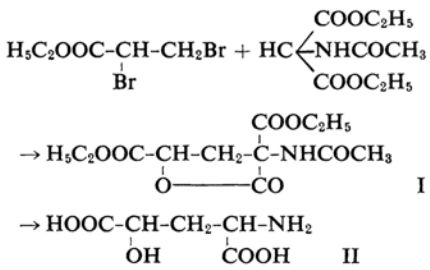
*Syntheses of γ-Hydroxyglutamic Acid**

By Takeo KANEKO, Yong Kyun LEE and Teruko HANAFUSA*1

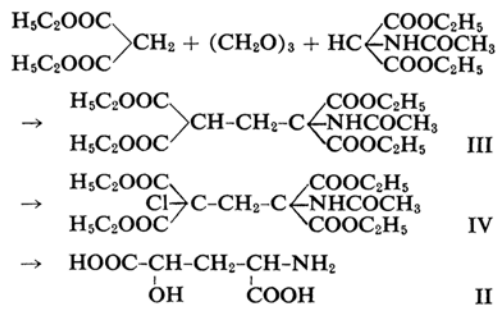
(Received September 14, 1961)

In 1955 Virtanen and Hietala[1] isolated γ-hydroxyglutamic acid from *Phlox decussata*, and Benoiton and Bouthillier[2] synthesized this hydroxyamino acid from ethyl *o*-acetylchlorolactate and ethyl acetamidocyanoacetate. Later, this synthetic method was improved by Benoiton et al.[3], who deduced the configuration of its optical isomers. Since the absolute configuration of the four optical isomers of β-hydroxyglutamic acid was determined in our laboratory[4], it has seemed to us of interest to study some of the properties of the stereo isomers of γ-hydroxyglutamic acid. First, attempts were made for a new synthesis of γ-hydroxyglutamic acid.

The hydroxyamino acid (II) was synthesized by hydrolysis of condensation product I of ethyl dibromopropionate with diethyl acetamidomalonate.



Two racemic diastereomers of II have been separated with procedures similar to those described by Benoiton et al.[3] When a crude hydrolyzate of the synthetic hydroxyamino acid was saturated with hydrogen chloride, one of the diastereomers, DL-γ-hydroxyglutamic acid (A) was precipitated asits lactone hydrochloride, m. p. 230~232°C (decomp.). From the mother liquor, another isomer, DL-γ-hydroxyglutamic acid (B), m. p. 166°C (decomp.), was obtained after treatment with pyridine and ethanol. After treatment of the amino acid A lactone hydrochloride with pyridine or silver carbonate, DL-γ-hydroxyglutamic acid (A) lactone, m. p. 245°C (decomp.), or DL-γ-hydroxyglutamic acid (A), m. p. 172~173°C (decomp.) was obtained*2.



Since the above synthesis, however, did not give a good yield, the next method was attempted. Tetraethyl α-acetamidomethylene-bismalonate (III), obtained by condensation of diethyl acetamido-malonate and diethyl malonate with paraformaldehyde according to the method of Hellmann and Lingens[5], was chlorinated with sulfuryl chloride in acetic acid to give, in a high yield, chlorinated compound IV, which was then hydrolyzed to γ-hydroxyglutamic acid with hydrochloric acid. The two diastereoisomeric DL-γ-hydroxyglutamic acids were isolated from the crude hydrolyzate in the same manner as has been described

* Studies on γ-Hydroxyglutamic Acid. I.

*1 Present address: Research Institute for Microbial Diseases, Osaka Univ., Dojima Nishimachi, Osaka.

1) A. I. Virtanen and P. K. Hietala, *Acta Chem. Scand.*, **9**, 175 (1955).

2) L. Benoiton and L. P. Bouthillier, *Can. J. Chem.*, **33**, 1473 (1955).

3) L. Benoiton, M. Winitz, S. M. Birnbaum and J. P. Greenstein, *J. Am. Chem. Soc.*, **79**, 6192 (1957).

4) T. Kaneko, R. Yoshida and H. Katsura, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **80**, 316 (1959).

*2 Presented at the 10th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1957.

5) H. Hellmann and F. Lingens, *Angew. Chem.*, **66**, 201 (1954).

above. The over-all yield from ethyl acetamido-malonate by this procedure was about 60% of the theoretical yield*3.

The solubility behaviors of the two racemates in hydrochloric acid and the melting points of *N*-benzyloxycarbonyl derivatives of the isomer A lactone and of B and its lactone were in good agreement with those reported by Benoiton et al.[3] It is clear from these facts that the two racemic modifications, A and B, correspond to the epimeric compounds (A and B)*4 obtained by the synthetic method. It failed to yield the hydrochloride of the isomer A because of its liability to form a lactone formation with hydrochloric acid.

