400. *Amino-acids and Peptides. Part II. Synthesis of α- and γ-L-Glutamyl-peptides by the Azide Route.*

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Carbobenzyloxy-α-L-glutamylhydrazide has been synthesised and its usefulness in preparing α-glutamyl-peptides examined. In general, the yields are somewhat lower than those obtained by the alternative anhydride route (preceding paper). Carbobenzyloxy-γ-L-glutamylhydrazide has been prepared in improved yield and purity and has been used for the synthesis of the following new peptides: γ-L-glutamyl-glycine, -L-glutamic acid, -L-aspartic acid, -L-tyrosine, and -glycylglycine. Preliminary results are reported of an investigation of the autohydrolysis of some α- and γ-glutamyl-peptides; there are indications that hydrolysis under these conditions may be preceded or accompanied by cyclisation to form pyrrolidone derivatives.

The α- and γ-hydrazides of carbobenzyloxy-L-glutamic acid should clearly provide unambiguous paths for the synthesis of α- and γ-L-glutamyl-peptides. In Part I (preceding paper) it was shown that the action of hydrazine on carbobenzyloxy-L-glutamic anhydride gives a mixture of α- and γ-isomers, which cannot be separated by crystallisation. The hydrazides have therefore been prepared by other routes, and characterised, and their use in synthesis has been examined.

α-Ethyl carbobenzyloxy-L-glutamate (*loc. cit.*) with hydrazine at room temperature afforded carbobenzyloxy-α-L-glutamylhydrazide (I). Curtius degradation in benzyl alcohol of the azide formed from this material gave γγ-dicarbobenzyloxyaminobutyric acid, in confirmation of the expected structure. The benzylidene derivative has m. p. 194—196°. Hydrogenation of (I) gave α-L-glutamylhydrazide.

$CH_2Ph\cdot O\cdot CO\cdot NH\cdot CH(CH_2\cdot CH_2\cdot CO_2H)\cdot CO\cdot NH\cdot NH_2$ (I.)

$CH_2Ph\cdot O\cdot CO\cdot NH\cdot CH(CH_2\cdot CH_2\cdot CO\cdot NH\cdot NH_2)\cdot CO_2H$ (II.)

The azide prepared from (I) was coupled with glycine ethyl ester in chloroform, yielding carbobenzyloxy-α-L-glutamylglycine ethyl ester. Coupling with diethyl L-glutamate and with L-tyrosine ethyl ester gave analogous derivatives, identical with those prepared by the anhydride route, but the yields in each case were lower.

For the preparation of carbobenzyloxy-γ-L-glutamylhydrazide (II), we treated with hydrazine Abderhalden and Nienburg's γ-ethyl carbobenzyloxy-L-glutamate (*Z. physiol. Chem.*, 1933, **219**, 155), the structure of which was proved by Bergmann and Zervas (*ibid.*, 1933, **221**, 51) and by Nienburg (*Ber.*, 1935, **68**, 2232). A preliminary report of this part of our work was presented in *Nature* (1949, **163**, 604). Hegedüs had meanwhile reported (*Helv. Chim. Acta*, 1948, **31**, 737) the synthesis of this hydrazide, m. p. 170—172°, by a similar route and in 44% yield. We obtained however an 80% yield of recrystallised product of m. p. 178—179°. The benzylidene derivative has m. p. 201—202°, depressed by admixture with that of the α-hydrazide. Curtius degradation in benzyl alcohol of the azide formed from (II) gave a product which, on hydrogenation and short acid hydrolysis, yielded L-αγ-diaminobutyric acid, isolated as the dipicrate. Examination of the solution obtained after hydrogenation failed to detect the presence of the aldehyde which would result from contamination of the original material by α-hydrazide.

The usefulness of carbobenzyloxy-γ-L-glutamylhydrazide has been shown by Hegedüs (*loc. cit.*) in a novel synthesis of glutathione. We have coupled the azide with the glycine ethyl ester in chloroform, obtaining carbobenzyloxy-γ-L-glutamylglycine ethyl ester, which on saponification gave carbobenzyloxy-γ-L-glutamylglycine; hydrogenation then yielded γ-L-glutamylglycine. The presence of a free α-amino-acid group in this dipeptide was shown by the reaction with ninhydrin under standard conditions (Van Slyke, Dillon, McFadyen, and Hamilton, *J. Biol. Chem.*, 1941, **141**, 627), which gave 1·0 mole of carbon dioxide. Through analogous intermediates γ-L-glutamyl-L-glutamic acid, -L-aspartic acid, -L-tyrosine, and -glycylglycine have been synthesised.

