A Synthesis of L-Proline from L-Glutamine

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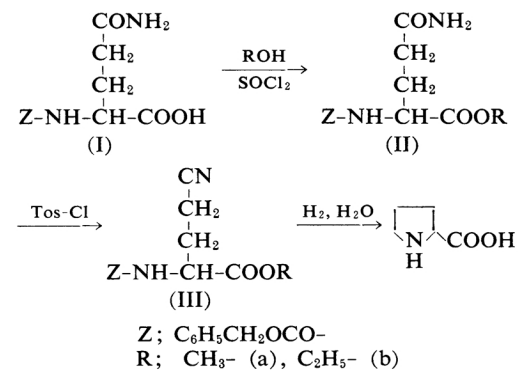
(Received July 28, 1962)

Since the resolution of racemic proline is difficult, it would be advantageous to develope a practical method for the synthesis of optically active proline. Two successful syntheses of L-proline in which L-glutamic acid was the starting material have been described to date. The method described by Pravda and Rudinger[1], which makes use of the selective reduction of the lactam group of tosyl-L-pyrrolidonecarboxylic acid derivatives by lithium aluminum hydride, resulted in an overall yield of 12% from L-glutamic acid in seven steps. Tanaka et al.[2] obtained L-proline in a 34% yield from methyl L-pyrroglutamate by means of sodium borohydride reduction.

In the course of an investigation of the dehydration products of L-glutamine derivatives, the author developed a novel method for the synthesis of optically active proline. It was found that catalytic hydrogenation of γ-cyano-α-L-carbobenzoxyaminobutyric acid esters in aqueous solvents produced L-proline in high yield under suitable conditions. Thus a single hydrogenation treatment in the presence of water effects pyrrolidine ring formation between the γ-carbon atom and the α-amino nitrogen atom without racemization by successive hydrogenation, hydrolysis and ring closure. The schematic representation of the synthesis of proline from carbobenzoxyglutamine is as follows:



Z; $C_6H_5CH_2OCO-$
R; CH_3- (a), C_2H_5- (b)

The author feels that the method will be of

1) Z. Pravda and J. Rudinger, *Colletion Czech. Chem. Commun.*, **20**, 1 (1955).
2) M. Tanaka, T. Kishi and S. Kinoshita, *J. Agr. Chem. Soc. Japan (Nippon Nōgei-Kagaku Kaishi)*, **34**, 782 (1960).

practical value for the synthesis of optically active proline because the starting material, L- or D-glutamine, is readily prepared from L- or D-glutamic acid, respectively, by a method previously reported by Akabori and Narita[3].

Results and Discussion

Preparation of Carbobenzoxy-L-glutamine Esters. — The methyl ester IIa was prepared from carbobenzoxy-L-glutamine[4-7] by the action of diazomethane[8], or by esterification with methanol in the presence of thionyl chloride at room temperature. In the latter case, increasing the reaction time or amount of thionyl chloride, or elevating the temperature resulted in increase of alcoholysis of the amide group to produce dimethyl carbobenzoxy-L-glutamate. The ethyl ester IIb was obtained by esterification with ethanol in the presence of thionyl chloride.

Taschner and Wasielewski[9] proposed the use of sulfuryl chloride as catalysts for the esterification of acylated amino acids. In the present work it was found that thionyl chloride is also a useful reagent under suitable conditions.

Dehydration of Carbobenzoxy-L-glutamine Esters.—Procedures for converting carboxamide residues of glutamine or asparagine derivatives to nitrile residue with suitable dehydrating reagents, *p*-toluenesulfonyl chloride, phosphorous oxychloride or *N,N'*-dicyclohexylcarbodiimide, were reported recently[10-12].

