Optical Resolution and Stereochemistry of γ-Hydroxyglutamic Acid[1]

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Four optical isomers of γ-hydroxyglutamic acid were prepared in pure states by optical resolution of two racemic diastereoisomers through brucine salt of *N*-benzyloxycarbonyl amino acid or strychnine salt of *N*-benzoyl derivative. Configurations of α- and γ-asymmetric carbon atoms of these isomers were estimated from contribution to molecular rotation and character of taste exhibition. These determinations were confirmed by chemical degradations to aspartic acid and malic diamide of the definite configurations. Cyclization of each racemic diastereomer to either lactone or lactam was also investigated.

γ-Hydroxyglutamic acid has been found in plants, *e.g., Phlox decussata*,[2] *Hemerocallis* species,[3] and *Linaria vulgaris*.[4] Occurrence of this amino acid was also reported as an intermediate in hydroxyproline metabolism,[5] and in bovine gallbladder bile as *N*-terminal residue in heptapeptide.[6] In the previous paper,[7] the authors devised a new method to synthesize this amino acid and reported a separation procedure of the resulting mixture of two diastereoisomers. Greenstein and Benoiton demonstrated an enzymic resolution of two diastereoisomers of this amino acid and estimated steric configurations of these isomers indirectly based on the stereochemistry of hydroxy acid derived from the amino acid.[8] In this paper, we present an optical resolution of both racemic diastereoisomers of γ-hydroxyglutamic acid by chemical method *via* salt formation with brucine or strychnine and an unambiguous determination of stereochemistry for each optical isomer through direct degradation to the compounds of established configurations. In addition, we now report some other properties of this amino acid, for instance, one example of the relationship of a stereostructure of amino acid with its taste, and also a trend of cyclization for each diastereomeric isomer to lactone or lactam.

For the optical resolution of one diastereoisomer A of γ-hydroxyglutamic acid with the higher melting point of 172—173 °C (decomp.),[7] it was converted to several *N*-acyl derivatives and its salt formation with various resolution reagents was tested. The best result was obtained in the case of brucine salt with *N*-benzyloxycarbonyl amino acid. Thus, one optical isomer (−)-A was obtained by acid hydrolysis of a sparingly soluble salt and the other isomer (+)-A from more soluble salt, both in optically pure states.

The resolution of another diastereoisomer B with the lower melting point of 166 °C (decomp.)[7] was carried out by fractional crystallization of strychnine salt with *N*-benzoyl amino acid. Optical isomers (−)-B and (+)-B were secured from a sparingly soluble salt and a non-crystalline soluble salt respectively.

TABLE 1. PROPERTIES OF OPTICAL ISOMERS OF γ-HYDROXYGLUTAMIC ACID

	Mp (°C) (decomp.)	$[\alpha]_D$ (°)	Concentration (%)	Solvent	Taste
L(−)-A	172—173	−12.5	2.0	H_2O	Strongly tasty
		+3.0	5.0	20% HCl	
D(+)-A	172—173	+12.7	3.0	H_2O	Tasteless
		−3.2	5.0	20% HCl	
D(−)-B	166	−19.5	2.0	H_2O	Tasteless
		−37.3	1.5	20% HCl	
L(+)-B	166	+20.0	2.0	H_2O	Weakly tasty
		+38.0	1.5	20% HCl	

Physical and physiological properties of the four optical isomers of γ-hydroxyglutamic acid thus obtained were summarized in Table 1. The taste of one isomer (−)-A is resembled to that of L-glutamic acid but with milder nuance and weaker strength. If this amino acid is obeyed to the empirical rule concerning with the relationship of stereochemistry of amino acid with taste exhibition which had been originally found by one of the authors,[9] configuration of α-asymmetric carbon atom of both isomers (−)-A and (+)-B exhibiting the meaty taste, should belong to L-amino acid series and that of the other tasteless isomers (+)-A and (−)-B to D-series. It is also observed that the presence of γ-hydroxy group in this amino acid effects the strength of taste as in the case of β-hydroxyglutamic acid.[10]

The molecular rotations of the four isomers both in 20% hydrochloric acid and aqueous solution are listed

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1) This work was partly presented at the 12th Annual Meeting of the Chemical Society of Japan, Kyoto, April, 1959.

