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The Synthesis of Some Peptides Related to the Active Site of Enzymes[1]

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The sequences, α-aspartyl-L-serylglycine and α-glutamyl-L-serylglycine, appear in the "active site" of many esteratic enzymes. The free tripeptides were synthesized in this study. In order to avoid difficulties encountered in more conventional synthetic routes, *N*-carbobenzoxy-L-aspartyl (β-benzyl ester)-*O*-benzyl-L-serylglycine dibenzyl ester and *N*-carbobenzoxy-L-glutamyl (γ-benzyl ester)-*O*-benzyl-L-serylglycine dibenzyl ester were synthesized. These protected peptides were converted to the free tripeptides upon hydrogenolysis, which removed all of the four protecting groups simultaneously. Several derivatives of these tripeptides were also synthesized.

The α-aspartyl-L-serylglycine sequence has been found in many esteratic enzymes in the so-called "active site."[3] The synthesis of this free tripeptide[4] is accordingly of interest for the preparation of derivatives including other peptides of defined constitution, as a standard reference substance in tests of catalytic activity with derivatives, and for investigation as a co-monomer in copolymerizations. For the latter objective, in particular, combination with histidine residues in the same macromolecule would be of interest.

The difficulties of preparation of the free tripeptide posed special requirements. One of these was protection of the unstable seryl residue during the syntheses. Another problem was avoidance of saponification of the ester of β-benzyl *N*-carbobenzoxy-L-aspartate, an intermediate in some projected syntheses. Bernhard et al.[5] found that the saponification of β-benzyl *N*-carbobenzoxy-L-aspartyl residues involved the formation of an imide intermediate. The most rapid reaction, both in the formation of the imide intermediate and its subsequent hydrolysis, was observed with *N*-carbobenzoxyaspartyl (β-benzyl ester)-serinamide. The hydroxyl group of the serine residue was found to be required for the most rapid hydrolysis of this benzyl ester. Bernhard et al. also isolated the intermediate imide compound. These findings suggested that alkaline conditions should be avoided during the synthesis and that protection of the hydroxyl group of the serine residue is desirable.

Attempts in this laboratory to saponify selectively the ethyl ester of *N*-carbobenzoxy-L-aspartyl (β-benzylester)-*O*-benzyl-L-serylglycine were unsuccessful. Hydrogenolysis of the saponified product resulted in hygroscopic peptides composed of aspartic acid, serine, and glycine. These were presumably mixtures of α- and β-aspartyl peptides which could result from saponification of an intermediate imide.

The ethyl esters of glycyl-α-L-aspartyl-L-serylglycine[7] and of L-aspartyl-*O*-acetyl-L-serylglycine[7] have been reported. However, saponification of the ethyl esters and of the *O*-acetate also is difficult to control.

In order to avoid all of the difficulties mentioned, *N*-carbobenzoxy-L-aspartyl-*O*-benzyl-L-serylglycine dibenzyl ester was synthesized because the four protecting groups would presumably be removable by hydrogenolysis, and alkaline treatments during synthetic steps would thus be avoided. A test of this hypothesis showed that the free tripeptide could indeed be prepared in this way. The α-L-glutamyl-L-serylglycine was then similarly prepared. This sequence has also been found in the active site of esteratic enzymes.[3]

Recently, the synthesis of an octapeptide corresponding to a sequence containing the active site of chymotrypsin has been reported.[8]

Experimental

β-Benzyl L-Aspartate.—β-Benzyl L-aspartate was prepared by the sulfuric acid method.[9] m. p. 221°C,

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2) Presented adress: Hokkaido University, Sapporo, Japan.