The author observed that carbobenzoxy-L-glutamine esters in pyridine solution were dehydrated to the corresponding cyano compounds in excellent yield by the action of *p*-toluenesulfonyl chloride, phosphorous oxychloride or thionyl chloride at 50°C for 30 to 50 min. *p*-Toluenesulfonyl chloride was the most suitable dehydrating reagent in these cases. Methyl γ-cyano-α-L-carbobenzoxyaminobutyrate (IIIa) was obtained as long crystalline needles, m. p. 52°C, from ethyl acetate-petroleum benzine mixture. The corresponding ethyl ester IIIb was obtained as crystalline leaflets, m. p. 60°C, from the same solvent system.

The infrared spectra of these cyano compounds showed characteristic nitrile absorption in the 2250 cm^{-1} region.

Hydrogenation of Methyl γ-Cyano-α-L-carbobenzoxyaminobutyrate. — Methyl γ-cyano-α-L-carbobenzoxyaminobutyrate was hydrogenated in a pressure bottle under various conditions as listed in the table.

After the catalyst was removed by filtration, the reaction mixture was hydrolyzed with hydrochloric acid, and the hydrolyzate was analyzed qualitatively by paper chromatography and quantitatively by colorimetry.

The main product was either proline or

TABLE I. HYDROGENATION OF METHYL γ-CYANO-α-L-CARBOBENZOXYAMINOBUTYRATE

Exp. no.	Catalyst	Solvent, ml.			Temp. °C	Time hr.	Yield of proline, %
		H_2O	MeOH	AcOH			
1	Silk-palladium 50 mg.	12	—	—	80	4	65
2	Silk-palladium 50 mg.	6	6	—	80	2	68
3	Silk-palladium 50 mg.	6	6	0.2	80	2	70
4	Silk-palladium 50 mg.	8	—	4	80	2	76
5	Silk-palladium 50 mg.	8	—	4	110	2	76
6	Silk-palladium 50 mg.	8	—	4	150	2	82
7	Silk-palladium 50 mg.	8	—	4	50	16	33
8	Silk-palladium 50 mg.	—	—	12	80	2	0*
9	Silk-platinum 10 mg.	8	—	4	80	2	0**
10	Silk-rhodium 10 mg.	8	—	4	80	2	0**
11	Raney nickel 100 mg.	8	—	4	80	2	0**
12	Raney nickel 100 mg.	—	—	12	80	2	0**

Each experiment was carried out in a 100 ml. stainless steel autoclave, with 0.5 g. substrate and initial pressure of hydrogen 70 kg./cm^2.

* Almost unreacted ** Main product was ornithine.

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9) E. Taschner and C. Wasielewski, *Ann.*, **640**, 137 (1961).
10) Z. Zaoral and J. Rudinger, *Collection Czech. Chem. Commun.*, **24**, 1993 (1959).
11) B. Liberek, *Chem. & Ind.*, **1961**, 987.
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ornithine, and the major side product was glutamic acid in all cases. One more ninhydrin positive spot which was believed to be δ-hydroxy-*n*-valine was present in some cases (Exp. no. 1–6, 11).

In order to determine the yield of proline by the ninhydrin method, ammonia formed in the hydrogenation was removed from the hydrolyzates by the addition of aqueous sodium hydroxide and evaporation under diminished pressure. The residue was dissolved in water and evaporated to dryness twice more, and the final residue was dissolved in water and adjusted pH to 7 with hydrochloric acid. The composition of this solution was determined colorimetrically by the ninhydrin method as usual[13]. The yield of proline was calculated from the optical densities at 440 and 570 mμ.

