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The Novel Synthesis of L-Hydroxyproline from D-Glutamic Acid

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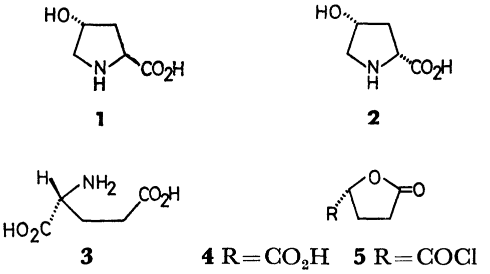
L-Hydroxyproline (**1**) and D-*allo*-hydroxyproline (**2**) were synthesized from D-glutamic acid (**3**) using the asymmetric center of **3** as that at C_4 of 4-hydroxyproline. When the aqueous solution of **1** or **2** was heated at 200 °C for 2 hr, a solution in the equilibrium state, containing 55% of **1** and 45% of **2**, was obtained. From this mixture, **1** was obtained by fractional crystallization.

Since 4-hydroxyproline was first synthesized in 1905 by Leuchs,[1] several synthetic routes have been studied by many researchers.[2-7] In these routes, comparable amounts of racemic diastereomers, *i.e.*, DL-hydroxyproline (DL-Hyp) and DL-*allo*-hydroxyproline (DL-*a*Hyp), have been formed. Therefore, difficult procedures, that is, the fractionation of DL-Hyp and DL-*a*Hyp and the optical resolution of DL-Hyp, were unavoidable, if one was to obtain L-hydroxyproline (**1**) from these racemic diastereomers.[8-9]

This paper will deal with a new synthesis of (4*R*)-4-hydroxyproline, *i.e.*, **1** and D-*allo*-hydroxyproline (**2**), from D-glutamic acid (**3**) using the asymmetric center of **3** as that at C_4 of 4-hydroxyproline, and with a new method of the interconversion between **1** and **2**. By these methods, **1** can be easily obtained without optical resolution, as will be described below.

Results and Discussion

Synthesis of L-*Hydroxyproline and* D-allo-*Hydroxyproline.* (*R*)-γ-Butyrolactone-γ-carboxylic acid (**4**) was prepared from **3** in an 85% yield by a reaction with nitrous acid in an aqueous solution. This reaction is known to proceed with a full retention of the configuration due to the participation of the neighboring carboxylate group.[10,11] The treatment of **4** with thionyl chloride afforded (*R*)-γ-butyrolactone-γ-carboxylic acid chloride (**5**) in a 96% yield. This reaction is considered to proceed with a full retention of its configuration by analogy with the conversion of *N*-trifluoroacetyl-L-proline to *N*-trifluoroacetyl-L-prolyl chloride.[12]



1 **2**

3 **4** R=CO₂H **5** R=COCl

6 R=CH₂OH **7** R=CH₂Cl

Formulae.

(*R*)-γ-Hydroxymethyl-γ-butyrolactone (**6**) was prepared from **5** in a 77% yield by reduction with sodium borohydride in dry diglyme. A small amount of 1,2,5-trihydroxypentane was also formed in this reduction. However, it was easily removed by silica gel chromatography.

In the case of sodium borohydride reduction, it is occasionally troublesome to decompose the reaction products and to remove the resulting boric acid.[13] However, in the present case, when methanol was poured into the reaction mixture we could easily decompose the reaction products and convert the boric acid into volatile trimethyl borate. The reduction mechanism of acid chloride with sodium borohydride has not been exactly reported, however, the reaction-equation seems to be as follows, by analogy with the reduction with lithium borohydride:[14]

$$4\,\mathrm{RCOCl} + 2\,\mathrm{NaBH_4} \longrightarrow \mathrm{NaCl} + \mathrm{BCl_3} + \mathrm{NaB(OCH_2R)_4} \qquad (1)$$

$$\mathrm{BCl_3} + \mathrm{CH_3OH} \longrightarrow \mathrm{B(OCH_3)_3} + 3\,\mathrm{HCl} \qquad (2)$$

