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Studies of Unnatural Amino Acids and Their Peptides. II. The Syntheses of DL-β-(4-Thiazolyl)-α-alanine and Its Peptides*1

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DL-β-(4-Thiazolyl)-α-alanine has been synthesized in a good yield by the condensation of 4-thiazolylmethyl chloride and diethyl acetamidomalonate, and by the subsequent hydrolysis of the product. The *N*-carbobenzoxy derivative of this amino acid could be prepared easily and transformed to its *p*-nitrophenyl ester and *t*-butoxycarbonylhydrazide, but it was found difficult to obtain the methyl ester or the benzyl ester directly from this amino acid. Carbobenzoxy-DL-β-(4-thiazolyl)-α-alanyl-glycine benzyl ester has been synthesized by several coupling methods: its yield decreased in the following order: *p*-nitrophenyl ester > dicyclohexylcarbodiimide (glycine ester was used as a free base) > azide > dicyclohexylcarbodiimide (glycine ester *p*-toluenesulfonate was used without triethylamine) > dicyclohexylcarbodiimide (glycine ester *p*-toluenesulfonate was used with triethylamine) > mixed anhydride. The alkaline hydrolysis of the protected dipeptide ester afforded the *N*-protected peptide in a good yield. Decarbobenzoxylation did not proceed smoothly by catalytic hydrogenation, in which hydrogen was absorbed slowly and incompletely, while it proceeded normally with hydrobromic acid in acetic acid.

As one of a series of studies of unnatural amino acids and their peptides,[1] the syntheses of DL-β-(4-thiazolyl)-α-alanine and its peptides will be reported in this paper. This amino acid has already been synthesized in several ways.[2,3] First, the authors prepared this compound by

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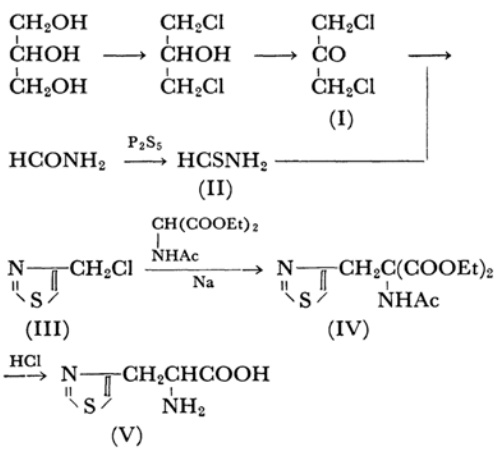
1) Part I: This Bulletin, **38**, 1461 (1965).

2) R. G. Jones, E. C. Kornfeld and K. C. McLaughlin, *J. Am. Chem. Soc.*, **72**, 4526 (1950).

3) W. T. Caldwell and S. M. Fox, ibid., **73**, 2935 (1951).

reproducing the method of Caldwell and Fox[3] (illustrated in Scheme 1), but they found the results unsatisfactory in some step. When 2 mol. of sodium ethyl acetamidomalonate were allowed to react with one mole of thiazolylmethyl chloride hydrochloride, according to the method of the literature,[3] the condensation product was somewhat difficult to separate from the unreacted acetamidomalonate present in a large excess, and the hydrolysate of the product was contaminated with glycine, as was confirmed by paper chromatography. Hence, we modified the method so as to use the thiazolylmethyl chloride as a free base and the equimolar sodium ethyl acetamidomalonate, as in the report of Jones et al.[2] In this way we succeeded not only in avoiding the



Scheme 1. The synthetic route of DL-β-(4-thiazolyl)-α-alanine.

contamination with acetamidomalonate, but also in raising the yield of the condensation product up to 58% of the theoretical amount in a large-scale reaction. The hydrolysis of the condensation product proceeded smoothly when it was boiled with 6 N hydrochloric acid for 8 hr., and the amino acid was obtained as hydrochloride in an almost quantitative yield.

In the case of an unnatural amino acid containing a heterocyclic ring, the methods generally used for the peptide synthesis are not always suitable because they may be accompanied by undesirable side reactions resulting from the heterocyclic ring. For instance, as has already been reported,[1] in order to remove the formyl group from formylfurylalanine the oxidative cleavage method can not be used because of the simultaneous destruction of the furan ring. Thus, the validity of some typical methods of peptide synthesis was examined for this amino acid.