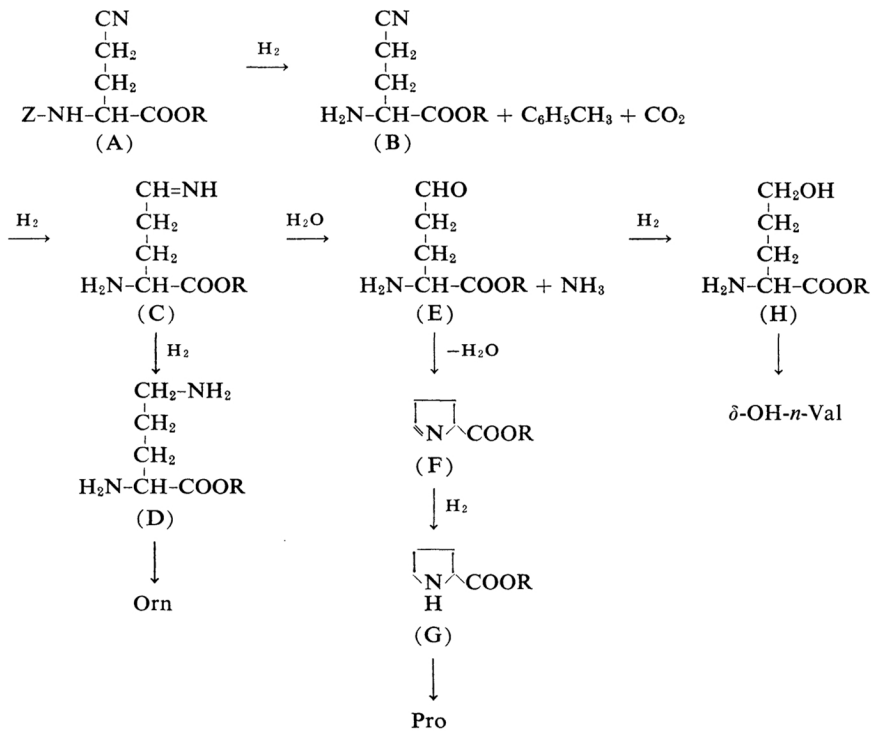
As catalyst for hydrogenation, silk-palladium[14], silk-platinum[15], silk-rhodium[16] and Raney nickel were used.

In aqueous solvent, silk-palladium catalyst produced proline as the main product with some ornithine, glutamic acid and δ-hydroxy-*n*-valine as by-products (Exp. no. 1–7). In acetic acid no hydrogenation occurred and glutamic acid was the only reaction product observed on paper chromatogram (Exp. no. 8). Rather low yield of proline and relatively high yield of ornithine was observed in water or water-methanol system (Exp. no. 1, 2). The pH of the reaction mixture was 9 and the high pH of the reaction media was believed responsible for the increased yield of ornithine. Water-acetic acid system increased the yield of proline (Exp. no. 3—6).

The three other catalysts, silk-platinum, silk-rhodium and Raney nickel, produced ornithine as almost the sole product in water-acetic acid mixture (Exp. no. 9—11). In these cases, only traces of proline were detected on paper chromatogram, and yield of ornithine was estimated to be over 80%.

The schematic representation of the hydrogenation yielding proline, ornithine, glutamic acid and δ-hydroxy-*n*-valine from γ-cyano-α-carbobenzoxyaminobutyric ester is shown in Scheme 1.

Hydrogenation of aldimine (C) to yield ornithine ester (D) is competitive with hydrolysis to yield aldehyde intermediate (E) which forms a pyrroline ring (F) by spontaneous ring closure. The pyrroline ring is hydrogenated to yield a pyrrolidine ring (G). The



Scheme 1. Reaction scheme of hydrogenation

13) S. Moore and W. H. Stein, *J. Biol. Chem.*, **211**, 907 (1954).
14) Y. Izumi, This Bulletin, **32**, 932, 936, 942 (1959).
15) A. Akamatsu, Y. Izumi and S. Akabori, ibid., **34**, 1067, 1302 (1961).
16) A. Akamatsu, Y. Izumi and S. Akabori, ibid., **35**, 1706 (1962).

aldehyde intermediate (E) is also hydrogenated to form δ-hydroxy-*n*-valine ester (H).

According to this scheme, Raney nickel, silk-platinum and silk-rhodium are highly active catalysts for the reduction of aldimine (C), but silk-palladium has low activity; consequently the hydrolytic reaction of aldimine to produce aldehyde (E) occurs predominantly.