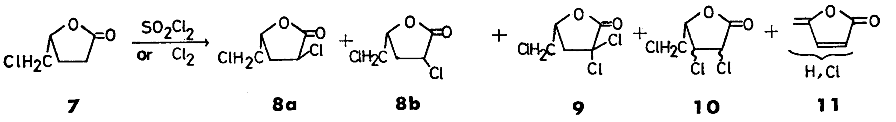
$$\mathrm{NaB(OCH_2R)_4} + 3\,\mathrm{CH_3OH} + \mathrm{HCl} \longrightarrow \mathrm{NaCl} + \mathrm{B(OCH_3)_3} + 4\,\mathrm{RCH_2OH} \qquad (3)$$

Fickett, Govner, and Leres reported that the optically-active alcohol, 2-chloropropanol, was obtained, with a retention of its configuration, from the optically-active acid chloride, 2-chloropropionyl chloride, by reduction with litium aluminium hydride.[15] By analogy with this reaction and by a comparision between the specific rotation of **6** and that of its (*S*)-isomer, which was synthesized by Koga, Taniguchi, and Yamada from (*S*)-γ-butyrolactone-γ-carboxylic acid methyl ester,[16] this reduction step may be considered to proceed with a full retention of configuration.

(*R*)-γ-Chloromethyl-γ-butyrolactone (**7**) was prepared from **6** in a 93% yield by reaction with thionyl chloride in the presence of pyridine. In this reaction, the configuration is considered to be retained.

At the next step, the halogenation of the α-position of **7** was examined. The α-position of the lactone or the ester is known to be hardly halogenated without the cleavage of the ester group.[17,18] However, in the present case the halogenation must be carried out without opening the lactone ring if we are to retain the configuration of the γ-position. A mixture of **7** and sulfuryl chloride was heated in a sealed glass tube. α-Monochloro-compounds (**8**), α,α-dichloro-compound (**9**), α,β-dichloro-compound (**10**) and polychloro-substituted protoanemonins (**11**) were formed. The gas-liquid chromatographic analyses (glc) showed two α-monochloro-compounds, *i.e.*, one with a shorter retention time (**8a**) and the other with a longer retention time (**8b**); their stereochemistries were deduced from the products of their amination, as will be discussed later. **11** was a mixture of tetra-, tri-, and dichloro-substituted protoanemonins. The component ratios of these products were determined by means of the ratios of their peak-areas in glc. A part of the results are shown in

Table 1. Chlorination of (*R*)-γ-chloromethyl-γ-butyrolactone (**7**) with sulfuryl chloride or gaseous chlorine



Expt. No.	$[SO_2Cl_2]$a) / [7]	Temp., C°	Time, hr	Products, % Unchanged 7	8a	8b	9	10	11
1	10.0	reflux	12	95	+b)	+	+	—c)	+
2	5.5	100	13	70	14	5	+	—	11
3	1.8	110	20	22	28	13	25	+	12
4	3.0	112	25	18	24	10	38	+	10
5	4.9	115	23	17	29	14	35	+	5
6	4.2	120	15	10	37	12	28	+	13
7	1.0	120	15	70	16	9	+	—	9
8	4.0	130	15	7	11	6	20	4	52
9	10.8	160	4.5	21	3	—	14	9	53
10d)		134	7.5	12	17	9	27	3	34

a) Molar ratio. b) Trace amount. c) Undetectable. d) Gaseous chlorine was bubbled into **7**.

Table 1. Hardly any reaction took place under 100 °C, and above 130 °C the amount of **11** rapidly increased. The amount of sulfuryl chloride required was more than twice the molar equivalent of **7**.

When **7** was chlorinated by gaseous chlorine, the same products (**8**—**11**) were formed, as is shown in Table 1. However, in this case **11** was more easily formed than in the case of the chlorination with sulfuryl chloride.

When **7** was heated for 15—25 hr with three—five times molar sulfulyl chloride at 110—120 °C, the total amount of α-chloro-compounds (**8a**, **8b**, and **9**) was the largest, as is shown in Table 1. However it was impossible to prepare only α-monochloro-compounds. Therefore, the selective conversion of **9** to **8** was examined. The chlorination products were hydrogenated in acetic acid under atmospheric pressure in the presence of sodium acetate and 5% Pd-C. The rate of the dehalogenation of **9** to **8a** and **8b** was much faster than that of **8** to **7**. Therefore, the reaction rate was followed by glc, and then the hydrogenation was stopped at the time when **9** was almost completely converted to **8** without the dehalogenation of **8**. The hydrogenated mixture generally contained 40—45% of **8a**, 30—40% of **8b**, and 2—3% of **9**.