We have successfully used the method of Boothe *et al.* (*J. Amer. Chem. Soc.*, 1949, **71**, 2304), coupling the azide with glycine in aqueous potassium hydrogen carbonate solution. With other amino-acids, we have experienced greater difficulty in obtaining pure products than when using anhydrous conditions. In general, γ-glutamyl derivatives show considerable reluctance to crystallise; this factor facilitates the isolation of α-glutamyl derivatives from mixed coupling products, but renders work upon the γ-isomers difficult.

An investigation of the autohydrolysis of α- and γ-glutamyl-peptides is now in progress and some preliminary results are of interest. Aqueous solutions of the peptides were heated at 100° and samples were examined by paper partition chromatography at various times. After 24 hours, little of the original peptide remained from γ-L-glutamyl-glycine, -L-tyrosine, -L-aspartic acid, -L-glutamic acid, or -glycylglycine; in each case, strong colours were formed with ninhydrin corresponding in position to the second-named amino-acid, the tripeptide yielding mainly glycylglycine but also some glycine. Only from the hydrolysis of γ-L-glutamyl-L-glutamic acid could a significant amount of glutamic acid be detected. From α-L-glutamylglycine and α-L-glutamyl-L-aspartic acid only small amounts of dipeptide remained after 24 hours; glycine and aspartic acid respectively were readily detected but again no glutamic acid appeared. Solutions of α-L-glutamyl-L-valine, -L-leucine, -L-tyrosine, and -L-glutamic acid showed after 24 hours not more than traces of any material giving a colour with ninhydrin. In a control experiment, after being heated for 24 hours at 100° an aqueous solution of L-glutamic acid still gave a normal though somewhat weaker chromatogram.

A possible explanation of these results is that autohydrolysis of α- and γ-glutamyl peptides is preceded or accompanied by cyclisation to form pyrrolidone derivatives, which do not give a ninhydrin reaction. In 0·5N-hydrochloric acid, where cyclisation is not to be expected, all the glutamyl-peptides examined gave chromatograms consistent with the presence of the normal hydrolysis products, including glutamic acid. These investigations are being continued.

Experimental.

All m. p.s are uncorrected. Combustion analyses are by Drs. Weiler and Strauss and by Mr. F. C. Hall.

Carbobenzyloxy-α-L-glutamylhydrazide.—α-Ethyl carbobenzyloxy-L-glutamate (2 g.; see preceding paper) in ethanol (3 ml.) was added drop-wise, with shaking, to hydrazine hydrate (3 ml.; 90%) during 5—10 minutes. After 2 days at room temperature, water (12 ml.) was added, followed by concentrated hydrochloric acid until the solution was acid to Congo-red. The solution was neutralised to Congo-red with saturated aqueous sodium acetate and left at 0° for 1 hour, and the *carbobenzyloxy-α-L-glutamylhydrazide* (1·5 g., 78%) filtered off and washed with water. The m. p. was 163—166°, raised by recrystallisation from boiling water to 168—170° (Found: C, 52·6; H, 5·7. $C_{13}H_{17}O_5N_3$ requires C, 52·9; H,

5·8%), $[\alpha]_D^{18}$ −23·4° (*c*, 8·66 in 0·5N-hydrochloric acid). The *benzylidene* derivative had m. p. 194—196° (Found: C, 62·2; H, 5·7; N, 11·0. $C_{20}H_{21}O_5N_3$ requires C, 62·6; H, 5·5; N, 11·0%).

Curtius Degradation of Carbobenzyloxy-α-L-glutamylhydrazide.—Carbobenzyloxy-α-L-glutamylhydrazide (0·5 g.) was converted into the azide in ether (20 ml.); after being dried (Na_2SO_4), the solution was added to benzyl alcohol (1·5 ml.). The ether was removed under reduced pressure and the remaining solution left overnight at room temperature, then heated at 100° for 2 hours. Ether (10 ml.) was added, and the γγ-dicarbobenzyloxyaminobutyric acid (0·3 g., 46%) filtered off. After recrystallisation from ethanol, it had m. p. 169—171°, unaltered by admixture with the product described in Part I.

α-L-Glutamylhydrazide.—Carbobenzyloxy-α-L-glutamylhydrazide in aqueous methanol was hydrogenated in the usual manner with palladium black as catalyst. The filtrate was evaporated to dryness under reduced pressure and the remaining oil triturated with ethanol, the *α-L-glutamylhydrazide* solidifying; it had m. p. 165—168°, raised by recrystallisation from aqueous ethanol to 169—171° (Found: C, 36·8; H, 6·6. $C_5H_{11}O_3N_3$ requires C, 37·2; H, 6·9%).

