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The Novel Synthesis of Two Diastereomers of γ-Hydroxyproline1)

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L-γ-Hydroxyproline is known as a component amino acid of limited proteins such as gelatine or collagen. Although several synthetic routes had been studied by many researchers, no satisfactory methods have been developed yet in view of the yield of the product and of the separation of two diastereomers formed. As far as literatures were concerned, γ-hydroxyproline had been synthesized through either 2-amino-5-halogeno-4-valerolactone2–8) or 2-halogeno-5-amino-4-valerolactone9) starting from epichlorohydrin. Alternatively, it had been also synthesized through 2,5-dichloro-4-valerolactone from either epichlorohydrin or alkyl halide.10,11) In every case, comparable amounts of racemic diastereomers, *i.e.*, DL- and DL-*allo*-γ-hydroxyproline have been formed. When these mixtures were attempted to be separated each other through their copper complexes, the purification of the more soluble DL-*allo* complex met often difficulty, though the less soluble DL-complex has been obtained in relatively pure state.

The authors found now a new synthetic method to lead to γ-hydroxyproline from 2-amino-5-bromo-4-valerolactone hydrobromide which was obtained as a diastereomeric mixture in good yield by heating 2,3-dibromopropylacetamidomalonate with hydrobromic acid. This mixture was readily separable from aqueous solution due to the difference in solubilities. This improvement in the synthetic route established a new way through which DL- and DL-*allo*-γ-hydroxyproline are available both in pure states.

Thus, diethyl acetamidomalonate (I) was condensed with equimolar allyl bromide (II) using sodium

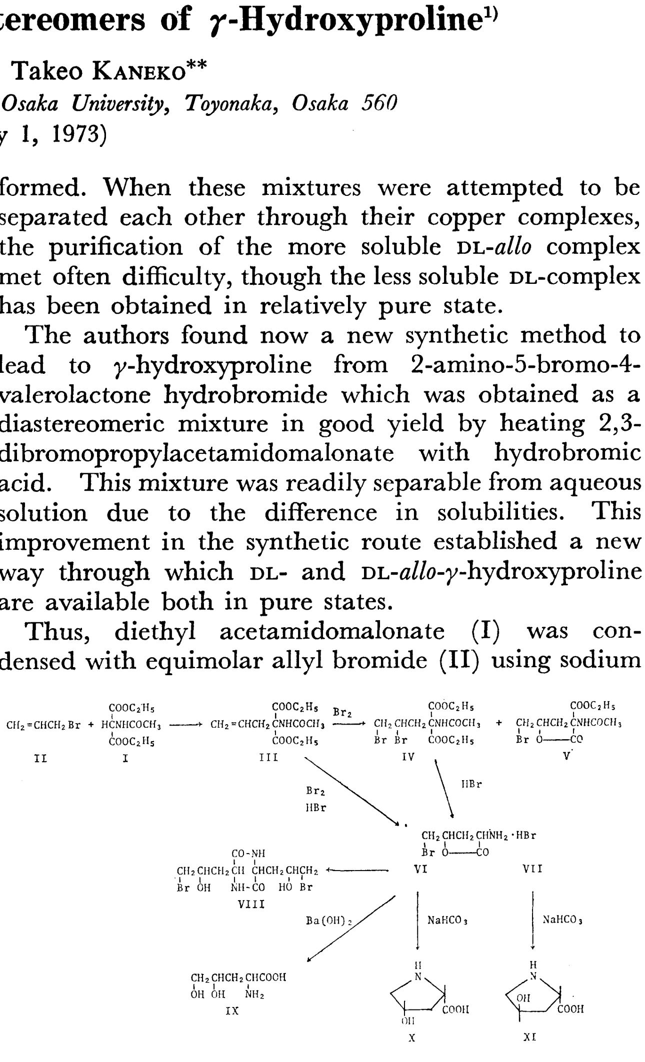

Fig. 1. Synthetic route for γ-hydroxyproline.

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alkoxide in absolute ethanol according to the method of Albertson.[12] To the resulting allylacetamidomalonate (III), bromine was added in absolute ether to afford crystals of dibromo compound (IV). When the mother liquor thus obtained was kept for long time, 5-bromo-4-valerolactone derivative (V) was crystallized out slowly. The bromination in chloroform or carbon tetrachloride resulted in an improved yield of V but not that of IV. When the dibromo compound IV was hydrolyzed with hydrobromic acid, a mixture of two racemic diastereomers of 2-amino-5-bromo-4-valerolactone hydrobromide was obtained in good yield. This mixture was separated successfully into a less soluble isomer (VI) and a more soluble isomer (VII) from aqueous solution. The mixture of VI and VII could be also obtained in satisfactory yield even when the bromination product was hydrolyzed directly without separation of IV. The amount ratio of VI and VII in any mixtures thus obtained was always close to 1 : 1.

