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New Synthesis of γ-Hydroxyglutamic Acid

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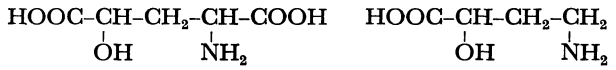
Synopsis. γ-Hydroxyglutamic acid was prepared easily via 3-ethoxycarbonyl-5-methoxycarbonyl-2-isoxazoline, which was the 1,3-dipolar adduct of methyl acrylate and ethyl cyanoformate *N*-oxide.

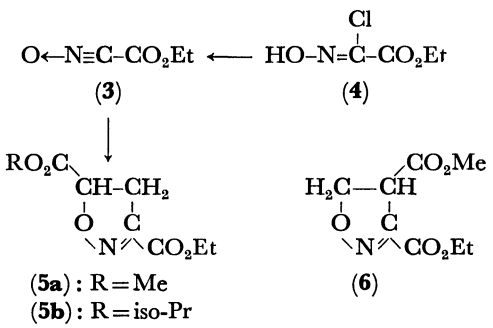
It has been reported that γ-hydroxy α-amino acids occur in some kinds of plants.[1a,b,c] γ-Hydroxyglutamic acid (**1**) has been found in the green parts of the plants, *Phlox decussa*[2] and *Linaria vulgaris*.[3] By enzymatic decarboxylation, this γ-hydroxyglutamic acid gives 4-amino-2-hydroxybutyric acid (**2**), which is known to accelarate the activity of antibiotics such as kanamycin.[4,5] γ-Hydroxyglutamic acid (**1**) is expected to have the biological activity.



(**1**) (**2**)

In this paper, we describe the new synthesis of **1**, *via* the 1,3-dipolar addition of ethyl cyanoformate *N*-oxide (**3**) and acrylic esters.

Glycine ethyl ester hydrochloride was treated with sodium nitrite to give ethyl chloro(hydroxyimino)acetate (**4**). It was reported that chloro oxime (**4**) smoothly yielded nitrile oxide (**3**) by the treatment with bases such as triethylamine.[6] Therefore, **4** was slowly added to a mixture of triethylamine and methyl acrylate in chloroform at room temperature. After standing for 2 days, the products were purified by distillation giving an oil. From the spectral properties, the product was found to be 3-ethoxycarbonyl-5-methoxycarbonyl-2-isoxazoline (**5a**) (71% yield). Another expected compound (**6**) was not detected.



(**3**) (**4**)

(**5a**): R=Me
(**5b**): R=iso-Pr

(**6**)

For the cleavage of N–O bond, **5a** was hydrogenated over platinum in the presence of 1 equivalent of hydrochloric acid. By the direct hydrolysis of the reduced product (**7**) with 20% hydrochloric acid, a disatereomeric mixture of lactam, lactone and free amino acid forms of γ-hydroxyglutamic acid was obtained. The reaction mixture was dissolved in a small volume of water, and the solution was saturated with hydrogen chloride at 0 °C. The precipitate, which was assigned to the *threo*-γ-hydroxyglutamic acid lactone (**8a**) hydrochloride[7] was obtained in 40% yield. By the ion exchange chromatography of the filtrate, the free *erythro*-γ-hydroxyglutamic acid (**1b**)[7] was isolated in 20% yield. Also the mixture of lactams, (**9a**) and (**9b**), was eluted. However, *erythro* lactone (**8b**) could not be isolated from the reaction mixture.