Carbobenzyloxy-α-L-glutamylglycine Ethyl Ester from the α-Hydrazide.—Carbobenzyloxy-α-L-glutamylhydrazide (0·85 g.) was converted into the azide in chloroform (15 ml.) in the normal manner. After being washed with water and dried (Na_2SO_4) for a few seconds, it was added at 0° portion-wise, with shaking, to a solution of glycine ethyl ester (from 3 g. of hydrochloride) in chloroform (15 ml.). The mixture was left for ½ hour at 0° and overnight at room temperature. It was then washed with 2N-hydrochloric acid and water, dried (Na_2SO_4), and evaporated under reduced pressure. The product solidified under light petroleum and was recrystallised from ethyl acetate–light petroleum, giving carbobenzyloxy-α-L-glutamylglycine ethyl ester (0·25 g., 24%); it had m. p. 120—122°, unchanged by admixture with the compound prepared by the anhydride ring-opening.

Diethyl Carbobenzyloxy-α-L-glutamyl-L-glutamate from the α-Hydrazide.—Carbobenzyloxy-α-L-glutamylhydrazide (0·79 g.) was converted into the azide in chloroform and added to diethyl L-glutamate (from 3·5 g. of hydrochloride) in chloroform. After 2 days at 0—5°, the solution was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated under reduced pressure. The product was crystallised from ethyl acetate–light petroleum, giving diethyl carbobenzyloxy-α-L-glutamyl-L-glutamate (0·3 g., 24%), m. p. 131—134°, raised to 134—136° by recrystallisation. Mixed with the compound from the anhydride ring-opening, it had m. p. 133—135°.

Carbobenzyloxy-γ-L-glutamyl-L-tyrosine Ethyl Ester from the α-Hydrazide.—Carbobenzyloxy-α-L-glutamylhydrazide (1 g.) was converted into the azide in chloroform solution and added to L-tyrosine ethyl ester (2·8 g.) in chloroform (15 ml.). The solution was left for ½ hour at 0° and overnight at room temperature, washed with dilute hydrochloric acid and water, dried, and evaporated under reduced pressure. Treatment of the resultant syrup with ethanol–ether gave carbobenzyloxy-α-L-glutamyl-L-tyrosine ethyl ester (0·2 g., 12%), m. p. 162—172°, raised to 172—176° by recrystallisation from ethanol–ether. The latter value was unaltered by admixture with the product from the anhydride ring-opening.

γ-Ethyl Carbobenzyloxy-L-glutamate.—γ-Ethyl carbobenzyloxy-L-glutamate was prepared as described by Abderhalden and Nienburg (*Z. physiol. Chem.*, 1933, **219**, 155) and, recrystallised from carbon tetrachloride, had m. p. 84—86°.

Carbobenzyloxy-γ-L-Glutamylhydrazide.—γ-Ethyl carbobenzyloxy-L-glutamate (3·4 g.) in ethanol (8 ml.) was added drop-wise during 5—10 minutes to hydrazine hydrate (3·5 ml.; 100%) and left for 48 hours at room temperature. Most of the ethanol was removed under reduced pressure and after addition of water the solution was acidified to Congo-red with hydrochloric acid. It was then neutralised with saturated aqueous sodium acetate; after 1 hour at 0°, the carbobenzyloxy-γ-L-glutamylhydrazide (3·2 g., 98%) was filtered off. It had m. p. 173—174°, raised by recrystallisation from boiling water to 178—179° (2·55 g., 80%) (Found: C, 52·7; H, 5·9; N, 14·0. Calc. for $C_{13}H_{17}O_5N_3$: C, 52·9; H, 5·8; N, 14·2%), $[\alpha]_D^{20}$ −13·4° (*c*, 6·4 in 0·5N-hydrochloric acid), $[\alpha]_D^{18}$ −5·1° (*c*, 10·0 in water containing 1 mole of sodium hydroxide). The *benzylidene* derivative had m. p. 200—201°, depressed to 181—186° by admixture with the α-analogue (Found: C, 62·5; H, 5·7; N, 11·2. $C_{20}H_{21}O_5N_3$ requires C, 62·6; H, 5·5; N, 11·0%).

Curtius Degradation of Carbobenzyloxy-γ-L-Glutamylhydrazide.—Carbobenzyloxy-γ-L-glutamylhydrazide (0·5 g.) was converted into the azide in the usual manner, and the dried ethereal solution added to benzyl alcohol (1·5 ml.). After removal of the ether under reduced pressure, the solution was heated at 100° for 2 hours, leaving an oil completely soluble in ether. A solution of it in aqueous methanol containing a little acetic acid was hydrogenated in the usual way. The filtrate was concentrated under reduced pressure to 2 ml., concentrated hydrochloric acid (2 ml.) added, and the mixture refluxed for 75 minutes. The solution was then evaporated to dryness under diminished pressure; the residue was dissolved in a little water, and saturated aqueous picric acid (40 ml.) added. After 2 days at 0°, the dipicrate of L-αγ-diaminobutyric acid (0·1 g.) was filtered off, having m. p. 177—179°, unchanged by recrystallisation from hot water.