The carbobenzoxy derivative of this amino acid was prepared by a usual method without any difficulty, while the attempts to esterify this amino acid with methyl alcohol and hydrogen chloride, or with benzyl alcohol and *p*-toluenesulfonic acid in the presence of benzene, resulted in failure.

The coupling reaction of carbobenzoxythiazolylalanine with another amino acid was demonstrated using glycine benzyl ester as an amine component. Though the alkyl ester of thiazolylalanine could not be obtained, carbobenzoxythiazolylalanine *p*-nitrophenyl ester could be prepared by condensing the protected amino acid with *p*-nitrophenol by dicyclohexylcarbodiimide. In the same way, the *t*-butoxycarbonylhydrazide of the carbobenzoxylated amino acid could be obtained by condensing both the components by the dicyclohexylcarbodiimide method, and this compound was used as an intermediate for the azide method. The results of some coupling reactions are summarized in Table I. The yield decreased in connection with the coupling methods in the following order: *p*-nitrophenyl ester > dicyclohexylcarbodiimide (glycine ester was used as a free base) > azide > dicyclohexylcarbodiimide (glycine ester *p*-toluenesulfonate was used without triethylamine) > dicyclohexylcarbodiimide (glycine ester *p*-toluenesulfonate was used with triethylamine) > mixed anhydride. It is noteworthy that, in the condensation of carbobenzoxythiazolylalanine with glycine benzyl ester *p*-toluenesulfonate with dicyclohexylcarbodiimide, a somewhat higher yield was obtained in the absence of triethylamine than in its presence.

The alkaline hydrolysis of the protected peptide ester proceeded smoothly, and carbobenzoxythiazolylalanylglycine was obtained in an excellent yield after the neutralization of the reaction mixture with the equivalent hydrochloric acid.

One of the most important problems is that of estimating the validity of the hydrogenolytic decarbobenzoxylation method of these amino acid derivatives, because they contain a sulfur atom as a constituent. Therefore, precise studies were made by using a quantitative hydrogenation apparatus. Carbobenzoxythiazolylalanine was hydrogenated in the apparatus, using palladium as a catalyst in the presence of sodium hydroxide to remove the carbon dioxide which was evolved during the reduction; the absorption-rate of hydrogen was then compared with that of carbobenzoxyphenylalanine. Though the reduction of carbobenzoxyphenylalanine proceeded smoothly and finished in a short time, that of carbobenzoxythiazolylalanine proceeded very slowly and was still incomplete even after 2 hr. On the basis of these results, we have reached the conclusion that the hydrogenolytic removal of the protecting group is unfavorable in these amino acid derivatives. It was shown, on the other hand, that the carbobenzoxy group was easily split off with hydrogen bromide in acetic acid, and a free peptide, which was proved chromatographically pure, was obtained

by the subsequenttreatment of the reaction product with an ion exchange resin.

Experimental

α, γ-Dichloroacetone (I).—α, γ-Dichlorohydrin was prepared from glycerol by passing hydrogen chloride through it at 100—110°C,[4] then it was oxidized to I with sodium dichromate, according to the method of Conant and Quayle.[4] B. p. 170—175°C.

Thioformamide (II).—This was prepared according to the method of Schmitz,[5] by the action of phosphorus pentasulfide on formamide in tetrahydrofuran; the oily, crude product was used for the next reaction without purification.

(4-Thiazolyl)-methyl Chloride Hydrochloride (III).—This was prepared according to the method of Caldwell and Fox[3] by condensing I and II in acetone. When the resulting crude material (65% yield) was sublimed at 130—140°C under reduced pressure, the pure substance was obtained as pale yellow needles melting at 167—168°C (85—90% yield).

Diethyl (4-Thiazolyl)-methyl Acetamidomalonate (IV).—(4-Thiazolyl)-methyl chloride, which was obtained as a viscous oil by treating III with alkali, was condensed with acetamidomalonate in ethanol, using sodium as the condensing agent, at 40°C for 5 days. The yield was 58%; m. p. 104—105°C (lit.[3] m. p. 104—105°C).