These mixtures were aminated with conc. ammonium hydroxide and hydrolyzed with 3 M hydrochloric acid or 1 M sodium hydroxide. The Hyp and *a*Hyp thus produced were treated by means of an ion-exchange column (acid form) and decolorizing resin and were estimated by means of an automatic amino-acid analyzer. The results are shown in Table 2. The chlorination of **7** with sulfuryl chloride, followed by hydrogenation, amination, and hydrolysis, gave Hyp and *a*Hyp in a 46% overall yield.

The optical purities of these amino acids were determined by Manning and Moore's method,[19] with some modifications. Thus, four dipeptides, *i.e.*, L-leucyl-L-hydroxyproline, L-leucyl-D-hydroxyproline, L-leucyl-L-*allo*-hydroxyproline and L-leucyl-D-*allo*-hydroxyproline, were prepared from the amino acid mixture by reaction with *N*-carboxy-L-leucine anhydride. Then, the optical purity of Hyp was determined from the amounts of L-leucyl-L-hydroxyproline and L-leucyl-D-hydroxyproline. On the other hand, for the determination of the optical purity of *a*Hyp, the amino acid mixture was

Table 2. Amination of (γ*R*)-α-chloro-γ-chloromethyl-γ-butyrolactone (**8a** and **8b**)

Expt. No.	[8b]a) / [8a]	Reaction conditionsb) Amination	Hydrolysis	4-Hydroxyprolines Yieldc) (%)	[1]d) / [2]	Content of enantiomer, % D-Hype)	L-*a*Hypf)
1	39/61	A	C	38	41/59	4.7	3.1
2	46/54	A	C	46	46/54	4.1	2.9
3	45/55	A	C	46	44/56	4.2	2.5
4	45/55	B	C	45	55/45	4.6	3.6
5	41/59	A	C	12	39/61		
6	41/59	A	D	10	40/60		

a) Molar ratio of **8b** to **8a** in the starting material of amination. b) A; at room temperature for 2 day in conc. ammonium hydroxide. B: at 100—110°C for 7.5 hr in conc. ammonium hydroxide. C; reflux for 2 hr in 3 M hydrochloric acid. D; reflux for 0.5 hr in 1 M sodium hydroxide. c) The yield is based on **7** by chlorination with sulfuryl chloride followed by hydrogenation, amination and hydrolysis in Expt. No. 1—4. In Expt. No. 5 and 6, that is based on **7** by chlorination with gaseous chlorine, amination, and hydrolysis. d) Molar ratio of **1** to **2** in the formed 4-hydroxyproline. e) [D-Hyp]/([D-Hyp]+[L-Hyp]). f) [L-*a*Hyp]/([L-*a*Hyp]+[D-*a*Hyp]).

reacted with *N*-carboxy-L-valine anhydride instead of *N*-carboxy-L-leucine anhydride, and the L-valyl-L-*allo*-hydroxyproline and L-valyl-D-*allo*-hydroxyproline thus formed were estimated by means of the automatic amino-acid analyzer. The Hyp was composed of about 4% of the D-isomer and about 96% of the L-isomer, while the *a*Hyp was composed of about 3% of the L-isomer and about 97% of the D-isomer, as is shown in Table 2.

By recrystallization from water–ethanol, **1** and **2** were obtained in a 40% yield based on **7** (*i.e.*, in a 23% yield based on **3**). In this mixture, D-hydroxyproline and L-*allo*-hydroxyproline were not found by the above-mentioned method used to determine the optical purity.

When the amination was carried out at room temperature, as is shown in Table 2, the molar ratio of **1** to **2** was in fair agreement with that of **8b** to **8a**. On the other hand, α-amino acid is known to be formed at room temperature from α-chlorocarboxylic acid with a full inversion of the configuration.[20] Therefore, it could unambiguously be deduced that **1** and **2** were formed from **8b** and **8a** respectively. Thus, **8a** must be (α*S*,γ*R*)-α-chloro-γ-chloromethyl-γ-butyrolactone and **8b** must be (α*R*,γ*R*)-α-chloro-γ-chloromethyl-γ-butyrolactone.