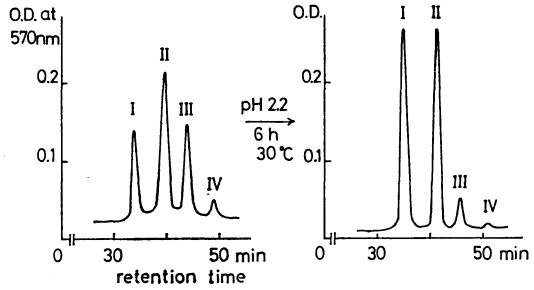
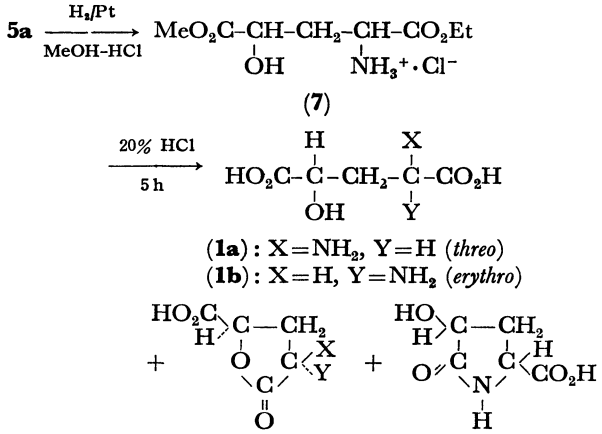


Fig. 1. Amino acid analysis of hydrolyzed product of **7**. Column: Yanagimoto SCX-1001; temp: 55 °C; elution buffer: citrate buffer, pH 3.25.

The product ratio of the amino acid and its lactone was determined by amino acid analyzer. In the amino acid chromatogram (Fig. 1), the reaction mixture showed four peaks (I—IV). After the buffered solution (sodium citrate buffer, pH 2.2) of the mixture was kept at room temperature for 6 hours, the peaks III and IV remarkably decreased, and the peaks I and II increased. When the solution of **8a** was allowed to stand in similar conditions, the peak III of **8a** gradually decreased, while the peak I increased. The peak II was found to correspond to **1b**. Thus, these four peaks (I—IV) were assigned to the compounds (**1a**), (**1b**), (**8a**), and (**8b**), respectively. After the peaks III and IV were diminished, the ratio of **1a** and **1b** was evaluated as 4: 6 from the



(**7**)

(**1a**): X=NH_2, Y=H (*threo*)
(**1b**): X=H, Y=NH_2 (*erythro*)

(**8a**): X=NH_2, Y=H (*threo*) (**9a**), (**9b**)
(**8b**): X=H, Y=NH_2 (*erythro*)

result of amino acid analysis.

In the hydrogenation on platinum, the isoxazolines are expected to change the ratio of the diastereomers by a bulky group on C-5 position. Therefore, 3-ethoxycarbonyl-5-isopropoxycarbonyl-2-isoxazoline (**5b**) was synthesized from isopropyl acrylate and the nitrile oxide (**3**). After hydrogenation and hydrolysis as described above, the ratio of **1a** and **1b** was determined as 4: 6. The isopropoxycarbonyl group has no effect on the ratio of the diastereomers. The lack of specificity of the hydrogenation must be due to facile rapture of the N–O bond prior to the reduction of the C=N bond.

In conclusion, the method described above is considered to have the general utility in the γ-hydroxyamino acid synthesis. This method for the γ-hydroxyamino acid synthesis is in progress.

Experimental

The melting points and boiling points are uncorrected. PMR spectra were recorded on a Hitachi R-24A instrument, TMS being used as an internal standard (δ, ppm). IR spectra were taken on a Hitachi EPI-S2 spectrometer. The amino acid chromatograms were recorded on a Yanagimoto amino acid analyzer LC-5S.

3-Ethoxycarbonyl-5-methoxycarbonyl-2-isoxazoline (5a). A solution of ethyl chloro(hydroxyimino)acetate[6] (**4**; 6.73 g) in dry chloroform (150 ml) was added to a solution of methyl acrylate (15 ml) and triethylamine (30 ml) in chloroform (50 ml) during 2.5 h at room temperature. The slightly brown solution was stirred at room temperature for two days, and the solution was washed with dilute hydrochloric acid and water. After drying over magnesium sulfate, the chloroform was evaporated under vacuum affording a brown liquid. Fractional distillation produced **5a** as a pale yellow liquid: Bp 107—121 °C/3 mmHg (7.0 g); NMR ($CDCl_3$): 1.35 (1H, t, J=7 Hz), 3.51 (2H, d, J=8 Hz), 3.78 (3H, s), 4.34 (2H, q, J=7 Hz), and 5.18 (1H, t, J=8 Hz); IR (film): 1740, 1600, 1260, 1120, 1020, and 920 cm^{-1}. Found: C, 47.77, H, 5.51, N, 6.84%. Calcd for $C_8H_{11}NO_5$: C, 47.76, H, 5.51, N, 6.96%.