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Table 2. Molecular rotation and calculated contribution of α-carbon atom of four stereoisomers of γ-hydroxyglutamic acid

Isomer	$[M]_D$		$[M]_{HCl}-[M]_{H_2O}$	α[a]		$\alpha_{HCl}-\alpha_{H_2O}$
	20% HCl	H_2O		20% HCl	H_2O	
(−)-A	+4.9	−20.4	+25.3	+33.5	+6.1	+27.4
(+)-A	−5.2	+20.7	−25.9	−33.1	−5.6	−27.5
(−)-B	−60.9	−31.8	−29.1	−33.1	−5.6	−27.5
(+)-B	+62.0	+32.6	+29.4	+33.5	+6.1	+27.4

a) $\alpha=1/2([M]_{\mp A}+[M]_{\pm B})$

in Table 2. It was noticed that, in the isomers (−)-A and (+)-B, a difference of two values in hydrochloric acid and aqueous solution was positive and that in (+)-A and (−)-B was negative. Furthermore, the same tendencies were recognized at the calculated values of contribution of α-carbon atoms to molecular rotation for each isomer. These facts may indicate that, according to Lutz and Jirgensons' rule,[11] the configuration of α-carbon atoms of (−)-A and (+)-B shlould be L-form and (+)-A and (−)-B must have D-configuration at α-carbon atoms.

In order to confirm the above estimation, one optical isomer of γ-hydroxyglutamic acid was degradated to aspartic acid by the following chemical procedure. Thus, *N*-benzyloxycarbonyl-(−)-A was oxidized with potassium permanganate followed by hydrolysis with 20% hydrochloric acid to yield aspartic acid which was identified with L-isomer from observed optical rotation value $[\alpha]_D^{15}$ +25.9° in hydrochloric acid. From this result, L-configuration of α-carbon atom of (−)-A was established in accordance with the deduction from the molecular rotation values and taste exhibition. Similarly, *N*-benzoyl-(−)-B was oxidized to give D-aspartic acid, indicating that its α-carbon atom possesses D-configuration.

For determination of the configuration of γ-carbon atom of this amino acid, one isomer (+)-A was oxidized with chloramine T to β-formyllactic acid which was then further oxidized to malic acid with bromine in a similar way to the degradation of β-hydroxyglutamic acid[12] as shown in Fig. 1. A specific rotation of diamide prepared from malic acid thus obtained was measured to be $[\alpha]_D^{18}$ +59.0° in methanol. This value corresponds to (R)-malic diamide of $[\alpha]_D^{18}$ +60.8°. Similarly, the same compound derived from (+)-B showed a specific rotation of $[\alpha]_D^{17}$ +60.0° in methanol. Therefore, it is concluded that γ-carbon atoms of (+)-A and (+)-B possess R-configuration and those of (−)-A and (−)-B belong to S-series.

Steric configurations of four optical isomers of γ-hydroxyglutamic acid deduced from the results obtained above are summarized and represented by Fischer's projection formula in Fig. 2. This conclusion coincides with the conclusion by Greenstein and Benoiton.[8]

If γ-hydroxyglutamic acid is cyclized either to lactone or to lactam, steric relationships of *cis-trans* isomerism

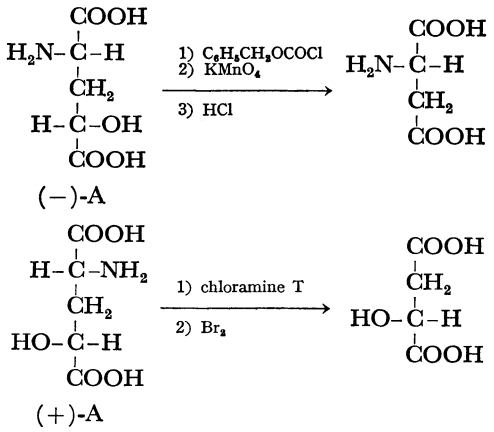

Fig. 1. Oxydative degradations of stereoisomers of γ-hydroxyglutamic acid.