Benzyloxycarbonyl-DL-γ-hydroxyglutamic acid (A) lactone was formed either by benzyloxycarbonylation of the lactone[3] or by treatment of benzyloxycarbonyl-DL-γ-hydroxyglutamic acid (A) with hydrochloric acid. The benzyloxycarbonyl-DL-γ-hydroxyglutamic acid (B) lactone was also obtained from the corresponding derivative of hydroxyamino acid B by treatment with hydrochloric acid.

Only benzoyl-amino acid A lactone was obtained after benzoylation of amino acid A, whereas both benzoylamino acid B and its lactone were obtained from amino acid B.

Experimental

Method 1.

α-Acetamido-α, γ-diethoxycarbonyl-γ-butyrolactone (I).—To a solution of 4.6 g. (0.2 mol.) of sodium in absolute ethanol, 43.4 g. (0.2 mol.) of ethyl acetamido-malonate were added. After evaporation of the mixture to dryness in vacuo, the residual sodium salt was suspended in anhydrous benzene, into which 54.6 g. (0.2 mol.) of ethyl dibromopropionate were stirred drop by drop at below 35°C. The reaction mixture was stirred until the solution became neutral, and then the reaction mixture was washed with water and dried. Benzene and unreacted ethyl dibromopropionate were removed by distillation, and diethyl acetamido-malonate was removed by vacuum distillation. By further distillation under 10^{-3} mmHg, an oily substance was distilled as 166~180°C with slight decomposition. Yield 35%.

Found: C, 50.38; H, 5.72; N, 4.85. Calcd. for $C_{12}H_{17}O_7N$: C, 50.17; H, 5.97; N, 4.88%.

Almost the same results were obtained from the reaction using dimethyl formamide in place of benzene.

DL-γ-Hydroxyglutamic Acid (II).—The lactone ester I was hydrolyzed and decarboxylated by boiling it with 6 N hydrochloric acid for several hours. After evaporation to dryness in vacuo, the hydrolyzate was dissolved in a minimal amount of water and the solution was saturated with hydrogen chloride to precipitate DL-γ-hydroxyglutamic acid (A) lactone hydrochloride m. p. 230°C (decomp.).

Found: C, 33.02; H, 4.73; N, 7.58; Cl, 19.74. Calcd. for $C_5H_8O_4NCl$: C, 33.07; H, 4.44; N, 7.75; Cl, 19.53%.

From the mother liquor, DL-γ-hydroxyglutamic acid (B) was obtained after treatment with pyridine and ethanol, m. p. 162~163°C (decomp.). Recrystallization from water gave m. p. 166°C. (decomp.).

Found: C, 36.62; H, 5.37; N, 8.56. Calcd. for $C_5H_9O_5N$: C, 36.81; H, 5.56; N, 8.59%.

The over-all yield from diethyl acetamido-malonate was about 20%.

Method 2.

Tetraethyl-1-acetamidopropane-1, 1, 3, 3-tetracarboxylate (III).—To 150 ml. of dried xylene, 67.2 g. (0.3 mol.) of diethyl acetamidomalonate, 67.2 g. (0.42 mol.) of diethyl malonate, 12.6 g. (0.42 mol.) of paraformaldehyde and 1.5 g. of powdered sodium hydroxide were added, and the mixture was refluxed for 7 hr. After cooling, the supernatant solution was separated from the sedimented residue by decantation, and the residue was washed twice with 20 ml. of ethyl acetate. The supernatant solution, combined with the washings, was evaporated in vacuo, and the residue was dissolved in 30 ml. of ethyl acetate. The ethyl acetate solution was washed with water, 5% sulfuric acid, water, 5% sodium bicarbonate and water in turn, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was crystallized from 30% ethanol; yield 92 g. (80%), m. p. 50~52°C. A pure sample recrystallized from 70% ethanol for elementary analysis melted at 58~59°C.

Found: C, 51.96; H, 7.14; N, 3.38. Calcd. for $C_{17}H_{27}O_9N$: C, 52.43; H, 6.99; N, 3.60%.

Tetraethyl-1-acetamido-3-chloropropane-1, 1, 3, 3-tetracarboxylate (IV).—Into a solution of 15.6 g. (0.04 mol.) of the tetracarboxylic acid ethyl ester III in 60 ml. of acetic acid, 7 g. (0.052 mol.) of sulfuryl chloride were stirred drop by drop at room temperature. Then the bath temperature was raised gradually, and the reaction mixture was heated for 1 hr. at 75°C. After the mixture had been evaporated to dryness in vacuo, the residue was dissolved in 100 ml. of ethyl acetate, washed several times with 5% aqueous sodium bicarbonate, dried, and concentrated in vacuo.