Hydrogenation of 5a and Subsequent Hydrolysis. The isoxazoline (**5a**) (3.02 g) was hydrogenated over platinum oxide (0.30 g) in methanol (100 ml)–3M hydrochloric acid (5 ml) at room temperature for 24 hr. The catalyst was filtered away, and the filtrate was concentrated to a colorless oil. The oil was refluxed in 20% hydrochloric acid (130 ml) for 5 h, and the yellow solution was treated with charcoal. After the charcoal was removed by filtration, the filtrate was concentrated giving a pale green oil (3.0 g), which partyl crystallized on standing. The oil (3.0 g) was dissolved in water (1.2 ml), and the solution was saturated with hydrogen chloride at 0 °C. The resulting mixture was allowed to stand in a refrigerator for two days. The precipitated *threo*-γ-hydroxyglutamic acid lactone hydrochloride (**8a**) (736 mg) was collected by filtration: Mp 228—230 °C (dec) [lit,[7] 228—230 °C (dec)]. IR (KBr): 3600—2400 (br), 1785, 1755, 1200, 1045 cm^{-1}; MS *m/e* 145 (M^+—HCl), 101 (145—CO_2), 100 (145—CO_2H), 84 (101—NH_3), 83 (100—NH_3), 72 (100—CO), 56 (100—CO_2), and 55 (83—CO).

The filtrate was concentrated, and a small volume of water was added. The solution was again saturated with hydrogen chloride. The second crop (343 mg) of **8a**, mp 225 °C (dec), was collected by filtration. The filtrate was concentrated producing a yellow oily residue (1.5 g), which was chromatographed on a cation exchange (Dowex-50, 100 g). Elution with water afforded a pale green oil (360 mg), from which the diastereomeric mixture of the lactams (**9a** and **9b**) was separated as semisolid by trituration (200 mg): IR (KBr): 3700—2300 (br), 1730, 1685, and 1100 cm^{-1}. This mixture afforded *threo* and *erythro* γ-hydroxyglutamic acid (by amino acid analysis) when it was heated with 6M hydrochloric acid at 100 °C for one day.

Further elution with 15% trimethylamine solution afforded a light yellow oil (1.05 g). The oil (965 mg) was dissolved in water (0.5 ml). Hydrochloric acid (3M) was added in small portions until the pH of the solution reached 3. The precipitated *erythro*-γ-hydroxyglutamic acid was collected by filtration (417 mg), mp 165—166 °C (dec) [lit,[7] 166 °C (dec)]. Analytical sample was obtained by recrystallization from water–ethanol. (Found: C, 36.82; H, 5.49; N, 8.67%. Calcd for $C_5H_9NO_5$: C, 36.81; H, 5.56; N, 8.59%).

3-Ethoxycarbonyl-5-isopropoxycarbonyl-2-isoxazoline (5b). In a 100-ml flask was placed isopropyl acrylate (1.96 g) in dry chloroform (5 ml), and to this mixture was added triethylamine (5 ml). A solution of the chloro oxime (**4**) (1.9 g) in chloroform (10 ml) was added to the mixture with stirring during 1 h at room temperature. The mixture was stirred at room temperature for 20 h, and diluted with chloroform. The solution was washed with several portions of water, and dried over sodium sulfate. The solution was concentrated affording a light brown oil, which was distilled under vacuum (1.63 g); bp 130—134 °C/3 mmHg. NMR ($CDCl_3$): 1.39 (6H, d, J=7 Hz), 1.43 (3H, t, J=7 Hz), 3.43 (2H, d, J=10 Hz), 4.32 (2H, q, J=7 Hz), 5.10 (1H, t, J=10 Hz), and 5.05 (1H, heptet, J=7 Hz). Found: C, 52.18; H, 6.67; N, 6.01%. Calcd for $C_{10}H_{15}O_5N$: C, 52.39; H, 6.60; N, 6.11%.

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