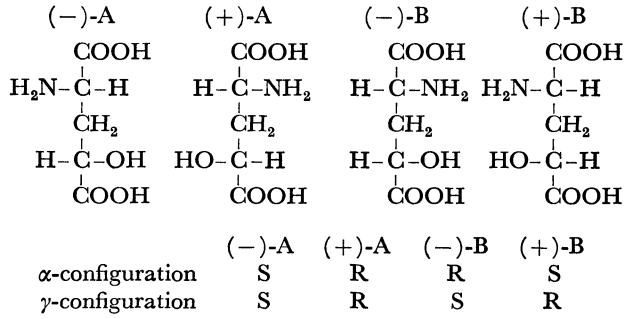

Fig. 2. Steric configurations of four optical isomers of γ-hydroxyglutamic acid.

concerning the ring planes will be resulted as shown in Fig. 3, each isomer corresponding to the four optical isomer mentioned in Fig. 2 respectively. In the lactone I and the lactam III, both derived from the isomer A, two substituents on the ring planes located each other at *cis* positions, while those in lactone II and lactam IV derived from the isomer B are at *trans* positions. It has been known that *allo*-γ-hydroxyproline, in which hydroxy and carboxy group are positioned at *cis* direction on pyrrolidine ring, can form bicyclic compound by lactonization. Since the lactams III and IV of γ-hydroxyglutamic acid can be considered as δ-oxo-γ-hydroxyproline, a relationship of stereochemistry of γ-hydroxyglutamic acid with cyclization seemed to be worthy of further investigation.

In fact, the racemic isomer A gave the lactone I hydrochloride which was easily crystallized.[7] When this hydrochloride was treated with pyridine, or the compound A was attempted to be esterified, a free lactone I was readily obtained.[7] On the contrary a

11) D. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930), **64**, 1221 (1931).

12) C. R. Harington and S. S. Randall, *Biochem. J.*, **25**, 1917 (1931).

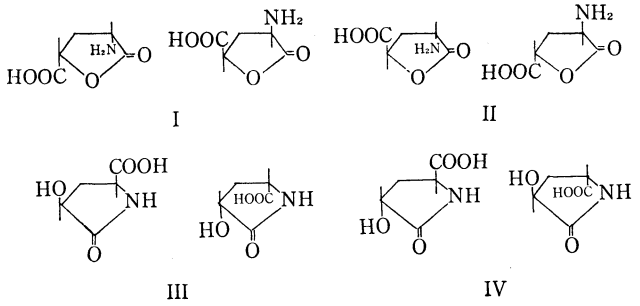

Fig. 3. Steric configurations of lactones and lactams derived from four optical isomers of γ-hydroxyglutamic acid.

free lactone II could not be obtained by the similar procedure from another racemic isomer B. In this case, esterification of the compound B gave mono- and diester hydrochloride, the latter of which could be converted to γ-hydroxyglutamic acid lactam ester with sodium hydrogencarbonate. The fact that isomer A can be easily lactonized compared with isomer B as demonstrated above, may be related to the resulting structure in the former lactone where amino and γ-carboxy groups in *cis* position could be interacted each other to stabilize the ring structure.

We next attempted to form the bicyclic compound of γ-hydroxyglutamic acid as shown in Fig. 4. The racemic isomer A was first converted to *N*-benzyloxycarbonyl derivative V which was readily cyclized to the lactone carboxylic acid VI with hydrochloric acid.[7] Methyl esterification of VI gave monomethyl ester VII and dimethyl ester VIII. When the lactone monoester VII was hydrogenated in dioxane using palladium