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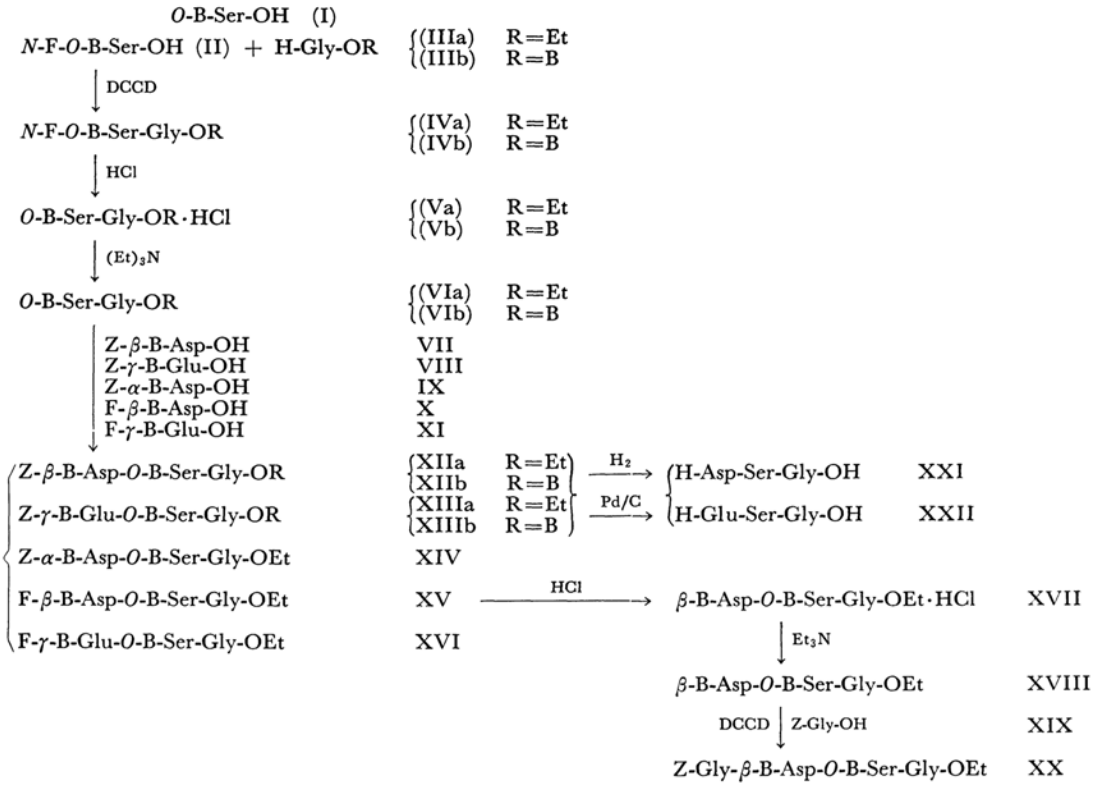

B: Benzyl F: Formyl Z: Carbobenzoxy Et: Ethyl

$[\alpha]_D^{26}$ +24.2° (*c* 1.18, 1 N HCl).

γ-Benzyl L-Glutamate.—γ-Benzyl L-glutamate was also prepared by the sulfuric acid method.[9] m. p. 174°C, $[\alpha]_D^{26}$ +21.8° (*c* 1.87, AcOH).

***O*-Benzyl-L-serine.**—*O*-Benzyl-L-serine was prepared from methyl acrylate by Okawa's method,[10] The *O*-benzyl-DL-serine was acetylated and the resulting *N*-acetyl *O*-benzyl-DL-serine was resolved by Takaacylase, m. p. 219—221°C (decomp.), $[\alpha]_D^{26}$ +5.0° (*c* 1.00, 3 N HCl).

β-Benzyl *N*-Carbobenzoxy-L-aspartate (VII).—β-Benzyl *N*-carbobenzoxy-L-aspartate was prepared by the usual Schotten-Bauman reaction.[11] m. p. 105—106°C, $[\alpha]_D^{27}$ +12.0° (*c* 1.50, AcOH).

γ-Benzyl *N*-Carbobenzoxy-L-glutamate (VIII).—γ-Benzyl *N*-carbobenzoxy-L-glutamate was prepared as described by Hanby et al.[12] m. p. 75—76°C, $[\alpha]_D^{27}$ −22.7° (*c* 1.41, N $KHCO_3$).