Interconversion between L-*Hydroxyproline and* D-allo-*Hydroxyproline and Isolation of* L-*Hydroxyproline.* To obtain **1** from the mixture of **1** and **2**, novel methods of interconversion and fractination were examined. Until now, the interconversion between **1** and **2** has been made by two methods: (A) by heating an aqueous solution of the amino acid in the presence of excess barium hydroxide at 200 °C for several hours[21] and (B) by treating the amino acid with an excess of acetic anhydride in refluxing glacial acetic acid.[22] The reaction mixtures at the equilibrium state contained about 50% of **1** in Method (A) and about 25% of **1** in Method (B).

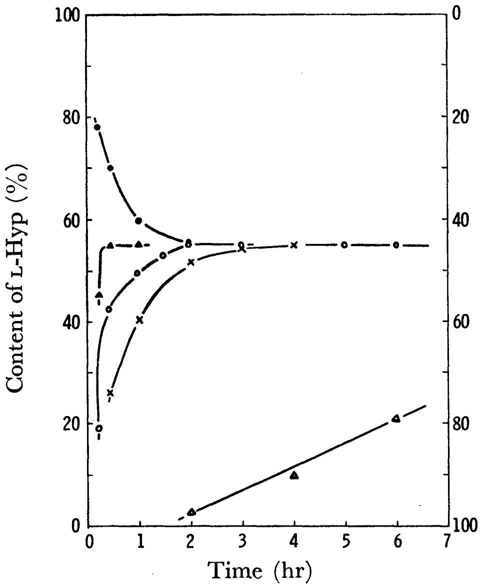


Fig. 1. Interconversion between L-hydroxyproline (**1**) and D-*allo*-hydroxyproline (**2**) in an aqueous solution: (△), **2** (starting material), 150 °C (reaction temperature); (×), **2**, 180 °C; (○), **2**, 200 °C; (▲), **2**, 220 °C; (●), **1**, 200 °C.

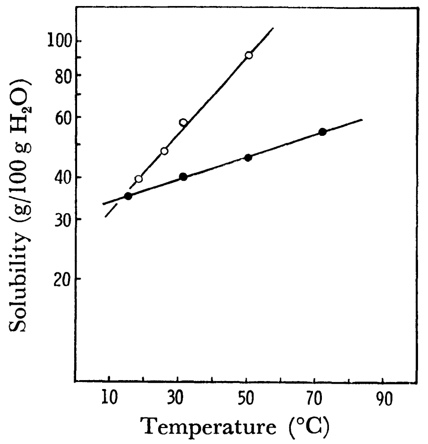


Fig. 2. Solubilities of L-hydroxyproline (●) and D-*allo*-hydroxyproline (○).

An aqueous solution of **1** or **2** was heated at 150, 180, 200, and 220 °C, and the contents of **1** and **2** in the solution were estimated using the optical rotation or an automatic amino-acid analyzer. The results are shown in Fig. 1. When the aqueous solution of **1** or **2** was heated at 200 °C for 2 hr, a solution at the equilibrium state, which contained 55% of **1** and 45% of **2**, was easily obtained.

The solubilities of **1** and **2** in water were also examined. The results are shown in Fig. 2. The temperature dependence of the solubility is apparently different between **1** and **2**. The solubility of **1** in water was constant regardless of the presence of **2**. Therefore, 9.00 g of a mixture of 55% of **1** and 45% of **2** gave 0.85 g of optically-pure **1** by fractional crystallization from 10 ml of water at 30 °C. Thus, the conversion of the mixture of **1** and **2** to **1** was carried out by a combination of interconversion and fractional crystallization in water. Also, by a method previously reported by the present authors, *i.e.*, the method through cyclic dipeptides, these mixtures could be easily converted to **1**.[23]

Experimental

The melting points are uncorrected. The IR spectra were recorded on a JASCO IR-S spectrometer. The NMR spectra were measured with a Varian T-60 or XL-100 spectrometer, using TMS as the internal standard. The mass spectra were obtained using a Hitachi RMU-6E spectrometer. The optical rotations were measured with a JASCO DIP-SL automatic polarimeter. The 4-hydroxyprolines were analyzed using a Hitachi KLA-3B automatic amino-acid analyzer (column size, 50×0.9 cm; resin, Hitachi Type 2612; column flow rate, 60 ml/hr; buffer, citrate (0.2 M) pH 3.25; temperature, 55 °C).

