Studies on Serine Peptides. II. Synthesis of Optically Active Serine Peptide Derivatives

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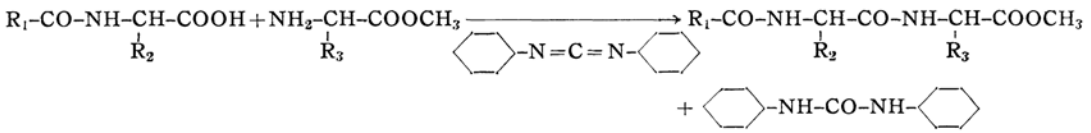
Although a number of peptides containing several kinds of the simpler amino acids have been prepared, little is known about the preparation of peptides which include more than one of trifunctional amino acids. This is especially true for peptides of L-serine and L-histidine.

In the previous paper[1], the author reported on the DL-serine peptides which include monofunctional amino acids.

In the present work, some derivatives of optically active peptides containing L-serine have been synthesized by means of the Sheehan's dicyclohexylcarbodiimide method[2], with O-benzyl-L-serine as starting material.



O-Benzyl-N-carbobenzyloxy-L-seryl-L-histidine methyl ester, O-benzyl-N-carbobenzyloxy-L-seryl-L-tyrosine methyl ester and bis-(O-benzyl-N-carbobenzyloxy-L-seryl)-L-cystine dimethyl ester have been synthesized in good yield.

Sheehan reported in his paper that the hydroxyl group of the serine was not affected by this coupling reagent, and L-seryl peptide was synthesized in 59% yield. But the relatively lower yield of the peptide shows that some side reactions may take place in the coupling procedure.

In the present experiments, a tyrosine peptide has been prepared in 76% yield. It seems that the free hydroxyl group of tyrosine is less reactive than that of serine.

It has been found from the above experiments that this coupling reagent is useful for peptide syntheses.

Experimental

N-N'-Dicyclohexyl Thiourea[3].—Into the solution of 100 g. of cyclohexylamine and 250 cc. of ethylalcohol was added 100 g. of carbondisulfide through the reflux condenser at the presence of two or three pieces of sodium hydroxide, and the mixture was refluxed for twenty-four hours on a waterbath. After cooling the reaction mixture, precipitated yellowish crystals were filtered. Recrystallization of the crystals from hot alcohol gave 100 g. of the pure material, m. p. 180–181°C.

Dicyclohexylcarbodiimide[4].—The intimate mixture of 100 g. of freshly pulverized mercuric oxide and 40 g. of dicyclohexylthiourea were placed in a suitable bottle of 500 cc. After adding 200 cc. of freshly distilled carbondisulfide, the mixture was shaken on a shuttle machine for 1.25 hrs. The precipitate was filtered and the

1) K. Okawa, Part I. This Balletin, 29, 486.
2) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, 77, 1067 (1955).
3) A. Skita und H. Rolfes, *Ber.*, 53, 1242 (1955).
4) R. Herbeck and M. Pezzati, *Ber.*, 71, 1933 (1938).

solvent was removed. The residual syrup was dissolved in petroleum ether and an insoluble precipitate was filtered. After removal of petroleum ether the residual gelatinous mass was distilled under reduced pressure. Colourless crystals (b. p. 132°C/4 mmHg) were obtained in 80% yield. Dicyclohexylcarbodiimide was stored in a sealed tube at 5°C.

O-Benzyl-N-carbobenzyloxy-L-serine[5].—Five grams of O-benzyl-N-carbobenzyloxy-L-serine were prepared from the 3.5 g. of O-benzyl-L-serine and 3.8 g. of carbobenzyloxychloride by the method as with O-benzyl-N-carbobengyloy-DL-serine, m. p. 98°C.

Syntheses of L-serine peptide derivatives.—All the condensation reactions carried out under anhydrous conditions.

O-Benzyl-N-carbobenzyloxy-L-seryl-L-tyrosine Methyl ester.—L-Tyrosine methyl ester hydrochloride (1.51 g.) was treated with 0.9 cc. of triethylamine in 10 cc. of tetrahydrofuran. After removal of triethylamine hydrochloride, the filtrate was mixed with 1.34 g. of carbodiimide and 1.65 g. of carbobenzyloxy derivative in 10 cc. of tetrahydrofuran at room temperature. Immediately, N-N'-dicyclohexylurea was precipitated from the above reaction mixture; then the coupling solution was kept for four hours in room temperature. To remove unchanged carbodiimide 0.15 cc. of acetic acid was added and the mixture was allowed to stand at this temperature for one hour, and filtered from the precipitate. Then, tetrahydrdofuran as the solvent was replaced with ethylacetate. Since a small amount of precipitate was formed in the ethylacetate solution, the precipitate was filtered off and petroleum ether was added to the solution until crystals of the peptide ester appeared. Recrystallization from ethylacetate-petroleum ether gave 1.8 g. of peptide, m. p. 111–112°C, in 72% yield. $[\alpha]_D^{15}=+15.5$ (28.4 mg./cc. in 99% EtOH).

Anal. Found: N, 5.74. Calcd. for $C_{28}H_{30}O_7N_2$: N, 5.53%.

O-Benzyl-N-carbobenzyloxy-L-seryl-L-histidine Methyl ester.—The free ester of histidine was obtained by the Fischer's method[6]. A methyl alcohol solution of histidine methyl ester dihydrochloride was treated with 13 cc. of 2 N-CH_3ONa solution and the reaction mixture filtered from the precipitated sodium hydrochloride. After removal of methyl alcohol, the free ester was dissolved in 15 cc. of tetrahydrofuran. The tetrahydrofuran solution was coupled with 3.3 g. of carbobenzyloxy derivative and 2.1 g. of carbodiimide in tetrahydrofuran. Under the same conditions as those of the above experiments, 4.2 g. of peptide ester were prepared in 87% yield, m. p. 110°C. $[\alpha]_D^{15}=+7.21$ (49.2 mg./cc. in 99% EtOH).

Anal. Found: N, 12.04. Calcd. for $C_{25}H_{30}O_6N_4$: N, 11.7%.

Bis-(O-benzyl-N-carbobenzyloxy-L-seryl)-L-cystine Dimethyl ester.—Into 20 cc. of the solution of cystine dimethyl ester in the tetrahydrofuran which was prepared from 2.2 g. of the dimethyl ester dihydrochloride and 1.8 cc. of triethylamine, were added 3.3 of carbobenzyloxy derivative and 2.6 g. of carbodiimide in 20 cc. of tetrahydrofuran. From the coupling mixture, 2.5 g. of crystals were obtained, m. p. 101–102°C, in 57% yield. $[\alpha]_D^{15}=+22.17$ (26.6 mg./cc. in ethylacetate.

Anal. Found: N, 6.33. Calcd. for $C_{44}H_{50}O_{12}N_4S_2$: N, 6.29%.

Summary

The derivatives of optically active L-seryl peptides containing trifunctional amino acids have been synthesized by the Sheehan's method in good yield.

In this method, the free hydroxyl group of the serine must have been substituted by a suitable protecting group, fx. benzyl rest, in order to obtain good results.

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5) K. Okawa and H. Tani, *J. Chem. Soc. Japan*, 75, 1197 (1950).

6) E. Fischer and L.H. Cone, *Ann.*, 363, 107 (1908).