DL-β-(4-Thiazolyl)-α-alanine (V).—IV was hydrolyzed by refluxing it with 6 N hydrochloric acid for 8 hr.; V-hydrochloride was thus obtained in a 93% yield. The free amino acid was obtained in a 87% yield by treating the hydrochloride with ion exchange resin (Amberlite CG-120). M. p. 226—229°C (decomp.) (darkened from ca. 200°C) (lit.[2] m. p. 227—230°C). R_f=0.40 (circular paper chromatography; solvent, *n*-butanol : acetic acid : water=5 : 4 : 1).

***N*-Carbobenzoxy-DL-β-(4-thiazolyl)-α-alanine (VI).**—Carbobenzoxy chloride (28.2 g.) was added, drop by drop, to a solution of V (24 g.) in a sodium hydroxide solution (sodium hydroxide 6 g.; water 150 cc.) with stirring under ice-cooling. The solution was kept alkaline by adding a sodium hydroxide solution. After the addition of carbobenzoxy chloride, stirring was continued for 1 hr. The clear reaction mixture was then extracted with ether, and the pH of the aqueous layer was adjusted to 4 with hydrochloric acid. The white crystals which formed were collected and washed thoroughly with water. The yield was 40 g. (94%); m. p. 122—124°C. For analysis it was recrystallized from dichloromethane; m. p. 130—131°C.

Found: C, 55.05; H, 4.71; N, 9.35. Calcd. for $C_{14}H_{14}O_4N_2S$: C, 54.89; H, 4.61; N, 9.15%.

***N*-Carbobenzoxy-DL-β-(4-thiazolyl)-α-alanine *p*-Nitrophenyl Ester (VII).**—Dicyclohexylcarbodiimide (1 g.) was added to the solution of VI (1.5 g.) and *p*-nitrophenol (0.7 g.) in dichloromethane (40 cc.); the mixture was then stirred for 3 hr. and allowed to stand overnight at room temperature. The dicyclohexylurea was filtered off, and the filtrate was washed with a cold potassium carbonate solution and water until the aqueous phase became colorless. The organic layer was dried with sodium sulfate and concentrated under reduced pressure. The residual solid was recrystallized from ethanol. The yield was 0.77 g. (37%); m. p. 101—103°C.

Found: C, 56.27; H, 3.99; N, 9.28. Calcd. for $C_{20}H_{17}O_6N_3S$: C, 56.20; H, 4.01; N, 9.83%.

***N*-Carbobenzoxy-DL-β-(4-thiazolyl)-α-alanine *t*-Butoxycarbonylhydrazide (VIII).** — Dicyclohexylcarbodiimide (2.1 g.) dissolved in dichloromethane (5 cc.) was added to a solution of VI (3.0 g.) and *t*-butoxycarbonylhydrazine (1.4 g.) in dichloromethane (ca. 50 cc.) under ice-cooling. When the reaction mixture was stirred for 1 hr., voluminous crystals were separated (probably due to the crystallization of *t*-butoxycarbonylhydrazine). By adding more dichloromethane (10 cc.) and methanol (26 cc.), almost all the crystals were dissolved; then the mixture was allowed to stand overnight at room temperature. Acetic acid (2 cc.) was added, and the mixture was stirred for 1 hr. The dicyclohexylurea was then filtered off, and the filtrate was concentrated to dryness. The resulting solid mass was recrystallized from methanol. The yield was 2.43 g. (59%). To remove the trace of dicyclohexylurea, the crystals were dissolved in a small volume of hot ethanol, and then water was added into this solution. The dicyclohexylurea which separated was filtered off, and then the filtrate was allowed to stand overnight at room temperature. The crystals obtained in this way melted sharply at 158.5—160°C, and their analytical data agreed well with the theoretical values.

Found: C, 54.25; H, 6.13; N, 13.17. Calcd. for $C_{19}H_{24}O_5N_4S$: C, 54.27; H, 5.75; N, 13.33%.