*Identification of the Chlorination Products of (*R*)-γ-Chloromethyl-γ-butyrolactone (7).* The reaction mixture was fractionated using a Shimadzu Gas Chromatograph GC-4A equipped with TCD and a glass column (5 ft×1/4 in. with 10% PEG-6000 on Chromosorb W (HMDS), 60—80 mesh; column temperature, 145 °C; flow rate of helium gas, 35 ml/min). The component ratios were determined by using a Hewlet Packard 402 High Efficiency Gas Chromatogram equipped with FID and a glass column (4 ft × 1/4 in. with 20% PEG-4000 on Chromosorb W (HMDS), 80—100 mesh; column temperature, 160 °C; flow rate of helium gas, 30 ml/min); the retention times (r.t.) will be shown below.

*(α*S, *γ*R*)-α-Chloro-γ-chloromethyl-γ-butyrolactone* (**8a**): r.t., 11.6 min; Mass spectrum *m/e* 119 (M–CH_2Cl, $C_4H_4O_2Cl$), 88, 83, 75 (C_3H_4Cl), 63 (C_2H_4Cl), 62 (C_2H_3Cl), 49 (CH_2Cl); IR (liquid film) 1960, 1795 (C=O), 1175, 1035 and 924 cm^{-1}.

*(α*R, *γ*R*)-α-Chloro-γ-chloromethyl-γ-butyrolactone* (**8b**): r.t., 15.6 min. The mass spectrum was identical with that of **8a**.

*(*R*)-α,α-Dichloro-γ-chloromethyl-γ-butyrolactone* (**9**): r.t., 8.4 min; Mass spectrum *m/e* 153 (M–CH_2Cl, $C_4H_3O_2Cl_2$), 125, 109 (C_3H_3Cl), 96 ($C_2H_2Cl_2$), 61 (C_2H_2Cl), 49 (CH_2Cl); IR (liquid film) 2970, 1810 (C=O), 1179, 1034 and 963 cm^{-1}; NMR (in $CDCl_3$) $ClCH_dH_e–C_\gamma H_c–C_\beta H_bH_a–$ δ_a 3.26, δ_b 2.97, 4.95, δ_d 3.79, δ_d 3.86, $J_{ab}=14.6$ Hz, $J_{ac}=5.5$ Hz, $J_{bc}=9.3$ Hz, $J_{de}=12.3$ Hz, $J_{dc}=4.9$ Hz, $J_{ec}=4.6$ Hz. The assignment of this five-spin system was confirmed by computor simulation.

*(γ*R*)-α,β-Dichloro-γ-chloromethyl-γ-butyrolactone* (**10**): r.t., 9.8 min. The mass spectrum was almost identical with that of **9**; however, they differed in the relative strengths of their peaks.

Polychloro-substituted Protoanemonins (**11**): r.t., 1.8—2.8 min; Mass spectrum *m/e* 268 ($C_5HO_2Cl_5$), 233 ($C_5HO_2Cl_4$), 222 ($C_5O_2Cl_4$), 198 ($C_5HO_2Cl_3$), 185 ($C_4O_2Cl_3$), 151 ($C_4HO_2Cl_2$), 123. In NMR spectrum, the signals appeared between δ 6.13 and 7.70.

