Amino Acid Synthesis from Nitromalonic Ester. II[1]. *Synthesis of Proline and Glutamic-γ-semialdehyde*

By Tôru OKUDA

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Akabori, Izumi and Okuda[1] have recently reported the synthetic preparations of DL-ornithine, starting from acrylonitrile and readily available diethyl nitromalonate (I) which has a reactive methine group. A new synthesis of DL-proline and the dimethyl acetal of glutamic-γ-semialdehyde (VI) which is biochemically interesting, but has been tedious for synthetic approach, from the same nitroester (I) and acrolein is described in this communication.

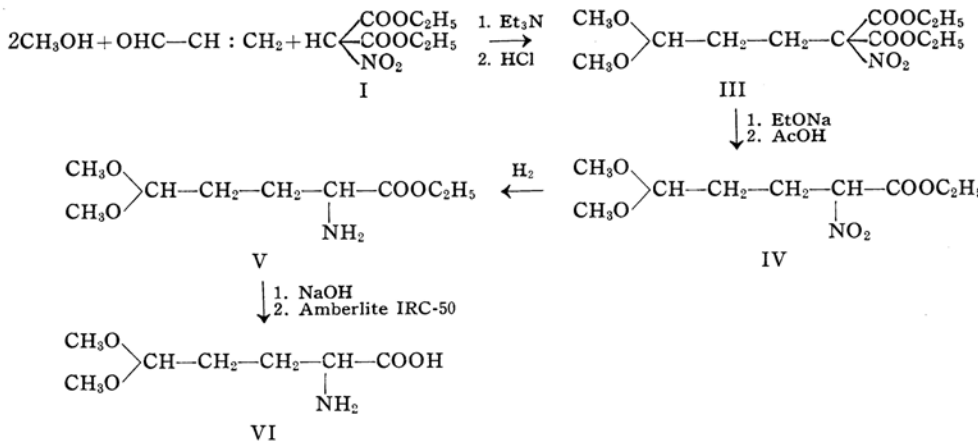
Since Albertson and Fillman[2] accomplished an excellent and practical work for the preparation of DL-proline, an amino acid that had been very difficult to synthesize, several similar or different methods[3-6] have appeared. Although inferior to their method in the yield, a simpler procedure to obtain this amino acid has been found. The addition reaction of diethyl nitromalonate (I) to acrolein proceeded very smoothly in the presence of basic catalyst such as triethylamine at lower temperature. The resulting γ,γ-dicarbethoxy-γ-nitrobutyraldehyde (II), of which the 2, 4-dinitrophenylhydrazone had already been prepared by Yamada et al[7] from the crude product for the characterization, was distilled without any decomposition in an yield of 94%. γ-Aminobutyraldehyde and the related compounds are reported to react in the cyclized form under the usual conditions[8,9]. The possibility that in the γ-aminaldehydes ring formation may be substantially complete, was proved by Vogel and Davis[10] by the fact that crude glutamic-γ-semialdehyde or Δ^1-pyrroline-5-carboxylic acid prepared from γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde was hydrogenated catalytically to proline.

Therefore, cyclization would be expected if γ-nitrobutyraldehyde (II) were reduced to the corresponding γ-amino compound. Accordingly, the catalytic hydrogenation of γ,γ-dicarbethoxy-γ-nitrobutyraldehyde (II) has been undertaken in glacial acetic acid containing an equimolecular amount of fused sodium acetate as condensing agent[11]. The possible intermediates, 5,5-dicarbethoxy-Δ^1-pyrroline and the ethyl ester of α-carbethoxyproline were not able to be isolated. On refluxing the hydrogenated product with hydrochloric acid and on the subsequent treatment with ammonium rhodanilate, DL-proline was obtained in an yield of 12–21%.

It is well-known that glutamic-γ-semialdehyde is a common intermediate in the enzymatic interconversions of glutamic acid, proline and ornithine. Several biochemical studies are currently underway on this compound in various laboratories, but the inaccessibility in a practical amount appears to restrict the use in experiments. The Vogel's method[10] to prepare the semialdehyde itself was re-investigated in laboratory scale, but appeared inadequate from the standpoint of preparation. Good and Mitchell[12] prepared the diethyl acetal of the glutamic-γ-semialdehyde with very poor yield by alkaliere hydrolisis of the diethyl acetal of γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde and paper-chromatographic separation.