The residue was crystallized from water and triturated with 4 ml. of 40% ethanol. After standing overnight, the mixture was filtered. Yield 16 g., m. p. 83~85°C. A pure sample was obtained after recrystallization from 10 ml. of 70% ethanol. Yield 15.2 g. (90%), m. p. 91~92°C.

Found: C, 48.17; H, 6.18; N, 3.30; Cl, 8.37. Calcd. for $C_{17}H_{26}O_9NCl$: C, 48.41; H, 6.13; N, 3.03; Cl, 8.40%.

DL-γ-Hydroxyglutamic Acid (II).—The chloro compound (IV, 10 g.) was hydrolyzed and decarboxylated by refluxing with 70 ml. of concentrated hydrochloric acid for 7 hr. The hydrolyzate was evaporated to dryness in vacuo after treatment with charcoal, and the residue was redissolved in 15 ml.

*3 Reported at the 12th Annual Meeting of the Chemical Society of Japan, Kyoto, April, 1959.

*4 α-Amino and γ-hydroxyl groups of the A form have a threo type configuration in the Fischer formulation, while those of the B form have an erythro type configuration[3].

of dilute hydrochloric acid and saturated with hydrogen chloride in the cold. After standing overnight, the precipitate was filtered and washed once with concentrated hydrochloric acid and twice with acetone. Yield 1.8 g., m. p. 228~229°C (decomp.). From the mother liquor, 0.2 g. of the same product was recovered. The total yield was 2.0 g. (46.5%). Pure γ-hydroxyglutamic acid (A) lactone hydrochloride, recrystallized by saturation of the aqueous solution with hydrogen chloride, melted at 230~232°C (decomp.).

Found: C, 33.24; H, 4.67; N. 7.88; Cl, 19.50. Calcd. for $C_5H_8O_4NCl$: C, 33.07; H, 4.44; N, 7.75; Cl, 19.53%.

The amino acid A lactone hydrobromide was obtained in a yield similar to that of the hydrochloride by hydrolysis of the chlorinated compound IV with hydrobromic acid, m. p. 230~232°C (decomp.).

Found: C, 26.49; H, 3.81; N, 6.50; Br, 34.82. Calcd. for $C_5H_8O_4NBr$: C, 26.55; H, 3.54; N, 6.20; Br, 35.36%.

γ-Hydroxyglutamic acid (A) lactone was obtained from the amino acid lactone hydrochloride by treatment with pyridine. It was recrystallized from water, m. p. 245°C (decomp.).

Found: C, 41.23; H, 4.89; N, 9.43. Calcd. for $C_5H_7O_4N$: C, 41.38; H, 4.86; N, 9.65%.

After treatment with silver carbonate, the lactone hydrochloride was converted almost quantitatively to γ-hydroxyglutamic acid (A), which was recrystallized from water and ethanol to give a monohydrate, m. p. 172~173°C (decomp.).

Found: H_2O, 10.5. Calcd. for $C_5H_9O_5N \cdot H_2O$, 9.9%. Found with the anhydrous form: C, 36.78; H, 5.40; N, 8.73. Calcd. for $C_5H_9O_5N$: C, 36.81; H, 5.56; N, 8.59%.

The R_f values were 0.13 (80% phenol) and 0.10 (80% phenol containing a small amount of ammonia). Racemic amino acid A had a weak taste of L-glutamic acid.

The mother liquor, from which γ-hydroxyglutamic acid (A) lactone hydrochloride had been isolated, was evaporated to dryness in vacuo. The addition of water and evaporation to dryness was repeated several times to remove hydrochloric acid. When pyridine and ethanol were gradually added to the residue dissolved in water, a precipitate formed. Yield 1.5 g. (40%), m. p. 158°C (decomp.). A pure sample of γ-hydroxyglutamic acid (B) was obtained by recrystallization from water and ethanol. M. p. 166°C (decomp.).

Found: C, 36.74; H, 5.40; N, 8.57. Calcd. for $C_5H_9O_5N$: C, 36.81; H, 5.56; N, 8.59%.

The R_f values were identical with those of the isomeric amino acid (A). Racemic amino acid B had only an acid taste.

The over-all yield from ethyl acetamidomalonate was about 55~60%.

***N*-Benzyloxycarbonyl-DL-γ-hydroxyglutamic Acid (A) and its Lactone.**—To 36 g. (0.2 mol.) of the amino acid A dissolved in 200 ml. of 2 N sodium hydroxide, 52 g. (0.3 mol.) of benzyloxycarbonyl chloride and 110 ml. of 4 N sodium hydroxide were added simultaneously at 10°C, and the mixture was stirred for 1 hr. Then the reaction mixture was washed three times with ether. The aqueous solution was acidified with 6 N hydrochloric acid, with Congo red paper as an indicator, and extracted ten times with ethyl acetate. When the ethyl acetate extract was dried and concentrated, colorless crystals were obtained. After recrystallization from ethyl acetate, 41.6 g. of benzyloxycarbonyl-DL-γ-hydroxyglutamic acid (A) were obtained. Yield 70%, m. p. 109~110°C.