The treatment of the less soluble salt VI with pyridine gave the dioxopiperazine (VIII) composing of two molecules of 5-bromo-4-hydroxy-2-aminovaleric acid, the structure of which was confirmed by elemental analyses and IR spectrum: ν_{max}, 3210 and 3050 cm^{-1} (NH), 1637 cm^{-1} (NHCO).

TABLE 1. FORMATION OF DL-ALLOHYDROXYPROLINE AND DL-γ-HYDROXYPROLINE FROM 2-AMINO-5-BROMO-4-VALEROLACTONE (VI, VII)

Basic reagent	Reaction		Yield (%)	
	Temp. (°C)	Time (hr)	DL-*allo* (from VI)	DL (from VII)
28% Aqueous ammonia	100	5	30	20
Ammonia (Saturated in abs. CH_3OH)	40	5	72	42
$NaHCO_3$(10% aq. sol.)	90	1	80	46
$Ba(OH)_2$ (0.2N sol)	100	2	54	30—35

For conversion of the aminobromolactone hydrobromide (VI or VII) into γ-hydroxyproline, various basic reagents, were examined as shown in Table 1. Among these reagents, sodium hydrogen carbonate gave best result giving 55% overall yield of hydroxyproline from I. When barium hydroxide was used in the above reaction, a compound giving deep purple color with ninhydrin and a positive test with periodic acid, was obtained. The structure of this compound was deduced to 2-amino-4,5-dihydroxyvaleric acid (IX) from its elemental analysis in addition to the properties mentioned above. From the less soluble salt (VI), very pure DL-*allo*-hydroxyproline was obtained solely, while the more soluble salt (VII) gave DL-hydroxyproline after purification of its crude material through copper complex.

According to the conventional method for separation of the final synthetic product, γ-hydroxyproline, through copper complex, it was difficult to secure the pure *allo* compound. The present method has advantage of ease of separation of DL-*allo*-γ-hydroxyproline in very pure form and also of DL-γ-hydroxyproline due to the less solubility of its copper complex. The stereochemistries of the lactone VI and VII were unambiguously deduced from those of hydroxyproline that was derived from each compound. Thus orientation of amino and bromomethyl groups must be *cis* in VI and *trans* in VII.

Experimental

All melting points are uncorrected.

Ethyl 2-Acetamido-4,5-dibromo-2-ethoxycarbonylvalerate (IV). To a solution of 5 g of diethyl allylacetamidomalonate[12] (III) in 40 ml of absolute ether, 3.5 g of bromine was added dropwise at -10—-15 °C for 1 hr. The reaction mixture was stirred at -10 °C for another 2 hr and then at 0 °C for 2 hr. Ether was removed under reduced pressure. The residue was triturated with a small amount of water to give crystals of IV. These were filtered and recrystallized from 90% aqueous ethanol, yield 5.4 g (67%), mp 83—84 °C.

Found: C, 34.63; H, 4.42; N, 3.32; Br, 38.44%. Calcd for $C_{12}H_{19}O_5NBr_2$: C, 34.55; H, 4.59; N, 3.36; Br, 38.32%.

2-Acetamido-5-bromo-2-ethoxycarbonyl-4-valerolactone (V). When the mother liquor from IV was kept for two weeks, crystals of V appeared. The crystals were filtered and recrystallized twice from 50% aqueous ethanol, mp 141—142 °C.

Found: C, 39.29; H, 4.48; N, 4.41; Br, 27.53%. Calcd for $C_{10}H_{14}O_5NBr$: C, 38.98; H, 4.58; N, 4.55; Br, 25.94%.

2-Amino-5-bromo-4-valerolactone Hydrobromide (VI and VII). Method A: The compound IV (30 g) was refluxed with 48% hydrobromic acid for 4 hr. Hydrobromic acid was removed by evaporation under reduced pressure. After the residue was kept in a refrigerator overnight, a solid mixture of VI and VII was filtered, yield 19 g (95%). It was dissolved in 5 ml of hot water and kept in a refrigerator overnight. The crystals of VI were separated out. These were filtered and washed with little acetone, yield 8 g. A solution of these crystals in 60 ml of methanol was treated with charcoal, and then concentrated to about 15 ml. Crystals formed were filtered and recrystallized from methanol, mp 244 °C (decomp.).