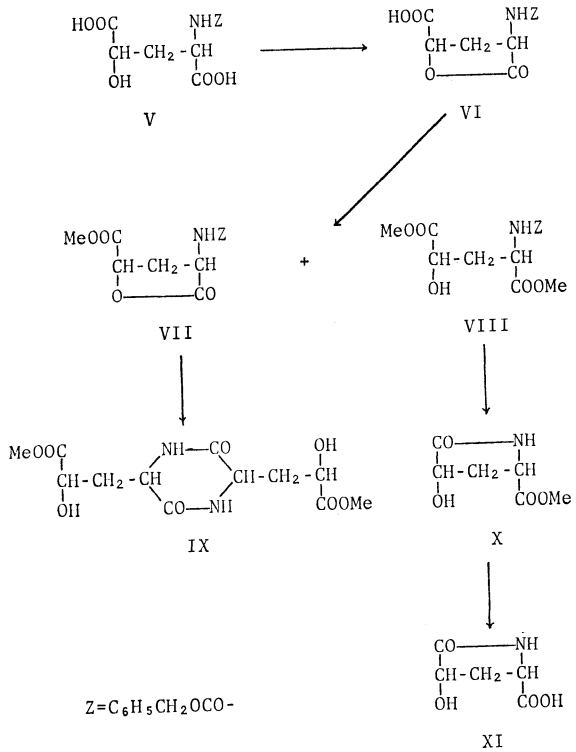

Fig. 4. Scheme for attempt at formation of bicyclic compound of γ-hydroxyglutamic acid.

black, a diketopiperazine IX was obtained unexpectedly. A similar diketopiperazine was also obtained from *N*-benzyloxycarbonyl-γ-hydroxy-DL-glutamic acid B lactone monomethyl ester. Hydrogenolysis of the diester VIII in aqueous methanol afforded directly a lactam ester X which was then saponified to yield a lactam carboxylic acid XI. Any trials for further lactonization of the lactam XI to make the bicyclic compound has not been succeeded.

Experimental

All melting points are uncorrected. Elemental analyses were carried out by Mr. Masakazu Okumiya and Miss Kiku Koike in our Department. For paper chromatography on Toyo paper No. 51, a developing solvent of *n*-butanol–acetic acid–water (4: 1: 2 v/v) was used.

Resolution of N-*Benzyloxycarbonyl Derivative of One Diastereoisomer A of γ-Hydroxy-*DL-*glutamic Acid.* To a solution of *N*-benzyloxycarbonyl derivative (25 g) of a racemic diastereoisomer A hydrate of γ-hydroxyglutamic acid with mp 172—173 °C (decomp.)[7] in 95% ethanol (350 ml), brucine (67 g) was added. The mixture was heated for 10 min to obtain the complete dissolution and then evaporated *in vacuo*. Water (30 ml) was added to the residue and evaporation was repeated twice to remove ethanol completely. Residual syrup was dissolved in water (600 ml) and kept in a refrigerator overnight to give crude crystals (45 g). The crystals were filtered and recrystallized three times from water (200 ml each) to give pure brucine salt (38 g). The salt was dissolved in water (600 ml) and decomposed by addition of 1 M sodium hydroxide to phenolphthalein alkaline on cooling. Crystals of brucine deposited were removed by filtration. The filtrate was neutralized with 6 M hydrochloric acid and concentrated under reduced pressure to 200 ml.

The solution was acidified to Congo red with 6 M hydrochloric acid and extracted with ethyl acetate repeatedly. The combined extract was dried over anhydrous sodium sulfate and concentrated to afford crystals of *N*-benzyloxycarbonyl-γ-hydroxy-L(−)-glutamic acid. This was recrystallized from ethyl acetate, yield, 8 g (66%), mp 124—125 °C (decomp.), $[\alpha]_D^{20}$ −18.0° (*c* 3.3, 99% ethanol).

Found: C, 52.62; H, 5.36; N, 4.65%. Calcd for $C_{13}H_{15}O_7N$: C, 52.52; H, 5.09; N, 4.71%.