β-Benzyl *N*-Formyl-L-aspartate (X).—β-Benzyl L-aspartate, 31.3 g. (0.13 mol.), was dissolved in 200 ml. of 98% formic acid. To this was added dropwise 78 ml. of acetic anhydride at 5—10°C under agitation during a period of 1 hr. After addition was complete, the mixture was stirred for 2 hr. at room temperature. The solution was evaporated to dryness under reduced pressure at 50°C in a water bath. The residue was dried in a vacuum desiccator overnight over sodium hydroxide. The dried residue was dissolved in 150 ml. of acetone and filtered to remove the insoluble material (1.05 g., m. p. 155—160°C). Petroleum ether was added to the acetone solution to crystallize the β-benzyl *N*-formyl-L-aspartate, 28.2 g. (81%), m. p. 122—125°C. This product was recrystallized from acetone and petroleum ether, m. p. 123—125°C, $[\alpha]_D^{25}$ +28.6° (*c* 1.75, ethanol).

Found: N, 5.61. Calcd. for $C_{12}H_{13}O_5N$: N, 5.58%. COOH titration,[13] Found: M=248, Calcd: M=251.2.

γ-Benzyl *N*-Formyl-L-glutamate (XI).—γ-Benzyl *N*-formyl-L-glutamate was synthesized in a way similar to that described above. m. p. 130—131°C, $[\alpha]_D^{25}$ +17.9° (*c* 1.98, ethanol).

Found: N, 5.32. Calcd. for $C_{13}H_{15}O_5N$: N, 5.28%. COOH titration: Found, M=264; Calcd. M=265.3.

***N*-Formyl-*O*-benzyl-L-serine (II).** — *N*-Formyl-*O*-benzyl-L-serine was prepared from *O*-benzyl-L-serine by use of a method similar to that described above. Yield, 78%, m. p. 123—128°C. This was recrystallized from ethyl acetate and petroleum ether, m. p. 129—130°C, $[\alpha]_D^{25}$ +54.8 (*c* 2.75, ethanol).

Found: N, 6.23. Calcd. for $C_{11}H_{13}O_4N$: N, 6.28%. COOH titration, Found: M=223; Calcd. M=223.2.

***N*-Formyl-*O*-benzyl-L-serylglycine Ethyl Ester (IVa).**—*N*-Formyl-*O*-benzyl-L-serine, 6.00 g., was dissolved in 60 ml. of tetrahydrofuran. To this was

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11) A. Berger and E. Katchalski, *J. Am. Chem. Soct.* **73**, 4084 (1951).
12) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, **1950**, 3239.
13) The carboxyl group was titrated with methanolic sodium methoxide in dimethylformamide or in dioxane by use of thymol blue as the indicator. Elemental Analyses were carried out by Micro-Tech Laboratories, INC., Skokie, Illinois, U. S. A.

added 2.77 g. of freshly distilled glycine ethyl ester (IIIa). To this solution, 5.90 g. of dicyclohexylcarbodiimide (DCCD) in 15 ml. of tetrahydrofuran was added dropwise under cooling with ice water. After 30 min. of cooling, the mixture was allowed to attain room temperature. Precipitated dicyclohexylurea was filtered after 24 hr. of standing, and the filtrate was evaporated under reduced pressure at 40°C. The residue was dissolved in ethyl acetate and filtered to remove dicyclohexylurea. The solvent was evaporated to 25 ml. and petroleum ether was added slowly to precipitate *N*-formyl-*O*-benzyl-L-serylglycine ethyl ester. The mixture was kept in the ice box for 3 hr., then filtered to collect the *N*-acyldipeptide ester, 6.2 g. (75%). This was recrystallized from 10 ml. of ethyl acetate, 15 ml. of ether and 35 ml. of petroleum ether, 5.0 g., m. p. 63—64°C, $[\alpha]_D^{25}$ +4.1° (*c* 2.48, ethanol).

Found: C, 58.44; H, 6.60; N, 9.03. Calcd. for $C_{13}H_{20}O_5N$: C, 58.44; H, 6.55; N, 9.09%.

