The Synthesis of α-L-Aspartyl-L-serylglycine[1]

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The tripeptide residue, α-L-aspartyl-L-serylglycine, has been found as part of the active site of many esteratic enzymes[2]. The synthesis of this tripeptide is accordingly of interest for studies of catalytic activity *per se*, as a standard intermediate for syntheses of related substances, and as a copolymerized monomer in three-dimensional association with histidine residues in the polymers[3].

The ethyl ester of the tripeptide has been synthesized in this study. Other workers[4] have reported the synthesis of the ethyl ester of glycyl-α-L-aspartyl-L-serylglycine and of the L-aspartyl-*O*-acetyl-L-serylglycylglycine[5]. Attempts in this laboratory to saponify the tripeptide ester led to impure products. The *O*-acetylserine residue may also be subject to racemization when the acetate is removed by the usual treatment. The substituted aspartyl residue is also known to undergo alkaline decomposition easily[6]. The absence of the desired tripeptide from the literature, as well as informal reports of unsuccessful attempts at synthesis, can be explained by the reasons given.

We have devised a route which depends solely upon benzyl or carbobenzyloxy substituents which are entirely removable by hydrogenolysis. This route accordingly avoids any alkaline dederivatization. The sequence is:

$$\text{F-Ser(Bz)-OH (I)} + \text{H-Gly-OBz (II)} \xrightarrow[(90\%)]{\text{DCCD}}$$

$$\text{F-Ser(Bz)-Gly-OBz (III)} \xrightarrow[(71\%)]{} \text{H-Ser(Bz)-Gly-OBz (IV)}$$

$$\text{Z-Asp(OBz(}\beta\text{))-OH (V)} + \text{IV} \xrightarrow[(47\%)]{\text{DCCD}}$$

$$\text{Z-Asp(OBz)-Ser(Bz)-Gly-OBz (VI)} \xrightarrow[\text{Pd-C (55\%)}]{H_2} \text{H-Asp-Ser-Gly-OH (VII)}$$

F = Formyl; Z = Carbobenzyloxy; Bz = Benzyl

Except for II and V, none of these compounds were found in the literature. The aspartic acid and serine residues were L throughout.

The *N*-formyl-*O*-benzyl-L-serine was prepared by formylation of *O*-benzyl-L-serine[7], m. p. 133~134°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%). This substance was reacted with benzyl glycinate and *N*, *N'*-dicyclohexylcarbodiimide (DCCD) in dioxane to yield the formyldipeptide ester (III), m. p. 91~92°C, $[\alpha]_D^{25} = +2.2°$ (*c* 2.43, acetic acid) (Found: C, 65.02; H, 5.92; N, 7.53. Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.85; H, 5.99; N, 7.56%). This compound

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6) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).

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was deformylated by standing at room temperature in dioxane: benzyl alcohol: hydrochloric acid (5.5 ml.: 5.5 ml.: 1.0 ml. 12 N hydrochloric acid) in an amount equivalent in hydrochloric acid to the formyl ester, for 48 hr. to yield the hydrochloride of IV, which was hygroscopic and not analyzed as such. The hydrochloride had, however, $[\alpha]_D^{25}$ of approximately +8.3° (*c* 6.3, water). The hydrochloride was treated with an equivalent amount of triethylamine in dioxane in an ice bath and filtered from triethylammonium chloride. To the cold filtrate was added β-benzyl *N*-carbobenzyloxy-L-aspartate (V) and DCCD to give the substituted tripeptide (VI), 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%). All four substituents were simultaneously removed by hydrogenolysis with palladium-charcoal in methanol containing an equimolar amount of hydrochloric acid. The hydrochloride of the desired tripeptide (VII) was obtained. The hydrochloride was dissolved in absolute ethanol, the ethanolic solution was filtered from a small amount of insoluble impurity, and the filtrate was treated with an equivalent of pyridine. The free tripeptide (VII) separated immediately. It had d. p. 174~176°C, $[\alpha]_D^{25} = -7.3°$ (*c* 1.54, water). (Found: C, 38.93; H, 5.64; N, 15.20. Calcd. for $C_9H_{15}O_7N_3$: C, 38.99; H, 5.45; N, 15.16%). [Found; on automatic amino acid analyzer (following hydrolysis with 6 N hydrochloric acid at 110°C for 12 hr.): aspartic acid, 0.97; serine, 0.98; glycine, 1.00]. The final tripeptide was found to yield a single spot with ninhydrin when chromatographed on paper in a phenol $-H_2O$ (4 : 1) solvent, $R_f = 0.17$, with R_f of glycine standard = 0.38.

The tripeptide has little activity for the hydrolysis of *p*-nitrophenyl acetate, and this is additive with that of the free histidine when the two are tested together in solution. Now that the tripeptide is available, it can be included in various kinds of copolymerization with histidine for further tests.

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