The mother liquor from crystals of the crude brucine salt mentioned above was concentrated to about 50 ml. Crystals deposited were removed by filtration. The filtrate was further concentrated and kept at room temperature for a few days. After removal of a small amount of crystals by filtration, the mother liquor was decomposed with aqueous sodium hydroxide as mentioned above to give *N*-benzyloxylcarbonyl-γ-hydroxy-D(+)-glutamic acid, yield, 6 g (50%), mp 124—125 °C (decomp.), $[\alpha]_D^{15}$ +18.0° (*c* 3.0, 99% ethanol).

Found: C, 52.61; H, 5.21; N, 4.68%.

*γ-Hydroxy-*L(−)*-glutamic acid.* *N*-Benzyloxycarbonyl-γ-hydroxy-L(−)-glutamic acid (2.5 g) obtained above was refluxed with 20% hydrochloric acid (25 ml) for 4 hr. The solution was evaporated *in vacuo*. Water was added to the residue and evaporation was repeated. Finally, the residue was dissolved in water and treated with silver carbonate to remove hydrochloric acid. The filtrate was again evaporated and the residue was crystallized from water and ethanol. Recrystallization from water gave pure γ-hydroxy-L(−)-glutamic acid, yield, 1 g, mp 172—173 °C (decomp.), $[\alpha]_D^{10}$ −12.5° (*c* 2.0, water), $[\alpha]_D^{10}$ +3.0° (*c* 5.0, 20% hydrochloric acid).

Found: C, 36.96; H, 5.52; N, 8.53%. Calcd for $C_5H_9O_5N$: C, 36.81; H, 5.56; N, 8.59%.

*γ-Hydroxy-*D(+)*-glutamic Acid.* In a similar manner to the above experiment, γ-hydroxy-D(+)-glutamic acid was obtained from its *N*-benzyloxycarbonyl derivative, mp 172—173 °C (decomp.), $[\alpha]_D^{10}$ +12.7° (*c* 3.0, water), $[\alpha]_D^{10}$ −3.2° (*c* 5.0, 20% hydrochloric acid).

Found: C, 36.96; H, 5.58; N, 8.56%.

Resolution of N*-Benzoyl Derivative of One Diastereoisomer B of γ-Hydroxy-*DL*-glutamic Acid.* To an aqueous solution (300 ml) of *N*-benzoyl derivative (18 g) of one racemic diastereoisomer B of γ-hydroxyglutamic acid with mp 166 °C (decomp.),[7] strychnine (47 g) was added on warming. Insoluble material was filtered off while hot and the filtrate was evaporated *in vacuo*. To the residue, ethanol (30 ml) was added, and evaporation was repeated twice. To the residual syrup, 95% ethanol (200 ml) was added and the mixture was kept in a refrigerator overnight to yield crystals of crude salt (29 g). This was recrystallized from 95% ethanol (100 ml) three times to give pure salt (22 g). The strychnine salt was dissolved in water (600 ml) and then decomposed with 1 M sodium hydroxide. Strychnine deposited was filtered off and the filtrate was acidified with 20% hydrochloric acid to Congo red. It was evaporated *in vacuo* to give *N*-benzoyl-γ-hydroxy-D(+)-glutamic acid lactone, yield, 6 g (66%), mp 224—225 °C, $[\alpha]^{15}$ +70.8° (*c* 1.3, 99% ethanol).

Found: C, 57.95; H, 4.61; N, 5.55%. Calcd for $C_{12}H_{11}O_5N$: C, 57.83; H, 4.45; N, 5.62%.

The mother liquor from the above crystalline strychnine salt was evaporated to remove the salt of D(+)-lactone deposited. The residual syrup was treated as above to give crude crystals of *N*-benzoyl-L(−)-lactone. They were recrystallized from 1 M hydrochloric acid, mp 224—225 °C, $[\alpha]_D^{10}$ −71.3° (*c* 1.5, 99% ethanol).

Found: C, 57.79; H, 4.38; N, 5.54%.