Found: C, 21.68; H, 3.30; N, 5.04; Br, 57.98%. Calcd for $C_5H_9O_2NBr_2$: C, 21.84; H, 3.30; N, 5.09; Br, 58.13%.

The mother liquor from VI was concentrated under reduced pressure. After addition of acetone, the solution was set aside for a few days to recover further crops of VI. However, easy soluble salt VII could not be obtained in crystalline state from the filtrate by this procedure.

Method B: To a solution of 20 g of III in 40 ml of chloroform, a solution of 15 g of bromine in 15 ml of chloroform was added at 20 °C with stirring for 1 hr. The stirring was continued for another 2 hr. Chloroform was removed under reduced pressure. To the syrupy residue obtained, 150 g of 48% hydrobromic acid was added. After refluxing for 3 hr, the solution was evaporated under reduced pressure and then cooled. The resulting crystals were filtered off to afford 19.6 g (92%) of a mixture of VI and VII hydrobromides. Through the treatment of the mixture as in the method A, 9.4 g of the less soluble salt VI in crystalline state, mp 222 °C (decomp.) and 9 g of the more soluble salt VII as syrup were obtained.

2,4-Bis(3-bromo-2-hydroxypropyl)-3,6-dioxopiperazine (VIII). Into a solution of 500 mg of VI in a small amount of water, 0.2 g of pyridine was added and the reaction mixture was allowed to stand at room temperature overnight. A crys-

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12) N. F. Albertson, *ibid.*, **68**, 450 (1946).

talline product was separated out, mp 183 °C (decomp.). After recrystallization from water, the melting point raised up to 186 °C (decomp.).

Found: C, 31.06; H, 4.20; N, 7.02; Br, 40.45%. Calcd for $C_{10}H_{16}O_4N_2Br_2$: C, 30.95; H, 4.16; N, 7.22; Br, 41.19%.

Hydrolysis of VI and VII. *a) By Aqueous Ammonia*: A mixture of 5 g of the less soluble salt VI and 110 ml of 28% aqueous ammonia was heated at 90—100 °C in a pressure bottle for 5 hr and then kept at room temperature for 24 hr. The ammonia and water were removed under reduced pressure and 30 ml of concentrated hydrochloric acid was added into the residue. The solution was heated at 80—90 °C for 1.5 hr and then evaporated under reduced pressure to obtain a syrupy residue. A solution of the residue in a small amount of water was made alkaline to phenolphthalein with 1M sodium hydroxide and then heated for about 10 min. After cooling, the solution was acidified with 1M hydrochloric acid and evaporated under reduced pressure. The inorganic materials were removed by treating several times with ethanol and finally with silver carbonate. The syrupy residue was crystallized from water and ethanol to give DL-*allo*-γ-hydroxyproline (X), yield 0.7 g, mp 238 °C (decomp.). Found: C, 45.99: H, 6.65; N, 10.44%. Calcd for $C_5H_9O_3N$: C, 45.79; H, 6.92; N, 10.68%.

The more soluble salt VII (5 g) was treated like as mentioned above. The resulting syrupy residue was dissolved in 50 ml of water and heated with cupric carbonate for 1 hr giving sky-blue copper complex, yield 1.5 g. A suspension of the copper complex in water was treated with hydrogen sulfide. The filtrate from copper sulfide was evaporated to the residue which was crystallized from water and ethanol giving pure DL-γ-hydroxyproline (XI), yield 0.5 g (20%), mp 246 °C (decomp.). Found: C, 45.80; H, 6.87; N, 10.69%

2-Amino-4,5-dihydroxyvaleric Acid (IX) A solution of 5 g of VI in 200 ml of 0.2N barium hydroxide was heated in a boiling water bath for 2 hr. From the hydrolyzate X was separated by usual way. The mother liquor from X was treated with cupric carbonate and the precipitate was recrystallized from water and ethanol to give a sky-blue copper complex. This copper compound was treated with hydrogen sulfide and filtered. The filtrate was evaporated and the residue was crystallized from water and ethanol giving IX, yield 0.7 g. This was recrystallized from 50% aqueous ethanol twice, mp 105 °C (decomp. at 175 °C).

Found: C, 36.10; H, 7.53; N, 8.19; H_2O, 9.15%. Calcd for $C_5H_{11}O_4N\cdot H_2O$: C, 35.92; H, 7.84; N, 8.38; H_2O, 10.78%.