Determination of the Optical Purity of 4-Hydroxyproline by Ion-exchange Chromatography. allo-*Hydroxyproline*: The borate buffer (0.45 M, 500 ml) was prepared by dissolving boric acid (13.9 g) in 497 ml of boiled, distilled water and then by adding 50% sodium hydroxide to bring the solution to pH 10.2 at 25 °C. In 15 ml of this borate buffer, 20 mg (0.152 mmol) of an amino acid mixture of hydroxyproline and *allo*-hydroxyproline was dissolved, and then a small amount of silicone grease was added to minimize foaming. To the vigorously-stirred solution we then rapidly added, at 0—2 °C, a solution of *N*-carboxy-L-valine anhydride (26 mg, 0.18 mmol) in dioxane (1.0 ml). After this mixture has been vigorously stirred for 3 min, 3 ml of 2 M hydrochloric acid was added rapidly to stop the reaction. The solution was filled up to 25 ml with a citrate buffer (0.2 M, pH 2.2), and the contents of L-valyl-L-*allo*-hydroxyproline and L-valyl-D-*allo*-hydroxyproline in the solution were estimated by comparison with authentic mixtures using a Hitachi KLA-5 automatic amino-acid analyzer (column size, 50 × 0.9 cm; resin, Hitachi Type 2613; column flow rate, 60 ml/hr; buffer, citrate (0.2 M), pH 3.25; temperature, 55 °C).

The elution times were 142 min for L-valyl-D-hydroxyproline and L-valine, 170 min for L-valyl-D-*allo*-hydroxyproline, 190 min for L-valyl-L-hydroxyproline, and 207 min for L-valyl-L-*allo*-hydroxyproline. The detectable amount of L-*allo*-hydroxyproline in the amino acid mixture was checked by using an authentic mixture. A 0.5% amount of L-*allo*-hydroxyproline in *allo*-hydroxyproline was detectable; however, any amount less than 0.5% of L-isomer was not detectable.

Hydroxyproline: Using *N*-carboxy-L-leucine anhydride (29 mg, 0.18 mmol), the contents of L- and D-hydroxyprolines in a mixture of hydroxyproline and *allo*-hydroxyproline were estimated in a manner similar to that described in the part of *allo*-hydroxyproline. The elution times were 155 min for L-leucyl-D-hydroxyproline, 220 min for L-leucyl-L-hydroxyproline, and 254 min for L-leucyl-L-*allo*-hydroxyproline, L-leucyl-D-*allo*-hydroxyproline, and L-leucine. Any amount less than 0.5% of D-hydroxyproline was not detectable.

*(*R*)-γ-Butyrolactone-γ-carboxylic Acid* (*4*). From D-glutamic acid ($[\alpha]_D^{27}$ −31.6° (*c* 10.2, 2 M HCl)), **4** was obtained in an 85% yield by the method of Austin and Howard:[24] bp 165.5—167.5 °C/0.30 mmHg; mp 73.5—74.0 °C (recrystallization from ethyl acetate–benzene–petroleum ether); $[\alpha]_D^{20}$ −15.9° (*c* 1.10, ethanol), +7.29° (*c* 1.03, water), −14.3° (*c* 1.19, methanol). Lit,[11] bp 150—155 °C/0.2 mmHg, mp 71—72 °C, $[\alpha]_D^{20}$ −2.8° (*c* 5.8, water), +10.6° (*c* 5.0, methanol) for (*S*)-isomer.

*(*R*)-γ-Butyrolactone-γ-carboxylic Acid Chloride* (*5*). A mixture of **4** (55.7 g, 0.429 mol) and thionyl chloride (102.0 g, 0.857 mol) was refluxed for 2 hr and then allowed to stand at room temperature overnight. After the removal of the excess thionyl chloride, the residue was distilled under reduced pressure to give 61.1 g (0.413 mol) of **5** in a 96% yield: bp 117.1 °C/4.5 mmHg; $[\alpha]_D^{30}$ +0.196° (*c* 4.34, dioxane), −44.5° (*c* 3.70, benzene); IR (liquid film) 2970, 1805—1780, 1458, 1490, 1355, 1138, 1060, and 910 cm^{-1}; NMR (in $CDCl_3$) δ 2.7 (4H, m, $–CH_2–CH_2–$) and 5.3 (1H, m, C_γ–H); Mass spectrum *m/e* 85 (M–COCl, $C_4H_5O_2$), 57, 36, 29. Found: C, 40.65; H, 3.49; Cl, 23.59%. Calcd for $C_5H_5O_3Cl$: C, 40.42; H, 3.39; Cl, 23.87%. Lit,[25] bp 136—137 °C/12 mmHg for the racemic form.