*γ-Hydroxy-*D(−)*-glutamic Acid.* *N*-Benzoyl-D(+)-lactone (2.3 g) obtained above was dissolved in 20% hydrochloric acid (25 ml) and refluxed for 4 hr. The solution was evaporated *in vacuo*. Evaporation after addition of water was repeated. The residue thus obtained was dissolved in a little water and treated with pyridine and ethanol to give crude crystals of γ-hydroxy-D(−)-glutamic acid. Recrystallization from water afforded pure crystals, yield, 1 g, mp 166 °C (decomp.), $[\alpha]_D^{15}$ −19.5° (*c* 2.0, water), $[\alpha]_D^{15}$ −37.3° (*c* 1.5, 20% hydrochloric acid).

Found: C, 36.70; H, 5.60; N, 8.58%. Calcd for $C_5H_9O_5N$: C, 36.81; H, 5.56; N, 8.59%.

*γ-Hydroxy-*L(+)*-glutamic Acid.* In a similar procedure to that of D(−)-amino acid, γ-hydroxy-L(+)-glutamic acid was obtained from *N*-benzoyl-L(−)-lactone, mp 166 °C (decomp.), $[\alpha]_D^{15}$ +20.0° (*c* 2.0, water), $[\alpha]_D^{15}$ +38.0° (*c* 1.5, 20% hydrochloric acid).

Found: C, 36.59; H, 5.70; N, 8.66%.

Determination of Configuration at α-Carbon Atom of γ-Hydroxyglutamic Acid. (i) Oxidation of N*-Benzyloxycarbonyl-γ-hydroxy-*L(−)*-glutamic Acid.* A derivative of one optical isomer (−)-A, *i.e.*, *N*-benzyloxycarbonyl-γ-hydroxy-L(−)-glutamic acid (1 g) was dissolved in water (50 ml). The solution was added dropwise into an aqueous solution (30 ml) of potassium permanganate (0.8 g) at 25—30 °C with stirring. The stirring was continued for additional 10 min, and then filtered. The filtrate was acidified with 6 M hydrochloric acid (3 ml) and then evaporated. The residue was dissolved in 6 M hydrochloric acid (15 ml) and refluxed for 3 hr. After cooling, the solution was washed with ether, and an aqueous layer was evaporated *in vacuo*. The residue was treated with ethanol to remove potassium chloride by filtration. The filtrate was evaporated again and the residue thus obtained was crystallized from pyridine and ethanol. Recrystallization from water gave crystals of L-aspartic acid. This sample was identified with authentic L-aspartic acid on paperchromatogram, yield, 200 mg, $[\alpha]_D^{15}$ +25.9° (*c* 1.7, 10% hydrochloric acid). Lit.,[13] $[\alpha]_D^{20}$ +25.8° (*c* 2, 6 M hydrochloric acid).

Found: C, 36.42; H, 5.41; N, 10.65%. Calcd for $C_4H_7O_4N$: C, 36.09; H, 5.30; N, 10.52%.

(ii) Oxidation of N*-Benzoyl-γ-hydroxy-*D(+)*-glutamic Acid Lactone.* Permanganate oxidation of *N*-benzoyl derivative of the isomer (−)-B gave D-aspartic acid in a similar procedure to that in (i). On paperchromatogram, the product was identified with the authentic aspartic acid. $[\alpha]_D^{15}$ −25.0° (*c* 1.0, 10% hydrochloric acid).

*Determination of Configuration at γ-Carbon Atom of γ-Hydroxyglutamic Acid. (i) Oxidation of γ-Hydroxy-*D(+)*-glutamic Acid.* One optical active isomer (+)-A, *i.e.*, γ-hydroxy-D(+)-glutamic acid (2.2 g) was dissolved in 1 M hydrochloric acid (15 ml). The solution was made slightly alkaline by addition of 5% aqueous sodium hydroxide on cooling, and aqueous solution (15 ml) of chloramin T (4.2 g) was added. The reaction mixture was warmed at 60 °C for 2 hr. *p*-Tosylamide deposited on cooling was filtered off. Combined filtrate and washings were extracted with ethyl ether and the aqueous layer was acidified with hydrochloric acid. Bromine water prepared from 5 g of bromine and 100 ml of water was added to the solution and the mixture was kept at room temperature for 48 hr until the bromine color almost disappeared. Excess bromine was decomposed by addition of aqueous sodium thiosulfate. The solution was concentrated *in vacuo* to 30 ml, and extracted with ethyl ether. After removal of ether from the extract, the residue was dissolved in methanol and esterified with diazomethane in the usual manner. The product was purified by distillation and a fraction of bp 100—110 °C/6 mmHg was collected (0.7 g). A solution of this dimethyl ester in methanol (30 ml) was saturated with ammonia, and kept at room temperature in a sealed tube for 4 days. The solvent was evaporated to yield crude R(+)-malic diamide (0.3 g, 17%). It was recrystallized from methanol, mp 157—158 °C, $[\alpha]_D^{15}$ +59.0° (*c* 1.0, methanol).

