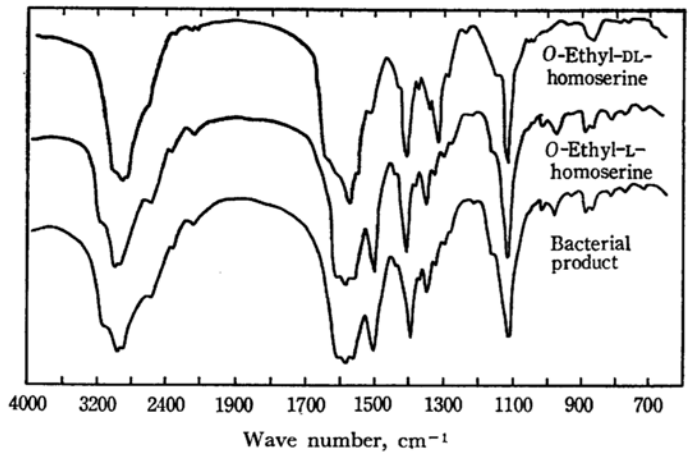

Fig. 1. Comparison of infrared spectra (in KRS-5) of bacterial product and synthetic DL- and L-*O*-ethylhomoserine.

procedure of Harrison and Diehl.[3]

***O*-Ethyl-DL-homoserine (V).** To 700 m*l* of an ethanolic solution of sodium ethylate (prepared from 30 g of sodium), 217 g of III and 1 g of potassium iodide were added. To the reaction mixture, 212 g of II were then added with heating over a 4-hr period, after which the reaction mixture was heated for a further 3 hr. The reaction mixture was removed from the sodium bromide by filtration, and the filtrate was evaporated to dryness. The residue was hydrolyzed with 1.5 *l* of 10% hydrochloric acid for 7 hr. The hydrolysate was evaporated to dryness *in vacuo* to remove the hydrochloric acid. The residue was dissolved in 300 m*l* of water and adjusted to pH 6.0 with sodium hydroxide. This solution was evaporated to dryness *in vacuo*, and the residue was extracted with 500 m*l* of 80% methanol. The solution was concentrated *in vacuo*, and the residue was dissolved with 50 m*l* of hot water. This solution was stored in a cold room, and then the crude material was collected and washed with aqueous acetone. The concentration of acetone was increased from 40% to 80%. The crystals which formed were dissolved in 100 m*l* of water, and a pure crystalline material was precipitated by the addition of 200 m*l* of acetone; yield, 95 g; mp 262°C.

Found: C, 49.25; H, 8.85; N, 9.58%. Calcd for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%.

Carbobenzoxy-*O*-ethyl-DL-homoserine (VI). Sodium hydroxide (50 g) in 100 m*l* of water and 63 g of carbobenzoxy chloride in 50 m*l* of dioxane were simultaneously added to a 400 m*l* aqueous solution of 50 g of V and 13 g of sodium hydroxide at 5°C over a period of about 3 hr. Stirring was then continued for another hour at the same temperature. The reaction mixture was washed twice with 150 m*l* of ether, and the aqueous layer was acidified to pH 3 with 5 N hydrochloric acid. The resulting oily precipitate was extracted with 300 m*l* of ethyl acetate. The ethyl acetate layer was extracted with 400 m*l* of 3.4% sodium bicarbonate solution.

The aqueous layer was acidified again and reextracted with 300 m*l* of ethyl acetate. The organic layer was washed twice with 30 m*l* of water to remove hydrochloric acid and dried over anhydrous sodium sulfate. The solution was then concentrated *in vacuo*. After the solution had been cooled in an ice bath, crystalline carbobenzoxy-*O*-ethyl-DL-homoserine appeared. The crystalline material was dissolved into 50 m*l* of benzene, after which 10 m*l* of petroleum ether were added to the solution. White, crystalline carbobenzoxy-*O*-ethylhomoserine was collected and dried in air; yield, 82 g; mp 65—66°C.

Found: C, 59.86; H, 6.77; N, 4.99%. Calcd for $C_{14}H_{19}NO_5$: C, 59.77; H, 6.81; N, 4.98%.

Carbobenzoxy-*O*-ethyl-L-homoserine Anilide (VII). The procedure was a modification of the method of Bergmann and Fraenkel-Conrat.[4]

An enzyme solution was prepared from 20 g of papaya latex by extraction with a mixture of 40 m*l* of a 0.5 M citrate buffer (pH 5.5) and 60 m*l* of water, and the suspension was filtered after incubation for one hour. Then 80 g of VI, 28 g of aniline, and 1 g of potassium cyanide were dissolved in 500 m*l* of water at 40°C. The solution was mixed with a papain-buffer solution at pH 7.0, and then the pH was adjusted to 5.5 with citric acid. After about ten minutes incubation at 38°C, synthetic anilide separated out as an oily material. It was dissolved by adding water to the incubated mixture, and the pH was readjusted to 5.5 with citric acid. After two days storage, 28 g of crude anilide separated out as crystals. These were filtered and washed with water and methanol. The crude material was recrystallized from 70 m*l* of aqueous ethanol (60%) (as white crystals); yield, 25 g; mp 105°C.

Found: C, 67.38; H, 6.83; N, 7.93%. Calcd for $C_{20}H_{24}N_2O_4$: C, 67.39; H, 6.79; N, 7.86%.

***O*-Ethyl-L-homoserine (VIII).** Twenty-five grams of VII were hydrolyzed with 10% hydrochloric acid for 7 hr. The lower layer of benzyl chloride separated off and was discarded. The upper layer was washed with ether and evaporated to dryness. The residue was dissolved in water and passed through Dowex

3) G. C. Harrison and H. Diehl, "Organic Syntheses," Coll. Vol. III, p. 370 (1955).

4) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).

50 (H^+ form). VIII was eluted with 2% aqueous ammonia from the exchanger, and the eluted solution was evaporated to dryness. The residue was recrystallized from aqueous ethanol (50%); yield, 4 g; mp 261°C. $[\alpha]_D^{30}$ −14° (*c* 2.5, water).

Found: C, 48.74; H, 9.37; N, 9.42%. Calcd for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%.

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