Experimental*

Carbobenzoxy-L-glutamine Methyl Ester (IIa).—Carbobenzoxy-L-glutamine (I), 28 g. (100 mmol.), was dissolved in 200 ml. of methanol containing thionyl chloride, 0.6 g. (5 mmol.), and the mixture was allowed to stand at 15°C for 48 hr. The methanol was distilled off under reduced pressure, and the residual crystalline product was washed thoroughly with cold water and dried. The crude product was recrystallized from methanol-ether. Aggregated colorless needles. Yield 25 g. (85%), m. p. 141~142°C (lit. 140~141°C[8]). $[\alpha]_D^{18}$ −23.6° (*c* 3, MeOH) (lit. $[\alpha]_D^{23}$ −19.4° (*c* 1, MeOH[8])).

Found: N, 9.26. Calcd. for $C_{14}H_{18}O_5N_2$: N, 9.52%.

From the mother liquor of recrystallization a colorless syrup was obtained which became a solid of very low melting point after several days. Elementary analysis and infrared spectra showed that this compound was the alcoholytic product of IIa, dimethyl carbobenzoxy-L-glutamate.

Found: C, 58.27; H, 6.26; N, 4.51. Calcd. for $C_{15}H_{19}O_6N$: C, 58.24; H, 6.19; N, 4.53%.

Carbobenzoxy-L-glutamine Ethyl Ester (IIb).—This compound was prepared from carbobenzoxy-L-glutamine, 14 g. (50 mmol.), and thionyl chloride 0.6 g. (5 mmol.), in 100 ml. of ethanol according to the procedure described for the preparation of the methyl ester IIa. Aggregated needles. Yield 80%, m. p. 125~126°C, $[\alpha]_D^{18}$ −20.6° (*c* 3, MeOH).

Found: C, 58.55; H, 6.53; N, 8.98. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.43; H, 6.54; N, 9.09%.

Methyl γ-Cyano-α-L-carbobenzoxyaminobutyrate (IIIa).—To a solution of carbobenzoxy-L-glutamine methyl ester (IIa), 18.4 g. (626 mmol.) in pyridine (120 g.), *p*-toluenesulfonyl chloride, 15 g. (788 mmol.) was added, and the mixture kept at 50°C for 30 min. The resulting dark red solution was concentrated under reduced pressure to obtain an oily residue, which was treated with 30 ml. of water and extracted three-times with ethyl acetate. The combined ethyl acetate extracts were washed successively with diluted hydrochloric acid, water, aqueous sodium bicarbonate and water, then decolorized with active charcoal and dried over magnesium sulfate. The concentrated ethyl acetate solution, to which petroleum benzine had been added just to the cloudy point, was stored in a refrigerator overnight. Long featherlike needles were collected and recrystallized from an ethyl acetate-petroleum benzine mixture to yield 14.2 g. (82%) of the ester (IIIa). M. p. 51~52°C, $[\alpha]_D^{21}$ −34.5° (*c* 3, MeOH).

Found: C, 60.82; H, 5.80; N, 10.04. Calcd. for $C_{14}H_{16}O_4N_2$: C, 60.86; H, 5.84; N, 10.14%.

* All melting points are not corrected.

Ethyl γ-Cyano-α-L-carbobenzoxyaminobutyrate (IIIb).—This compound was prepared as described for the methyl ester IIIa from carbobenzoxy-L-glutamine ethyl ester (IIb). Crystalline leaflets. Yield 70%, m. p. 59~60°C, $[\alpha]_D^{18}$ −30.7° (*c* 3, MeOH).

Found: C, 61.85; H, 6.15; N, 9.51. Calcd. for $C_{15}H_{18}O_4N_2$: C, 62.05; H, 6.25; N, 9.65%.