Found: C, 36.60; H, 6.28; N, 21.20%. Calcd for $C_4H_8O_3N_2$: C, 36.36; H, 6.10; N, 21.20%.

*(ii) Oxidation of γ-Hydroxy-*L(+)*-glutamic Acid.* From one optical isomer (+)-B, *i.e.*, γ-hydroxy-L(+)-glutamic acid, R(+)-malic diamide was obtained by the similar procedure to that in (i), mp 157—158 °C, $[\alpha]_D^{17}$ +60.0° (*c* 1.0, methanol).

Found: C, 36.43; H, 6.14; N, 21.37%.

*γ-Hydroxy-*DL*-glutamic Acid B Lactone Hydrochloride.* The racemic isomer B was dissolved in a small amount of concentrated hydrochloric acid and then kept over phosphorus pentoxide in a vacuum desiccator to yield needles of the hydrochloride, mp 187—189 °C (decomp.).

Found: C, 30.04; H, 5.05; N, 6.83; Cl, 17.27%. Calcd for $C_5H_{10}O_5NCl$: C, 30.08; H, 5.05; N, 7.02; Cl, 17.48%.

*γ-Hydroxy-*DL*-glutamic Acid B Mono- and Diethyl Ester Hydrochloride.* To a suspension of the racemic isomer B (2 g) in anhydrous ethanol (50 ml), dry hydrogen chloride was saturated. The mixture was kept at room temperature overnight and then concentrated. To the residue, a small amount of acetone was added to yield crystals. Recrystallization from ethanol and acetone gave pure monoester, yield, 1.6 g (57%), mp 168 °C (decomp.).

13) T. Kaneko and H. Katsura, "Chemistry of Proteins," Vol. 1, Kyoritsu-Shuppan, Tokyo (1969), p. 113.

Found: C, 37.06; H, 6.20; N, 6.36; Cl, 16.14%. Calcd for $C_7H_{14}O_5NCl$: C, 36.93; H, 6.20; N, 6.15; Cl, 15.58%.

The mother liquor from crystals of the monoester was evaporated. The residue was crystallized to afford diester, yield, 0.8 g (26%), mp 90—95 °C (decomp.).

Found: C, 41.13; H, 6.99; N, 5.46; Cl, 14.59%. Calcd for $C_9H_{18}O_5NCl$: C, 42.28; H, 7.10; N, 5.48; Cl, 13.87%.

*γ-Hydroxy-*DL*-glutamic Acid B Lactam Ethyl Ester.* The diethyl ester (1.5 g) obtained above was dissolved in water (15 ml), and sodium hydrogencarbonate (0.6 g) was added. The mixture was extracted with ethyl acetate five times. The combined extract was dried with sodium sulfate and then concentrated to a syrup. It was crystallized from ethyl acetate and petroleum ether and then recrystallized from the same solvents, yield, 0.4 g (40%), mp 70—73 °C.

Found: C, 48.30; H, 6.52; N, 7.82%. Calcd for $C_7H_{11}O_4N$: C, 48.35; H, 6.40; N, 8.09%.