O-Benzyl-L-serylglycine Ethyl Ester Hydrochloride (Va).—*N*-Formyl-*O*-benzyl-L-serylglycine ethyl ester (IVa), 4.62 g., was dissolved in 18.8 ml. of methanol. To this was added 16.5 ml. of 1 N hydrochloric acid in methanol under cooling (2 ml. of concentrated hydrochloric acid was diluted to 24 ml. with methanol). The solution was allowed to stand at room temperature for 48 hr. The solvent was evaporated under reduced pressure, and the residual syrup was dissolved in 20 ml. of water. The insoluble material was filtered and the filtrate was twice extracted with ether. The aqueous solution was then lyophilized. A slightly yellowish vitreous dipeptide ester hydrochloride was obtained; 4.68 g. (98%). This peptide ester hydrochloride was used for subsequent syntheses without further purification.

N-Carbobenzoxy-L-aspartyl (β-benzyl ester)-O-benzyl-L-serylglycine Ethyl Ester (XIIa).—*O*-Benzyl-L-serylglycine ethyl ester hydrochloride (Va), 14.05 g., was dissolved in 150 ml. of dry dioxane. To this solution, 4.80 g. of triethylamine was added under cooling. The resultant triethylamine hydrochloride was filtered. In this filtrate, 15.85 g. of β-benzyl *N*-carbobenzoxy-L-aspartate was dissolved. To the mixture, a solution of 9.17 g. of DCCD in 50 ml. of dioxane was added slowly under cooling. After 5 min., dicyclohexylurea began to precipitate. The reaction mixture was allowed to stand overnight. Precipitated urea was filtered out and the filtrate was evaporated at 40—45°C under diminished pressure. The residual syrup was dissolved in 180 ml. of ethyl acetate at 45°C and filtered. The filtrate was washed twice with each of the following: 0.3 N hydrochloric acid, 5% sodium hydrogen carbonate, and water. The washed solution was dried over anhydrous sodium sulfate. The ethyl acetate was evaporated to about 80 ml. and then petroleum ether was added solwly to precipitate the protected tripeptide ester. The mixture was kept in the refrigerator overnight. The crystals were filtered and washed with ether and dried; 19.80 g., m. p. 117—120°C. From the mother liquor an additional 1.50 g. of crystals was obtained; yield 78%. This was recrystallized from ethyl acetate, acetone, and petroleum ether; m. p. 121—124°C, $[\alpha]_D^{25}$ −5.6° (*c* 1.51, ethanol).

Found: C, 64.05; H, 6.10; N, 6.79. Calcd. for $C_{37}H_{37}O_9N$: C, 63.96; H, 6.02; N, 6.78%.

N-Carbobenzoxy-L-glutamyl (γ-benzyl ester)-O-benzyl-L-serylglycine Ethyl Ester (XIIIa).—The protected tripeptide ester (XIIIa) was prepared in a way similar to that described above. After washing the ethyl acetate solution with 0.3 N hydrochloric acid, a yield of 10.1 g. (74%) of XIIIa was obtained. This was recrystallized by dissolving in acetone - ethyl acetate mixture and by precipitating with petroleum ether. By this recrystallization procedure, two fractions of different melting points were isolated. One (A), (0.21 g.), has a m. p. 133—134°C and the other (B), (5.10 g.), melted at 147—148°C. The compound A melted at 133—134°C, then solidified, and the solid remelted at 146—147°C. Accordingly, compound A is probably converted to compound B by heating. Elemental analyses of compound A and B each agree with the theoretical value.

Compound A, Found: C, 64.59; H, 6.27; N, 6.81.
Compound B, Found: C, 64.83; H, 6.06; N, 6.68.
Calcd. for $C_{34}H_{39}O_9N_3$: C, 64.54; H, 6.31; N, 6.63%.

N-Formyl-L-aspartyl (β-benzyl ester)-O-benzyl-L-serylglycine Ethyl Ester (XV).—A solution of 23 g. of *O*-benzyl-L-serylglycine ethyl ester hydrochloride (Va) in 200 ml. of dioxane was treated with 11.0 ml. of triethylamine. The free dipeptide ester (VIa) was condensed with 19.1 g. of β-benzyl *N*-formyl-L-aspartate (X) by use of 16.6 g. of DCCD. A yield of 33.0 g. (89%) of protected tripeptide ester (XV) was obtained, m. p. 83—86°C. This was recrystallized from ethyl acetate; m. p. 91—93°C, $[\alpha]_D^{25}$ −5.8° (*c* 1.67, AcOH).

