Studies on Serine Peptides. I. Optical Resolution of O-Benzyl-DL-serine and Synthesis of DL-Serine Peptides

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The serine peptides are important in organic and biological chemistry. For example, these peptides readily undergo hydrolysis with dilute acids or alkalis and acyl migration occurs in the acidic condition. Some of these peptides have been considered to have growth-promoting effect or enzymatic activity. However, there have been few papers available concerning the synthesis of the serine peptides[1], mainly because this amino acid has a free hydroxyl group which induces side reaction in the peptide synthesis.

The author tried in the present work to synthesize the serine peptide from the O-benzyl-serine[2] in which the hydroxyl group was protected with a benzyl rest. An optically active O-benzyl-L-serine was prepared by the enzymatic resolution of the N-acetyl derivative with the takadiastase. The O-benzyl-L-serine was easily obtained in 78% yield from the reaction mixture, since this amino acid was slightly soluble in water.

Price reported in his paper that the optical active L-serine was obtained from the N-acetyl-DL-serine in 40% yield[3].

The O-benzyl-L-serine showed a specific rotation of +5.86 in 1 N-hydrochloric acid solution at 15°C, and the L-serine which was prepared quantitatively by the acid hydrolysis of the benzyl derivative, showed a rotation of +14.6 in 1N-hydrochloric acid solution at the same temperature.

At the preliminary experiment, the O-benzyl-L-serine peptides have been synthesized by the N-carboxylic acid anhydride method (N. C. A. method) of Bailey[4], but satisfactory results were not obtained except in the case of a seryl-glycine peptide.

With O-benzyl-DL-seryl-L-glutamic acid, O-benzyl-DL-seryl-L-leucine, and O-benzyl-DL-seryl-L-phenylalanine, so many by-products were formed during the reaction period that the purification of the products was very difficult.

O-Benzyl-DL-seryl-L-leucine ethyl ester and DL-seryl-L-leucine have been synthesized by the Fischer's acid chloride method.

It has been found from the above experiments that the O-benzyl-serine can be utilized as the starting material in the peptide synthesis.

Experimental

Enzymatic Resolution of O-benzyl-DL-serine.—O-Benzyl-N-acethyl-DL-serine.—Into a mixture of 33 g. of O-benzyl-DL-serine and 83 cc. of 2N-sodium hydroxide solution were added separately 25 cc. of acetic anhydride and 163 cc. of 2N-sodium hydroxide solution under cooling. The reaction mixture was neutralized with 1 N-hydrochloric acid solution, when white crystals precipitated. Recrystallization from hot water gave 37 g. of the crystals in 80% yield, m.p. 139–140°C.

Anal. Found: C, 60.59; H, 6.67. Calcd. for $C_{12}H_{15}O_4N_1$: C, 60.76; H, 6.38%.

Carboxypeptidase Preparation.—Twenty six grams of Takadiastase were extracted with distilled water at 0°C. The mixture was filtered using Celite. This extracted solution was used directly in the enzymatic resolution. The acetyl derivative (33 g.) was dissolved in 24 cc. of 6N-sodium hydroxide solution. To this solution were added the above enzymatic extract and 60 cc. of phosphate buffer which brought the total mixture to pH 6.8. In order to insure complete hydrolysis, the digests were usually set up in the late afternoon and allowed to stand at 37°C. until early next morning. The L-amino acid crystallized in high yield and usually filled the digestion flask. The mixture is brought to pH 5.0 by the addition of glacial acetic acid. The L-amino acid was filtered by suction, and washed with cold water. The mother liquor was combined with washings and concentrated in vacuo to a low bulk. A small amount of the L-amino acid appeared during this concentration. The crystals were combined with the main product and recrystallized from hot water with the aid of Celite. Optically active 11 g. of crystals were obtained in 78% yield, $[\alpha]_D^{15} = +5.86$. (in 1N-hydrochloric acid) The residual concentrated solution was brought to pH 2.0 with conc. hydrochloric acid. The acetyl derivative was extracted several times into ethylacetate. The extracts were combined, dried for a few minute over anhydrous sodium sulfate, and finally filtered. The ethylacetate was removed in vacuo. To the oily residue was added twice their volume of petroleum ether and it was allowed

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1) A. Miekely and J. Fruton, *J. Biol. Chem.*, **146**, 463 (1942); D. Wooley, *J. Biol. Chem.*, **159**, 753 (1948); R.F. Fischer and R.R. Whetson, *J. Am. Chem. Soc.*, **76**, 5076 (1954); M. Botunik, *Zhur. Obschcliei, Khim*, **23**, 1716-19 (1953).