The method reported here affords a simple and practical synthesis of the dimethyl acetal of the semialdehyde (VI) through the following reaction diagram:

1) Paper I in this series, S. Akabori, Y. Izumi and T. Okuda, *J. Chem. Soc. Japan*, **77**, 490 (1956).
2) N. F. Albertson and J. L. Fillman, *J. Am. Chem. Soc.*, **71**, 2818 (1949).
3) P. E. Gagnon, J. L. Boivin and P. A. Boivin, *Can. J. Research*, **28B**, 207 (1950).
4) H. Plieninger, *Chem. Ber.*, **83**, 2710 (1950).
5) J. Caprová-Jirků, J. V. Koštir and M. Vondráček, *Chem. Listy*, **44**, 19 (1950).
6) R. Gaudry and L. Berlinquet, *Can. J. Research*, **28B**, 245 (1950).
7) S. Yamada, I. Chibata and T. Tsurui, *J. Pharm. Soc. Japan*, **73**, 123 (1953).
8) C. Schöpf and F. Oechler, *Ann.*, **523**, 1 (1936).
9) C. Schöpf and H. Steuer, ibid., **558**, 124 (1947).
10) H. J. Vogel and B. D. Davis, *J. Am. Chem. Soc.*, **74**, 109 (1952).
11) "Organic Reactions", Vol. IV, John Wiley & Sons, Inc. New York, N. Y. 1948, p. 201.
12) N. Good and H. K. Mitchell, *J. Am. Chem. Soc.*, **74**, 4953 (1952).
13) Ulpiani, *Gazz. chim. ital.*, **34**, 174 (1904).
14) D. I. Weisblat and D. A. Lyttle, *J. Am. Chem. Soc.*, **71**, 3078 (1949).



When the above-described addition of diethyl nitromalonate (I) to acrolein was carried out in methanolic solution and the reaction mixture was then directly treated with a small amount of dry hydrogen chloride, the acetal diester (III) was readily obtained in one step in fairly good yield. After the decarbethoxylation of III by the Ulpiani's method[13,14,1], the resulting acetal monoester (IV) was hydrogenated catalytically under pressure in methanol. The ester of the acetal amino acid (V) was readily purified by vacuum distillation with an yield of about 80%. On treatment of the amino acid ester (V) with aqueous sodium hydroxide and then with Amberlite IRC-50 exchange resin to remove sodium ions, the dimethyl acetal of glutamic-γ-semialdehyde (VI) was obtained as white crystals. Although the diethyl acetal of the semialdehyde was reported to be very soluble in ethanol[12], the dimethyl acetal was found to be less soluble like other common amino acids.

Experimental

γ,γ-Dicarbethoxy-γ-nitrobutyraldehyde (II).—In a three-necked flask fitted with a drying tube, a thermometer, a dropping funnel and a stirrer was placed a solution of 102.5 g. (0.50 mole) of diethyl nitromalonate (I) in 450 ml. of ethanol containing 0.2 ml. of triethylamine. A mixture of 29 g. (0.52 mole) of acrolein and 30 ml. of ethanol was added dropwise to this with vigorous stirring over the course of about one hour at such a rate that the temperature remained below 40°C. After additional stirring for two hours the solvent was removed in vacuo and the residual oil was distilled. One hundred twenty-three grams of γ, γ-dicarbethoxy-γ-nitro-butyraldehyde was obtained as yellowish oil, b. p. 134-137°C/1 mm.

Anal. Found: N, 5.31. Calcd. for $C_{10}H_{15}O_7N$: N, 5.36%.

2,4-Dinitrophenylhydrazone of the aldehyde (II) prepared by usual method melted at 74-76°C after recrystallization from methanol-water. (*lit.* 74-76°C[7])

Anal. Found: N, 15.75. Calcd. for $C_{16}H_{19}O_{10}N_5$: N, 15.87%.

DL-Proline.—In the pressure bottle of an apparatus for catalytic reduction were placed 26.1g. (0.10 mole) of freshly distilled γ,γ-dicarbethoxy-γ-nitrobutyraldehyde (II), 9 g. of fused sodium acetate, 130 ml. of glacial acetic acid and 10 g. of Raney nickel. An initial pressure of 90 atm. of hydrogen was applied. When, after about twenty-four hours, 0.4 mole of hydrogen had been absorbed, the reaction was stopped and the catalyst was removed by filtration. After removing the acetic acid in vacuo, 50 ml. of concentrated hydrochloric acid was added to the syrupy residue and the mixture was cooled overnight in an ice box. Precipitated Sodium chloride was filtered off and the filtrate was refluxed for five hours. The dark solution was concentrated to a syrup under reduced pressure, then taken up in water and charcoaled. Then the pH of the solution was adjusted to 2.6-3.0 by adding aqueous sodium hydroxide and the solution was treated with methanolic solution of 25 g. of ammonium rhodanilate by the usual method. Recrystallization of the crude product from isopropyl alcohol-ether gave 2.5 g. of white crystals of DL-proline, m. p. 206° (dec.). Yield, 21%.

Anal. Found: N, 11.84. Calcd. for $C_5H_9O_2N$: N, 12.17%.