Found: C, 61.52; H, 6.48; N, 7.90. Calcd. for $C_{27}H_{33}O_8N_3$: C, 61.47; H, 6.30; N, 7.97%.

L-Aspartyl (β-benzyl ester)-O-benzyl-L-serylglycine Ethyl Ester Hydrochloride (XVII).—β-Benzyl ester of *N*-formyl-L-aspartyl-L-serylglycine ethyl ester (XV), 16.0 g., was dissolved in 60 ml. of absolute ethanol. To this solution, 40 ml. of 1 N ethanolic hydrogen chloride was added. The mixture was allowed to stand at room temperature for three days. The solvent was evaporated under reduced pressure and the residue was dissolved in 30 ml. of water. The insoluble material was filtered off and the filtrate was extracted with ethyl acetate to remove the unreacted *N*-formyl derivative. The aqueous solution was lyophilized; yield, 3.60 g. (22%).

N-Carbobenzoxy-glycyl-L-aspartyl (β-benzyl ester)-O-benzyl-L-serylglycine Ethyl Ester (XX).—A solution of 2.1 g. of carbobenzoxyglycine (XIX) in 15 ml. of dioxane was added to 50 ml. of acetonitrile solution of L-aspartyl (β-benzyl ester)-*O*-benzyl-L-serylglycine ethyl ester (XVIII) which was prepared from 5.3 g. of L-aspartyl (β-benzyl ester)-*O*-benzyl-L-serylglycine ethyl ester hydrochloride (XVII) and an equimolar amount of triethylamine. The two compounds were condensed by 2.1 g. of DCCD under stirring and cooling. After standing overnight the reaction mixture was treated in the same way as described above. The protected tetrapeptide, 4.0g. (60%), was obtained from ethyl acetate solution by addition of ether, m. p. 68—73°C. This was recrystallized twice from ethyl acetate and ether, m. p. 85—89°C, $[\alpha]_D^{25}$ −13.0° (*c* 2.17, dioxane).

Found: C, 61.55; H, 6.11; N, 8.35. Calcd. for $C_{35}H_{40}O_4N_4$: C, 62.12; H, 5.96; N, 8.28%.

α-Benzyl N-Carbobenzoxy-L-aspartate (IX).—

This compound was prepared by the method of Bergmann et al.[14] The crude product (m. p. 71—78°C) was recrystallized repeatedly from ethyl acetate by petroleum ether. The material which melted at 82—85°C was used for further synthesis, $[\alpha]_D^{25}$ −15.3° (*c* 1.60, ethanol).

Found: N, 3.95. Calcd. for $C_{19}H_{19}O_6N$: N, 3.93%. COOH titration, Found: M=358; Calcd.: M=357.5.

***N*-Carbobenzoxy-L-aspartyl (*α*-benzyl ester)-*O*-benzyl-L-serylglycine Ethyl Ester (XIV).**—Compound Va, 4.00 g., in 30 ml. of tetrahydrofuran, was treated with 1.28 g. of triethylamine. The precipitated amine salt was removed by filtration. To the filtrate, 4.50 g. of *α*-benzyl *N*-carbobenzoxy-L-aspartate (IX) was dissolved. DCCD (2.88 g.), in 10 ml. of tetrahydrofuran, was added slowly to the above mixture while stirring and cooling. The reaction mixture was treated in the same way as described earlier. The product obtained weighed 6.0 g. (77%). This was recrystallized from ethyl acetate (45 ml.) and petroleum ether (40 ml.). Yield, 4.1 g., m. p. 134—136°C (sintering at 128°C).

Found: C, 64.05; H, 6.10; N, 6.76. Calcd. for $C_{33}H_{37}O_9N_3$: C, 63.96; H, 6.02; N, 6.78%.

Glycine Benzyl Ester (IIIb).—The *p*-toluensulfonate of glycine ester was prepared by the method of Zervas, et al.[15] Yield, 80—90%, m. p. 130—132°C. Free glycine benzyl ester was prepared by treating with an excess of anhydrous potassium carbonate and ether while cooling and stirring. The resulting free ester was used for further synthesis without purification.