2) K. Okawa and H. Tani, *J. Chem. Soc. Japan*, **75**, 1199 (1954).

3) V. Price, *J. Biol. Chem.*, **179**, 1169 (1949).

4) J. Bailey, *J. Chem. Soc.*, **1950**, 3461.

to stand over night in an ice bath, then white precipitate was obatined. After recrystallization from hot water, 13.6 g. of N-acetyl-D-derivative was prepared in fine crystals. in 80% yield, m.p. 103°C.

D- and L-Serine.—D- and L-Serine were obtained by acid hydrolysis of benzyl derivatives with 5N-hydrochloric acid for half an hour. The D- and L-form exhibit $[\alpha]^{15}_{D}$ of −14.6 and +14.6 in 1N-hydrochloric acid solution, respectively.

O-Benzyl-DL-serine N.C.A.—This was prepared by the reaction of carbonylchloride with O-benzyl-DL-serine in dioxane at 40°–50°C by the method of Leuchs[5,6]. Several times recrystallization from ethylacetate-petroleum ether gave white crystals, m.p. 76°-77°C, in 82% yield.

Preparation of Serine Peptides.—The peptide coupling experiments were carried out under anhydrous conditions. All solvents were stored over suitable dry reagents.

O-Benzyl-DL-seryl-glycine.—Glycine ethylester hydrochloride (0.7 g.) was treated with 3.3 cc. of 1.66N-ammonium-chloroform solution and the reaction mixture filtered from the precipitated ammonium-chloride. After removal of a slight excess of ammonia by the concentration of the solvent in vacuo, 6.6 cc. of ethylacetate were added and the solution was cooled to 65°C in solid carbondioxide-aceton bath. After addition of triethylamine (0.5 cc.) to the ester solution, a solution of DL-serine N. C. A. (0.9 g.) in 6.6 cc. of ethylacetate was precooled to 65°C and slowly run into the mixture. The reaction mixture was allowed to stand at this temperature for six hours, the mixture was then warmed to room temperature and the solvent removed in vacuo. The O-benzyl-DL-seryl-glycine ethylester was hydrolized with 11.1 cc. of 0.37 N-barium hydroxide, neutralized with 11.1 cc. of 0.37 N-sulfuric acid and concentrated in vacuo. White crystals were obtained. Recrystallization gave 0.15 g. of O-benzyl-DL-seryl-glycine, m.p. 202°C, in 65% yield.

Anal. Found: N, 10.95. Calcld. for $C_{12}H_{15}O_4N_2$: N, 11.10%.

DL-Seryl-glycine.—O-Benzyl-DL-seryl-glycine (0.3 g.) was suspended in 30 cc. of methyl alcohol. After addition of palladium oxide (0.3 g.) and 0.2 cc. of hydrochloric acid, the mixture was reduced for six hours. Then palladium oxide was removed and the reaction mixture was concentrated in vacuo. Recrystallization of residual precipitate gave 0.23 g. of seryl-glycine hydrochloride, m.p. 195°C, with decomposition, in 95% yield.

Anal. Found: N, 14.24. Calcd. for $C_5H_{11}O_4N_2Cl$: N, 14.16%.

O-Benzyl-DL-seryl-L-glutamic acid.—Glutamic acid ethylester was prepared from 1.5 g. of the hydrochloride. The free ester was treated with 1.1 g. of O-benzyl-DL-serine N. C. A. and 0.1 cc. of triethylamine under the same conditions. White precipitate was obtained in 50% yield.

Anal. Found: N, 8.36. Calcld. for $C_{15}H_{20}O_6N_2$: N, 8.60%.