N-*Benzyloxycarbonyl-γ-hydroxy-*DL*-glutamic Acid A Lactone Methyl Ester (VII).* To a solution of *N*-benzyloxycarbonyl-γ-hydroxy-DL-glutamic acid A lactone (VI)[7] (2 g) in methanol (5 ml), ethyl ether solution of diazomethane was added at 0 °C until yellow color remained. The solvent was removed under reduced pressure to deposite crystals. They were recrystallized from 70% aqueous methanol, yield, 1.4 g (70%), mp 106.5—108 °C.

Found: C, 57.82; H, 5.23; N, 4.71%. Calcd for $C_{14}H_{15}O_6N$: C, 57.33; H, 5.16; N, 4.78%.

*γ-Hydroxy-*DL*-glutamic Acid A Lactam Methyl Ester (X).* When the esterification of VI (15 g) in methanol (35 g) was carried out using excess diazomethane in the above experiment, a syrup of the expected *N*-benzyloxycarbonyl-γ-hydroxy-DL-glutamic acid A dimethyl ester (VIII) was obtained. Through a solution of the syrup thus obtained in a mixture of methanol (100 ml) and water (50 ml), hydrogen was passed at 45 °C in the presence of palladium black (500 mg). The catalyst was removed by filtration and the solvent was removed to give crystals. Recrystallization from water gave crystals with 1 mol of crystallization water, yield, 4 g (47%), mp 70.5—71.5 °C. After drying at the refluxing temperature of acetone for 15 hr, the melting point rised up to 109—110 °C.

Found: C, 45.26; H, 5.69; N, 8.68%. Calcd for $C_6H_9O_4N$: C, 45.28; H, 5.70; N, 8.80%.

*γ-Hydroxy-*DL*-glutamic Acid A Lactam (XI).* The lactam methyl ester X (2.5 g) was dissolved in 5% aqueous sodium hydroxide (1.5 ml) and allowed to stand at room temperature overnight. The solution was passed through Dowex-50 (H^+ form) column, and the acidic eluate was concentrated *in vacuo* to deposit crystals, yield, 1.4 g (61%), mp 182 °C (decomp.).

Found: C, 41.25; H, 4.94; N, 9.46%. Calcd for $C_5H_7O_4N$: C, 41.38; H, 4.86; N, 9.65%.

*Diketopiperazine (IX) of γ-Hydroxy-*DL*-glutamic Acid A Methyl Ester.* Into a solution of VII (1 g) in anhydrous dioxane (40 ml), hydrogen was passed in the presence of palladium black (300 mg). After the hydrogenation had been finished, the catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to give crystals. They were recrystallized twice from dioxane and then from water, yield, 0.8 g (73%), mp 192 °C (decomp.); ν_{max} 3400, 1675 cm^{-1}. A diketopiperazine structure was deduced for this product from its IR spectrum and elemental analyses in addition to the observation of negative ninhydrin test.

Found: C, 45.32; H, 5.89; N, 8.89%. Calcd for $C_{12}H_{18}O_8N_2$: C, 45.28; H, 5.70; N, 8.80%.

N-*Benzyloxycarbonyl-γ-hydroxy-*DL*-glutamic Acid B Lactone Methyl Ester.* *N*-Benzyloxycarbonyl-γ-hydroxy-DL-glutamic acid B lactone[7] (2.8 g) was esterified with diazomethane in the usual manner. The product was recrystallized from 70% aqueous methanol, yield, 1.9 g (65%), mp 109—110 °C.

Found: C, 57.37; H, 5.20; N, 4.88%. Calcd for $C_{14}H_{15}O_6N$: C, 57.33; H, 5.16; N, 4.78%.

*Diketopiperazine (IX) of γ-Hydroxy-*DL*-glutamic Acid B γ-Methyl Ester.* Hydrogenolysis of the lactone methyl ester (1 g) obtained above in anhydrous dioxane using palladium black in the usual manner afforded the diketopiperazine which was recrystallized from 99% aqueous ethanol, yield, 0.7 g (64%), mp 204 °C (decomp.).

Found: C, 45.33; H, 5.73; N, 7.91%. Calcd for $C_{12}H_{18}O_8N_2$: C, 45.28; H, 5.70; N, 7.91%.