***N*-Formyl-*O*-benzyl-L-serylglycine Benzyl Ester (IVb).**—To the mixture consisting of 11.2 g. of *N*-formyl-*O*-benzyl-L-serine, 9.1 g. of glycine benzyl ester in 50 ml. of dioxane, 11.0 g. of DCCD in 20 ml. of dioxane was added under cooling. The mixture was allowed to stand overnight. The reaction mixture was treated in the same way as was described earlier. The resultant dipeptide benzyl ester was dissolved in ethyl acetate and precipitated with ether, yield, 15.0 g. (81%), m. p. 89—90°C. The precipitate was further purified by treating it twice with ethyl acetate and ether, 13.0 g., m. p. 91—92°C, $[\alpha]_D^{25}$ +2.2° (*c* 2.43, AcOH), $[\alpha]_D^{25}$ +0.26° (*c* 3.0, ethanol).

Found: C, 65.02; H, 5.92; N, 7.53. Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.85; H, 5.97; N, 7.56%.

***O*-Benyzl-L-serylglycine Benzyl Ester Hydrochloride (Vb).** — *N*-Formyl-*O*-benzyl-L-serylglycine benzyl ester (IVb), 2.0 g., was dissolved in 6.4 ml. of dioxane and 6 ml. of 1 N ethanolic hydrochloric acid. This solution was allowed to stand at room temperature for 3 days. The reaction mixture was evaporated under reduced pressure at 40°C. The resudue was dissolved in water and the insoluble material was filtered off; the filtrate was extracted with ethyl acetate. The aqueous solution was lyophilized. Yield, 1.40 g. (69%). $[\alpha]_D^{27}$ +8.3° (*c* 6.3, water).

***N*-Carbobenzoxy-L-aspartyl-*O*-benzyl-L-serylglycine Dibenzyl Ester (XIIb).**—*β*-Benzyl *N*-carbobenzoxy-L-aspartate (VII), 6.0 g., was mixed with *O*-benzyl-L-serylglycine benzyl ester (VIb) in 60 ml. of dioxane which was prepared from 7.3 g. of its hydrochloride (Vb) and 2.7 ml. of triethylamine. To this mixture, 4.0 g. of DCCD in 10 ml. of dioxane was added under cooling and stirring. The mixture was allowed to stand overnight at room temperature. The reaction mixture was processed in the usual manner. The protected tripeptide ester was crystallized from ethyl acetate and ether. The crystals were washed with petroleum ether and dried, yield, 5.3 g., (41%), m. p. 119—125°C. This was recrystallized from ethyl acetate and ether, m. p. 129—131°C, $[\alpha]_D^{25}$ −4.7° (*c* 1.43, dioxane).

Found: C, 67.41; H, 5.99; N, 6.25. Calcd. for $C_{38}H_{39}O_9N_3$: C, 66.95; H, 5.77; N, 6.16%.

***α*-Aspartyl-L-serylglycine (XXI).** — *N*-Carbobenzoxy-*α*-L-aspartyl-*O*-benzyl-L-serylglycine dibenzyl ester (XIIb), 1.36 g., was dissolved in 60 ml. of methanol and 5 ml. of 0.4 N hydrochloric acid. This solution was mixed with 3.5 g. of 5% palladium on charcoal. Hydrogenolysis of the mixture was carried out at room temperature for 5 hr. After the reaction was completed, the catalyst was filtered and the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in a small amount of water and filtered. The filtrate was lyophilized. A yield of 550 mg. of material was obtained. The tripeptide hydrochloride was dissolved in absolute alcohol and the insoluble part was filtered off. To the filtrate was added 0.03 ml. of pyridine and the solution was kept in the refrigerator overnight. The precipitated free tripeptide was collected by centrifugation and washed with absolute alcohol and ether and dried. This was recrystallized by dissolving in water and precipitating with absolute ethanol; yield, 250 mg. (45%). The crystals are not hygroscopic. m. p. 174—176°C (decomp.), $[\alpha]_D^{25}$ −7.3° (*c* 1.54, water).