*(*R*)-γ-Hydroxymethyl-γ-butyrolactone* (*6*). A solution of **5** (24.8 g, 0.168 mol) in dry diglyme (50 ml) was added at 0—5 °C to a solution of sodium borohydride (4.12 g, 0.109 mol) in dry diglyme (175 ml). The mixture was then stirred for 30 min at room temperature. To the mixture, methanol (70 ml) was added. After the filtration of sodium chloride, the filtrate was concentrated under reduced pressure. The resulting syrup was placed on a silica gel column containing 100 ml of WAKO GEL C-200 and eluted with 2.5% ethanol in chloroform. **6** was eluted faster than 1,2,5-trihydroxypentane, so the two products were easily separated. After the removal of the solvent from the elute containing **6**, the residue was distilled under reduced pressure to give 14.7 g (0.127 mol) of **6** in a 76% yield: bp 101.0—102.0 °C/0.048 mmHg; $[\alpha]_D^{30}$ −53.5° (*c* 3.17 chloroform), −33.5° (*c* 3.12, ethanol); IR (liquid film) 3400, 1765, 1350, 1185, 1060, 935, and 803 cm^{-1}; NMR (in $CDCl_3$) δ 1.95—2.75 (4H, $–CH_2–CH_2–$), 3.80 (2H, $–CH_2OH$) and 4.63 (1H, C_γ–H); Mass spectrum *m/e* 116 (M^+), 85 (M–CH_2OH), 57, 31 (CH_2-OH). Found: C, 51.90; H, 6.67%. Calcd for $C_5H_8O_3$: C, 51.72; H, 6.94%. Lit,[16] $[\alpha]_D^{26}$ +31.1° (ethanol) for the (*S*)-isomer.

*(*R*)-γ-Chloromethyl-γ-butyrolactone* (*7*). Thionyl chloride (26.9 g, 0.226 mol) was added to a mixture of **6** (23.2 g, 0.200 mol) and dry pyridine (17.6 g, 0.222 mol) at 40—50 °C. The mixture was stirred for 1 hr at 60 °C. To the mixture, chloroform (200 ml) was then added. The resulting chloroform solution was washed with water (50 ml) (three times), saturated sodium bicarbonate (20 ml), and water (50 ml), dried with anhydrous sodium sulfate, and decolorized with active charcoal. After the removal of the chloroform, the residue was distilled under reduced pressure to give 25.0 g (0.187 mol) of **7** in a 94% yield; bp 94.0—94.2 °C/2.0 mmHg; $[\alpha]_D^{27}$ −12.9° (*c* 3.03, chloroform), −33.2° (*c* 3.51, ethanol); IR (liquid film) 2965, 1775, 1169, 1040, 915, and 738 cm^{-1}; NMR (in $CDCl_3$) δ 1.95—2.77 (4H, $–CH_2–CH_2–$), 3.70 (2H, CH_2Cl), 4.76 (1H, C_γ–H); Mass spectrum *m/e* 134 (M^+, 2%), 85 (M–CH_2Cl, 100%), 57, 55, 29. Found: C, 44.85; H, 5.17; Cl, 26.65%. Calcd for $C_5H_7O_2Cl$: C, 44.63; H, 5.24; Cl, 26.35%.

L-Hydroxyproline (*1*) *and* D-allo-*Hydroxyproline* (*2*). A mixture of **7** (1.98 g, 14.8 mmol) and sulfuryl chloride (6.04 g, 44.7 mmol) was heated at 112 °C for 25 hr in a glass tube.

After the removal of the excess sulfuryl chloride from the mixture under reduced pressure, chloroform (50 ml) was added to the residue. The resulting chloroform solution was washed with water (10 ml), saturated sodium bicarbonate (10 ml), and water (10 ml), and dried with anhydrous sodium sulfate. The removal of the chloroform gave 3.39 g of a syrup. The results of glc analysis showed that the sryup contained 24% of **8a**, 10% of **8b**, 38% of **9**, a trace amount of **10**, 10% of **11**, and 18% of unchanged **7**.