***N*-Carbobenzoxy-DL-β-(4-thiazolyl)-α-alanylglycine Benzyl Ester (IX).**—*The Azide Method.*— VIII (1.8 g.) was added to methanolic hydrogen chloride (50 cc.); then, after the evolution of carbon dioxide had ceased, the clear solution was evaporated under reduced pressure. The resulting, strongly hygroscopic solid was dissolved in 1 N hydrochloric acid (4.3 cc.), and ethyl acetate (20 cc.) was added. A solution of sodium nitrite (0.3 g.) in water (2 cc.) was added at −10°C and, then, 2 min. later, 50% potassium carbonate (10 cc.) was added at the same temperature. Then the organic layer was separated, and the aqueous layer was extracted with ethyl acetate (20 cc.). The combined organic layer was dried with sodium sulfate. On the other hand, glycine benzyl ester *p*-toluenesulfonate (2 g.) was dissolved in a small volume of water, made alkaline by the addition of 50% potassium carbonate, and extracted with ethyl acetate 3 times (total volume: 40 cc.). After dried with sodium sulfate, the ester solution was combined with the azide solution and kept in a refrigerator for 2 days. After filtered and washed successively with water, a 0.5% sodium bicarbonate solution, and water, the ethyl acetate solution was dried with sodium sulfate. The solid mass (1.3 g.), obtained by evaporating off the solvent, melted at 110—112°C. After recrystallization from ethanol, it melted at 114—115°C (1.13 g., 58%).

Found: C, 61.24; H, 5.24; N, 8.89. Calcd. for $C_{23}H_{23}O_5N_3S$: C, 60.91; H, 5.11; N, 9.27%.

The p-Nitrophenyl Ester Method.—To a solution of VII

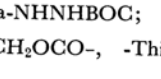
4) J. B. Conant and O. R. Quayle, "Organic Syntheses," Coll. Vol. I, 292, 211 (1948).

5) Wm. R. Schmitz, U. S. Pat. 2682558; *Chem. Abstr.*, **49**, 9029e (1955).

TABLE I. RESULTS OF THE COUPLING REACTIONS OF CARBOBENZOXYTHIAZOLYLALANINE AND GLYCINE BENZYL ESTER

Coupling method	Z-Thia-OH g.	H-Gly-OBz·TosOH g.	Et_3N cc.	Form of amine component	Product g.	Yield, %	M. p., °C
Azide	1.8[a]	2	—	Free base	1.13	58	114—115
p-Nitrophenyl ester	0.57[b]	0.5	0.3	Salt+Et_3N	0.45	74	111.5—113
Mixed anhydride	4.2	5.3	2.2	Salt+Et_3N	2.2	35	114—114.5 (sintered at 112)
DCC	1.5	1.8	—	Free base	1.58	71	113—114 (sintered at 112)
DCC	2.9	3.5	1.6	Salt+Et_3N	1.55	36	106—111.5
DCC	1.5	1.8	—	Salt	0.91	41	111—114 (sintered at 108)

a=Z-Thia-NHNHBOC; b=Z-Thia-ONP

Z: $C_6H_5CH_2OCO$-, -Thia-:  , Bz: $-CH_2C_6H_5$, Tos: $CH_3C_6H_4SO_2$-,

DCC: Dicyclohexylcarbodiimide, BOC: $(CH_3)_3COCO$-, ONP: $-OC_6H_4NO_2$

(0.57 g.) in dichloromethane (40 cc.), there was added glycine benzyl ester *p*-toluenesulfonate (0.5 g.) and triethylamine (0.3 cc.); then the mixture was allowed to stand at room temperature overnight. After washed thoroughly with 5% hydrochloric acid, 0.5% sodium bicarbonate, and water, the solution was dried with sodium sulfate and evaporated under reduced pressure, leaving a viscous, pale yellow oil. By cooling the ethyl acetate solution of the oil, white needle crystals were obtained.

The Mixed Anhydride Method.—To a solution of VI (4.2 g.) and triethylamine (2 cc.) in dichloromethane (60 cc.), ethyl chloroformate (1.37 cc.) was added, drop by drop, at −30°C with stirring. The mixture was then kept at −15——10°C for 5 min. To the somewhat reddish-brown solution thus obtained, a mixture of glycine benzyl ester *p*-toluenesulfonate (5.3 g.) and triethylamine (2.2 cc.) in dichloromethane (20 cc.) was added with stirring, then allowed to stand overnight at room temperature. The dichloromethane solution was washed successively with water, 5% hydrochloric acid, 0.5% sodium bicarbonate, and water, and dired with sodium sulfate. After the solvent had been evaporated off, a yellowish-brown semi-solid (5.1 g.) was obtained. Recrystallization from ethyl acetate afforded needle crystals.