Found: C, 52.34; H, 5.24; N, 4.57. Calcd. for $C_{13}H_{15}O_7N$: C, 52.52; H, 5.09; N, 4.71%.

When the benzyloxycarbonyl-amino acid (A) was treated with 1 N hydrochloric acid, its lactone was obtained in crystalline form. This melted at 165~166°C after two recrystallizations from 1 N hydrochloric acid. No depression of the melting point was observed when it was mixed with another sample of benzyloxycarbonyl-DL-γ-hydroxyglutamic acid (A) lactone (m. p. 165~166°C; lit.[3] m. p. 168~169°C), which was obtained in a yield of 75% from DL-γ-hydroxyglutamic acid (A) lactone hydrochloride in the same manner as described above, and recrystallized from water and ethanol.

Found: C, 55.95; H, 4.85; N, 4.99. Calcd. for $C_{13}H_{13}O_6N$: C, 55.91; H, 4.70; N, 5.02%.

***N*-Benzyloxycarbonyl-DL-γ-hydroxyglutamic Acid (B) and its Lactone.**—*N*-Benzyloxycarbonyl-DL-γ-hydroxyglutamic acid (B) was obtained in a manner similar to that reported by Benoiton et al.[3] M. p. 132~133°C (decomp.) (lit.[3] 132~133°C).

Found: C, 52.53; H, 5.12; N, 4.58. Calcd. for $C_{13}H_{15}O_7N$: C, 52.52; H, 5.09; N, 4.71%

The above *N*-benzyloxycarbonyl-DL-amino acid (B) was converted to its lactone by recrystallization using 1 N hydrochloric acid, m. p. 152~153.5°C (decomp.) (lit.[3] m. p. 152~153°C).

Found: C, 55.55; H, 4.84; N, 4.89. Calcd. for $C_{13}H_{13}O_6N$: C, 55.91; H, 4.70; N, 5.02%.

Benzoyl-DL-γ-hydroxyglutamic Acid (A) Lactone.—To a solution of 3.6 g. (0.02 mol.) of DL-γ-hydroxyglutamic acid (A) lactone hydrochloride and 10 g. of sodium bicarbonate in 100 ml. of water, 4.2 g. (0.03 mol.) of benzoyl chloride were added drop by drop at 10°C; the reaction mixture was then stirred vigorously for one and a half hours at 10~15°C. Then the solution was acidified slightly with 6 N hydrochloric acid, using Congo red paper, and the benzoic acid which precipitated was filtered off. The filtrate was washed twice with ether, and the aqueous layer was evaporated to dryness in vacuo. To the residue dissolved in 10 ml. of water, 4~5 drops of concentrated hydrochloric acid were added, and the precipitate formed was filtered and washed with water. A crude sample of benzoyl-DL-γ-hydroxyglutamic acid (A) lactone was obtained by recrystallization of the precipitate from ethanol; yield 3.1 g. (62%). For elementary analysis, it was further recrystallized from ethanol. m. p. 230~232°C.

Found: C, 58.03; H, 4.61; N, 5.71. Calcd. for $C_{12}H_{11}O_5N$: C, 57.83; H, 4.45; N, 5.62%.

Benzoyl-DL-γ-hydroxyglutamic Acid (B) and its Lactone.—Benzoylation of DL-γ-hydroxyglutamic acid (B) was carried out in the same manner as described above from 9.8 g. (0.06 mol.) of DL-γ-hydroxyglutamic acid (B), 17 g. (0.12 mol.) of

benzoyl chloride, 35 g. of sodium bicarbonate and 200 ml. of water. A crude sample of the benzoyl-amino acid (B) was recrystallized from water. Yield, 11 g. (68%), m. p. 145°C (decomp.).

Found: C, 53.80; H, 5.02; N, 5.44. Calcd. for $C_{12}H_{13}O_6N$: C, 53.93; H, 4.87; N, 5.24%.

When concentrated hydrochloric acid was added to an aqueous solution of benzoyl-amino acid (B) and heated for a short time, its lactone was deposited. A pure sample of benzoyl-DL-γ-hydroxyglutamic acid (B) lactone was obtained after recrystallization from hot water; m. p. 202~203°C (decomp.).

Found: C, 57.42; H, 4.40; N, 5.49. Calcd. for $C_{12}H_{11}O_5N$: C, 57.83; H, 4.45; N, 5.62%.

The authors wish to express their thanks to Mr. Masakazu Okumiya and Miss Kiku Koike for their microanalyses.

Department of Chemistry
Faculty of Science
Osaka University
Nakanoshima, Osaka