O-Benzyl-DL-seryl-L-leucine.—Under the same conditions, 0.37 g. of crystals were obtained in 10% yield from 2.5 g. of leucine ethylester hydrochloride and 2.2 g. of O-benzyl-DL-serine N. C. A.

Anal. Found: N, 9.48. Calcd. for $C_{16}H_{24}O_4N_2$: N, 9.10%.

O-Benzyl-DL-seryl-L-phenylalanine.—Under the same conditions, 0.17 g. of crystals were obtained in 10% yield, from 1.4 g. of phenylalanine ethylester and 1.1 g. of O-benzyl-DL-serine N. C. A.

Anal. Found: N, 8.19. Calcld. for $C_{19}H_{22}O_4N_2$: N, 8.18%.

O-Benzyl-N-carbobenzyloxy-DL-serine.—O-Benzyl-N-carbobenzyloxy-DL-serine was prepared in the previous experiments by the author[7]. The carbobenzyloxy derivative (2.8 g.) was synthesized in 80% yield from 2 g. of O-benzyl-DL-serine and 2.68 g. carbobenzyloxychloride.

O-Benzyl-O-carbobenzyloxy-DL-seryl-L-leucine ethylester.—N-Carbobenzyloxy derivative was treated with 2.5 g. of phosphorus pentachloride in 30 cc. of absolute ether. After dissolution of N-carbobenzyloxy derivative, excessive phosphorus pentachloride was filtered off and the filtrate was concentrated in vacuo at 0°C. The concentrated syrup was extracted twice with petroleum ether in order to remove phosphate oxychloride, and the residual syrup was dissolved in 20 cc. of ethylacetate. To the 20 cc. of ethylacetate solution of L-leucine ethylester which was prepared from 3.3 g. of the hydrochloride, was added the acid chloride solution. After removal of the leucine ethylester hydrochloride, the reaction mixture was washed twice with petroleum ether, and then allowed to stand over night in an ice box. White precipitate was obtained. Recrystallization from ethylacetate-petroleum ether gave 2.7 g. of peptide, in 70% yield, m.p. 78°C.

Anal. Found: N, 5.80. Calcld. for $C_{25}H_{34}O_6N_2$: N, 5.95%.

O-Benzyl-N-carbobenzyloxy-DL-seryl-L-leucine.—The peptide ester (2.6 g.) was hydrolized with mixed reagent (4 cc. of 1N-sodium hydroxide and 5 cc. of aceton). The reaction mixture was neutralized with 1N-hydrochloric acid being obtained in white crystals. Recrystallization from ethylacetate-petroleum ether gave 1.0 g. of peptide, m.p. 92°C, in 72% yield.

DL-Seryl-L-leucine.—N-Carbobenzyloxy peptide was suspended in 30 cc. of methyl alcohol. Into the methyl alcohol solution were added 0.1 cc. of acetic acid and 0.3 g. of palladium oxide. The mixture was reduced under the ordinary pressure in the hydrogen atmosphere at room temperature. After removal of palladium oxide, the reaction mixture was concentrated in vacuo; then white crystals were obtained. Recrystallization gave 0.12 g. of seryl-L-leucine, m.p. 189°C., in 71% yield.

Anal. Found: N, 12.63. Calcld. for $C_9H_{18}O_4N_2$: N, 12.80%.

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[5] H. Leuchs, *Ber.*, **39**, 857 (1906).

[6] K. Okawa and H. Tani, *J. Chem. Soc. Japan*, **75**, 1199 (1950).

[7] K. Okawa and H. Tani, *J. Chem. Soc. Japan*, **75**, 1199 (1954).

Summary

The optically active O-Benzyl-L-serine was prepared in 78% yield by the enzymatic resolution of the N-acetyl derivative with Takadiastase.

DL-Seryl-glycine was prepared by the N. C. A. method of Bailey and DL-seryl-L-leucine was synthesized by the Fischer's acid chloride method respectively.

As was expected, it was found that O-benzyl-serine can be utilized as the starting material in the peptide syntheses.

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