Ethyl δ,δ-Dimethoxy-α-carbethoxy-α-nitrovalerate (III, Dimethyl Acetal of γ,γ-Dicarbethoxy-γ-nitrobutyraldehyde).—102.5 g. (0.50 mole) of diethyl nitromalonate (I), 29 g. (0.52 mole) of acrolein and 360 ml. of methanol instead of ethanol were treated in the same manner as described above. About 3 g. of dry hydrogen chloride was dissolved in the mixture and then 70 g. of anhydrous sodium sulfate was added. After standing at room temperature for forty-eight hours, the reaction mixture was neutralized with aqueous sodium bicarbonate and concentrated under reduced pressure. The residue was then poured into about 1 l. of ice water, the aqeous phase was extracted three times with ether or isopropyl ether and the extracts were combined

with the oil. The combined ether solution was washed twice with water and dried over sodium sulfate in an ice box. After removal of the ether, the residue was distilled at 0.5 mm. and the fraction boiling between 145–150°C was collected as the product. The dimethyl acetal of γ,γ-dicarbethoxy-γ-nitrobutyraldehyde (III) thus obtained was yellowish and weighed 130 g. Yield, 84.6%.

Anal. Found: N, 4.62. Calcd. for $C_{12}H_{21}O_8N$: N, 4.56%.

Ethyl δ,δ-Dimethoxy-α-nitrovalerate (IV, Dimethyl Acetal of γ-Carbethoxy-γ-nitrobutyraldehyde).—Ethyl δ,δ-dimethoxy-α-carbethoxy-α-nitrovalerate (III), 42 g. (0.136 mole), in 400 ml. of anhydrous isopropyl ether was cooled in an ice-bath. A solution of 3.25 g. (0.141 mole) of sodium in 150 ml. of absolute alcohol was added slowly, with vigorous stirring, over a period of two hours. Precipitation of the sodium salt of IV and probably some sodium ethylate occured to form a very thick slurry. This was stirred further for five hours and allowed to stand overnight in an ice-box and then filtered. The salt was washed twice with 50 ml. portions of ether, transferred to a separatory funnel, together with the salt obtained from the mother liquor, covered with 200 ml. of isopropyl ether and acidified with 45ml. of 20% aqueous acetic acid. Vigorous shaking was continued until all the solid had disappeared. The ether layer was separated and the aqueous phase extracted with ether. The ether solutions were combined and washed with two 50 ml. of water. Drying, concentration and vacuum-distillation gave 30 g. (94%) of oily IV, which boiled at 118–123°C/2 mm.

Anal. Found: C, 45.94; H, 6.91; N, 5.86. Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.29; N, 5.96%.

Hydrolysis of the acetal group of IV and the subsequent treatment with 2, 4-dinitrophenyl hydrazine gave yellow crystals that melt at 106–108°C. (*lit.* 110–111°C[15]).

Anal. Found: C, 41.93; H, 4.26; N, 18.91. Calcd. for $C_{13}H_{15}O_8N_5$: C, 42.28; H, 4.07; N, 18.97%.

Ethyl δ,δ-Dimethoxy-α-aminovalerate (V).—The reduction of 47 g. (0.2 mole) of IV was carried out in a stainless steel autoclave using 200 ml. of methanol and 4 g. of Raney nickel catalyst at 50–70°C for three hours. The autoclave was heated as rapidly as possible to avoid the formation of undesirable by-products. The catalyst was removed by filtration and the methanol was distilled off in vacuo. Thirty-three grams of V was obtained as colorless liquid by distillation; b. p. 105–107°C at 2 mm. Yield, 80.5%.

Anal. Found: C, 51.87; H, 9.23; N, 6.66. Calcd. for $C_9H_{19}O_4N$: C, 52.68; H, 9.27; N, 6.34%.

Dimethyl Acetal of Glutamic-γ-semialdehyde (VI).—Five grams of ethyl δ,δ-dimethoxy-α-aminovalerate (V) was dissolved in 10 ml. of 10% aq. sodium hydroxide solution and the mixture was heated at 60°C for about one hour. The aqueous solution of the sodium salt of the dimethyl acetal of glutamic-γ-semialdehyde was treated with Amberlite IRC-50 and then evaporated to dryness in vacuo. The crude product which melts at 206–209°C was dissolved in a small volume of warm water, and then charcoaled. Addition of ethanol and ether and cooling gave 2.5 g. of white crystals of VI melting at 215–217 °C (dec.). This amino acid is very soluble in water, less soluble in ethanol and insoluble in ether and benzene.

Anal. Found: C, 47.08; H, 8.14; N, 7.78. Calcd. for $C_7H_{15}O_4N$: C, 47.46; H, 8.47; N, 7.91%.

15) O. A. Moe and D. T. Warner, *U. S. Patent.*, 2, 599, 653 (June 10, 1952).

Summary

1. A synthesis of DL-proline from diethyl nitromalonate and acrolein by intramolecular reductive alkylation is described.

2. A new method for preparation of the dimethyl acetal of glutamic-γ-semialdehyde from the same starting materials in an over-all yield of 38% (based on diethyl nitromalonate) is also presented.

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Laboratory of Organic Chemistry
Faculty of Science
Osaka University, Kita-ku, Osaka