L-Proline.—In a 100 ml. autoclave were placed methyl γ-cyano-α-L-carbobenzoxyaminobutyrate (IIIa), 2.76 g. (10 mmol.), water, 15 ml., acetic acid, 10 ml. and silk-palladium catalyst, 138 mg. An initial pressure of 80 kg./cm² of hydrogen was applied. After 2 hr. of shaking at 120°C, the reaction was stopped, and the catalyst was removed by filtration. The reaction mixture was concentrated under reduced pressure, then hydrolyzed with 60 ml. of 3 N hydrochloric acid for 3 hr. After the hydrolyzate was evaporated in vacuo to remove excess hydrochloric acid, the residue was dissolved in 300 ml. of deionized water and passed through a column (16×400 mm.) of Amberlite CG-45 (OH cycle) then CG-50 (H cycle). The combined effluents and washings were evaporated to dryness. The residue was taken up in absolute ethanol and the proline precipitated by addition of ether. Repeated recrystallization gave 0.75 g. (65%) of non-hygroscopic white crystals of optically and chromatographically pure L-proline. The identity of thus obtained L-proline with authentic L-proline was also confirmed by infrared spectra. M. p. (decomp.) 216~218°C (lit. 226~228°C[1]), $[\alpha]_D^{23}$ −83.0° (*c* 1, H_2O) (lit. $[\alpha]_D^{24}$ −84.6°[1]).

Found: N, 12.02. Calcd. for $C_5H_9O_2N$: N, 12.17%.

D-Ornithine Hydrochloride.—In a 100 ml. autoclave were placed methyl γ-cyano-α-D-carbobenzoxyaminobutyrate (prepared starting from D-glutamic acid) (IIIa), 5.00 g. (18.1 mmol.), acetic acid, 30 ml. and Raney nickel catalyst prepared from 2 g. of the alloy*. High pressure hydrogenation at 90 kg./cm² of initial pressure at 100°C was continued for 2 hr. After removal of the catalyst by filtration and the acetic acid by vacuum evaporation, the residue was hydrolyzed with concentrated hydrochloric acid for 3 hr. under reflux. After excess of hydrochloric acid was removed by vacuum evaporation, the residue was diluted to 100 ml. and adjusted pH to 3 by addition of sodium bicarbonate. Hydrogen sulfide gas was bubbled to precipitate traces of nickel ion, the filtrate was evaporated to dryness. The residue was extracted three times with 95% ethanol. The combined ethanol extract was treated with pyridine to obtain crude crystals of D-ornithine hydrochloride which was recrystallized three times from water-ethanol. Yield 2 g. (63%), m. p. (decomp.) 227~228°C (lit. 236~237°C[10]), $[\alpha]_D^{20}$ −21.7° (*c* 3, 6 N HCl) (lit. −22.5°[10]).

Found: N, 16.48. Calcd. for $C_5H_{13}N_2O_2Cl$: N, 16.61%.

* The alloy was added to 20 ml. of 20% aqueous sodium hydroxide solution, and the mixture was warmed at 80°C for 20 min. The sodium hydroxide solution was decanted, and the catalyst was washed three times with water and twice with acetic acid by decantation.

Summary

1. Novel syntheses of optically active proline and ornithine from optically active glutamine are described.

2. Carbobenzoxy-L-glutamine esters were dehydrated to γ-cyano-α-L-carbobenzoxyaminobutyric acid esters with *p*-toluenesulfonyl chloride, phosphorous oxychloride or thionyl chloride.

3. Hydrogenation of γ-cyano-α-L-carbobenzoxyaminobutyric acid esters in aqueous solvent systems were effected by silk-palladium catalyst to yield mainly L-proline.

4. By hydrogenation in the presence of Raney nickel in non-aqueous solvent, L-ornithine was obtained.

The author wishes to express his hearty thanks to Professor Shiro Akabori and Dr. Yoshiharu Izumi for their cordial guidance and encouragement throughout this work. He is also grateful to Miss Kiku Koike and Miss Masako Kawakita for microanalysis.

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