Found: C, 38.93; H, 5.45; N, 15.16. Calcd. for $C_9H_{15}O_7N_3$: C, 38.99; H, 5.45; N, 15.16%.

By paper chromatography in 80% phenol, the peptide showed an R_f=0.17. Amino acid composition analyzed by the Phoenix K 5000 amino acid analyzer showed 97 aspartic acid: 98 serine: 100 glycine.

***N*-Carbobenzoxy-L-glutamyl-*O*-benzyl-L-serylglycine Dibenzyl Ester (XIIIb).**—*O*-Benzyl-L-serylglycine benzyl ester hydrochloride (Vb), 2.36 g. in 20 ml. of dioxane, was treated with 0.66 g. of triethylamine. Precipitated amine hydrochloride was removed by filtration. To the filtrate, 2.50 g. of *γ*-benzyl *N*-carbobenzoxy-L-glutamate (VIII) and 1.45 g. of DCCD in 5 ml. of dioxane were added. The reaction mixture was kept at room temperature overnight. This was treated in the way described earlier. The protected tripeptide ester was crystallized from ethyl acetate and ether, 2.45 g., m. p. 108—111°C. This solid was recrystallized from ethyl acetate, m. p. 109—112°C, $[\alpha]_D^{25}$ −1.83° (*c* 1.81, dioxane).

Found: C, 67.31; H, 5.97; Calcd. for $C_{39}H_{41}O_9N_3$: C, 67.32; H, 5.94%.

***α*-L-Glutamyl-L-seryl-glycine (XXII).**—*N*-Carbobenzoxy-L-glutamyl-*O*-benzyl-L-serylglycine dibenzyl ester (XIIIb), 1.35 g., was dissolved in a mixture of 60 ml. of methanol, 30 ml. of ethanol, 2.15 ml. of 1 N hydrochloric acid, and 3 ml. of water. To this mixture, 3.0 g. of 5% palladium on charcoal was added. Hydrogenolysis was carried out at room temperature for 10 hr. After the reaction was completed, the catalyst

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15) L. Zervas, M. Winitz and J. P. Greenstein, *J. Org. Chem.*, **22**, 1515 (1957).

was removed by filtration and the filtrate was evaporated to dryness under reduced pressure at 40°C. The residue was dissolved in a small amount of water. The insoluble material was filtered and the filtrate was lyophilized. The dried residue (tripeptide hydrochloride) was dissolved in 15 ml. of ethanol and 1.4 millimole of pyridine was added slowly. The tripeptide crystallized at once. The crystals were collected by centrifugation, and were washed with ether and dried, yield, 0.25 g. (44%). This solid was recrystallized by dissolving in 2.5 ml. of water and precipitating by 15 ml. of absolute ethanol. Tripeptide, 202 mg., was obtained, m. p. 191—192°C (decomp.), $[\alpha]_D^{27}$ +8.62° (*c* 1.24, water).

Found: C, 41.23; H, 5.93; N, 14.18. Calcd. for $C_{10}H_{17}N_3O_7$: C, 41.23; H, 5.88; N, 14.43%.

The tripeptide is not hygroscopic. By paper chromatography in 80% phenol, the peptide showed an R_f=0.35. The amino acid composition analyzed showed 100 glutamic acid: 98 serine: 99 glycine.

Summary

Syntheses of the free tripeptides found in the active site of various enzymes,[3] α-L-aspartyl-L-serylglycine and α-L-glutamyl-L-serylglycine, are described. Difficulties in the synthesis included the rapid saponification of β-benzyl *N*-carbobenzoxy-L-aspartyl residues.[5] Also, although many protected tri- and tetra-peptide esters were synthesized, attempts to obtain pure products by saponification were unsuccessful.

The synthesis was accomplished by using intermediates protected only with groups that could be simultaneously removed by hydrogenolysis. The intermediates thus employed were the *N*-carbobenzoxy-α-L-aspartyl-*O*-benzyl-L-serylglycine dibenzyl ester (XIIb) and the α-L-glutamyl analog (XIIIb). The syntheses of these two intermediates are also described.