This syrup was dissolved in 30 ml of acetic acid and hydrogenated in the presence of sodium acetate (1.0 g) and 5% Pd–C (250 mg) at atmospheric pressure. The conversion of **9** to **8a** and **8b** was followed by glc. When 230 ml of hydrogen gas had been absorbed, the reaction was stopped. After the filtration of the catalyst and the sodium acetate, the filtrate was concentrated under reduced pressure. Toluene (10 ml) was added to the residue. The toluene solution was evaporated to remove acetic acid under reduced pressure. The syrup (2.88 g) thus obtained was found by glc to contain 43% of **8a**, 37% of **8b**, 2% of **9**, 4% of **10**, 9% of **11**, and 5% of **7**.

This syrup was dissolved in 30 ml of conc. ammonium hydroxide, and the solution was stirred for 2 days at room temperature. The excess of ammonium hydroxide was evaporated under reduced pressure, 90 ml of 3 M hydrochloric acid was added, and the solution was refluxed for 2 hr. The solution was then evaporated under reduced pressure. The residue was dissolved in water (30 ml), and the aqueous solution was placed in an ion-exchange column containing 100 ml of IR-120 resin (acid form). The resin was washed with water, and the absorbed amino acids were eluted with 0.2 M ammonium hydroxide. The elute was concentrated under reduced pressure and decolorized by the use of decolorizing resin (Hokuetsu Tanso Co.'s HS decolorizing resin). By analysis using the automatic amino-acid analyzer, the solution was found to contain 0.409 g (31.2 mmol) of hydroxyproline, 0.478 g (36.5 mmol) of *allo*-hydroxyproline, and trace amounts of unidentified ninhydrin positive substances. The optical purity of the 4-hydroxyproline thus obtained was determined by the above-mentioned method. The hydroxyproline was composed of 95.9% of the L-isomer and 4.1% of the D-isomer, while the *allo*-hydroxyproline was composed of 97.1% of the D-isomer and 2.9% of the L-isomer.

The solution was evaporated to dryness under reduced pressure. The residue was recrystallized from water–ethanol to give 0.784 g (5.98 mmol) of a mixture composed of 44.8% of **1** and 55.2% of **2** in a 40% yield based on **7**: mp 239 °C (decomp.); $[\alpha]^{31}_{D}$ $-1.72\pm0.25°$ (*c* 2.03, water). Found: C, 45.71; H, 7.02; N, 10.89%. Calcd for $C_5H_9O_3N$: C, 45.79; H, 6.91; N, 10.68%. In the mixture thus obtained, D-hydroxyproline and L-*allo*-hydroxyproline were not found by the above-mentioned method used to determine the optical purity.

Interconversion between L-*Hydroxyproline (1) and* D-allo-*Hydroxyproline (2).* A solution of **2** (0.500 g, 3.82 mmol) in water (5 ml) was sealed in a glass tube and heated for 2 hr at 200 °C. The tube was then cooled and opened, and the solution was filled up to 50 ml. The specific rotations, $[\alpha]^{30}_{D}$ (*c* 1.00, water), of the solution and authentic **1** and **2** were -16.0, -76.0, and $+58.6°$ respectively. Therefore, the solution contained 55.4% of **1** and 44.6% of **2**. The analytical results obtained by means of the automatic amino-acid analyzer showed that the solution was composed of 56.2% of **1** and 43.8% of **2**. The experiments were similarly carried out while varying the heating time. The following results were obtained: 19.1% of **1** at 15 min, 42.4% of **1** at 30 min, 49.5% of **1** at 1 hr, 52.1% of **1** at 1.5 hr, 55.4% of **1** at 2 hr, 55.8% of **1** at 3 hr, 54.9% of **1** at 5 hr, and 55.5% of **1** at 6 hr. The experiments were similarly done at 150, 180, 200, and 220 °C by using **1** or **2** as the starting material.

Solubility of L-*Hydroxyproline (1) or* D-allo-*Hydroxyproline (2) in Water.* A suspension of **2** (10 g) in water (10 ml) was stirred at 32.0±0.1 °C overnight. The supernatant solution (1.585 g) was then taken up and dried at 80 °C until the weight became constant. The rsidue weighed 0.580 g. Therefore, the solubility of **2** in water at 32 °C was determined to be 57.7 g/100 g-water. The solubilities of **1** or **2** in water were similarly determined at different temperatures.

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