The Dicyclohexylcarbodiimide Method. — To a solution of VI (1.5 g.) in dichloromethane (30 cc.) there was added a dichloromethane solution of glycine benzyl ester (prepared from 1.8 g. of glycine benzyl ester *p*-toluenesulfonate by neutralizing with potassium carbonate and extracting with dichlorometane). After dicyclohexylcarbodiimide (1.2 g.) in dichloromethane (3 cc.) had been added, the reaction mixture was stirred for 3 hr. at room temperature and then allowed to stand overnight at this temperature. After dicyclohexylurea had been filtered off, the solution was washed successively with 5% hydrochloric acid, 0.5% sodium bicarbonate, and water, and dried with sodium sulfate. After the solvent had been evaporated off, the pale yellow, syrupy residue was crystallized from ethyl acetate (the yield was 1.58 g. (71%)). Instead of free glycine benzyl ester, a mixture of glycine benzyl ester *p*-toluenesulfonate and the equimolar triethylamine, in one case, and glycine benzyl ester *p*-toluenesulfonate alone, in the other case, were used.

All of these results are summarized in Table I, together with those of the other coupling methods.

***N*-Carbobenzoxy-DL-thiazolylalanyl-glycine (X).**—To a methanolic solution (400 cc.) of IX (3.3 g.), 1 N sodium hydroxide (10 cc.) was added; the solution was then allowed to stand at 25°C for 1 hr. with occasinal shaking. About a half of the solvent was then distilled off under reduced pressure, and water was added. After filtration, the solution was neutralized with 1 N hydrochloric acid (10 cc.). The resulting white precipitates were collected, washed thoroughly with water, and air-dried. The yield was 2.3 g. (87%); m. p. 179—181°C (from ethanol).

Found: C, 52.63; H, 4.97; N, 11.40. Calcd. for $C_{16}H_{17}O_5N_3S$: C, 52.88; H, 4.72; N, 11.56%.

The Hydrogenation of Carbobenzoxy-DL-phenylalanine and Carbobenzoxy-DL-thiazolylalanine.—A solution of carbobenzoxy-DL-phenylalanine (0.5 g., 1.67 mmol.) in aqueous methanol (methanol, 25 cc.; water, 10 cc.) and 1 N sodium hydroxide (4 cc.) was placed in a hydrogenation flask containing a palladium catalyst (0.1 g.) in water (20 cc.) which had previously been equilibrated with hydrogen. After the air in the apparatus had been completely replaced with hydrogen, stirring was started; the decreasing volume of the hydrogen was measured against the time. The same experiment was then carried out with carbobenzoxy-DL-thiazolylalanine (0.5 g., 1.63 mmol). In the latter case only about one-third of the theoretical amount of hydrogen was absorbed, even after 2 hr., while in the former case the hydrogenation was complete after 15 min.

DL-Thiazolylalanyl-glycine (XI).—To a 25% solution of hydrogen bromide in acetic acid (3 cc.), X (0.5 g.) was added. After the evolution of carbon dioxide had ceased, water was added and the mixture was extracted with ether. The aqueous layer was then decolorized, the pH was adjusted to ca. 6 with aqueous ammonia, and the solution was allowed to pass through an Amberlite CG-120 (H^+ form) column. After the column had been thoroughly washed with water, the absorbed free peptide was eluted with aqueous ammonia. The eluate was then decolorized, concentrated to a small volume under reduced pressure, and finally lyophilized. The yield was 0.31 g. (92% calculated as hemihydrate); m. p. 158—160°C (sintered at 154°C). Paper chromatography gave only one spot: R_f=0.34.

Found: C, 39.93; H, 5.37; N, 16.84. Calcd. for $C_8H_{11}O_3N_3S\cdot{}^1/_2H_2O$: C, 40.33; H, 5.08; N, 17.64.