The solution obtained by hydrogenation did not reduce Fehling's solution or give a precipitate with *p*-nitrophenylhydrazine in dilute hydrochloric acid.

Carbobenzyloxy-γ-L-glutamylglycine Ethyl Ester.—Carbobenzyloxy-γ-L-glutamylhydrazide (2 g.) was converted into the azide in chloroform solution and added to glycine ethyl ester (from 7·5 g. of hydrochloride) in chloroform. The solution was treated in the same manner as that from the α-compound and yielded, after removal of the solvent under reduced pressure, an oil which solidified after some hours under light petroleum. *Carbobenzyloxy-γ-L-glutamylglycine ethyl ester* (1·25 g.; 50%) had m. p. 97—104°, raised to 107—109° by recrystallisation from ethyl acetate–light petroleum (Found: C, 55·7; H, 5·9. $C_{17}H_{22}O_7N_2$ requires C, 55·7; H, 6·1%). The mixed m. p. with the α-analogue was 97—106°.

Carbobenzyloxy-γ-L-glutamylglycine.—Carbobenzyloxy-γ-L-glutamylglycine ethyl ester (1·5 g.) was dissolved in N-potassium hydroxide (10 ml.) and left for 2 hours at room temperature. The solution was acidified with 5N-hydrochloric acid and extracted with 4 successive portions (each 20 ml.) of ethyl acetate. The combined extracts were washed with small quantities of dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated under reduced pressure. The remaining *carbobenzyloxy-α-L-glutamylglycine* (0·7 g., 51%) was washed on to the filter with a little ethyl acetate. It had m. p. 159—161°, unchanged

by recrystallisation from ethyl acetate–ethanol–light petroleum (Found: C, 53·5; H, 5·3; N, 8·3. $C_{15}H_{18}O_7N_2$ requires C, 53·2; H, 5·4; N, 8·3%).

γ-L-Glutamylglycine.—Carbobenzyloxy-γ-L-glutamylglycine (0·7 g.) in aqueous methanol was hydrogenated in the normal manner in the presence of palladium black. The filtrate was evaporated under reduced pressure to small volume and excess of ethanol added. After ½ hour, the *γ-L-glutamylglycine* (0·3 g., 74%) was filtered off; after reprecipitation from water with ethanol, it had m. p. 193—194° (Found: C, 41·5; H, 5·9; N, 13·7. $C_7H_{12}O_5N_2$ requires C, 41·2; H, 5·9; N, 13·7%), $[\alpha]_D^{14}$ +11·1° (*c*, 2·52 in water). With ninhydrin at 100° for 15 minutes at pH 2·5, 1·0 mole of carbon dioxide was evolved.

Diethyl Carbobenzyloxy-γ-L-glutamyl-L-glutamate.—Carbobenzyloxy-γ-L-glutamylhydrazide (1 g.) was converted into the azide in chloroform (15 ml.) and added portionwise at 0° to diethyl L-glutamate (from 4 g. of hydrochloride). It was left for some hours at 0° and overnight at room temperature. The solution was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in a little ethyl acetate, and light petroleum added until a little oil separated. The solution was then poured off and excess of light petroleum added to it; the precipitated *diethyl carbobenzyloxy-γ-L-glutamyl-L-glutamate* slowly hardened to a waxy consistency under fresh petroleum. It had m. p. 62—68° (Found: C, 56·1; H, 6·3; N, 6·5. $C_{22}H_{30}O_9N_2$ requires C, 56·6; H, 6·5; N, 6·0%).

γ-L-Glutamyl-L-glutamic Acid.—Diethyl carbobenzyloxy-γ-L-glutamyl-L-glutamate (0·4 g.) was dissolved in N-sodium hydroxide (3 ml.) and left for 2 hours at room temperature. Subsequent treatment in the same manner as in the hydrolysis of carbobenzyloxy-γ-L-glutamylglycine ethyl ester gave carbobenzyloxy-γ-L-glutamyl-L-glutamic acid as an oil which did not crystallise under light petroleum. It was hydrogenated in the usual manner in aqueous methanol, and after evaporation of the filtrate under reduced pressure to 1—2 ml., acetone (10 ml.) was added. *γ-L-Glutamyl-L-glutamic acid* separated as an oil which solidified after some minutes. It was dissolved in water (2 ml.) and acetone (10 ml.) added. After 2 minutes, the supernatant solution was poured off and more acetone (20 ml.) added to it. The acid was filtered off after 1 hour at 0°, and had m. p. 188—191°. Drying at 100°/18 mm. was found necessary to remove water (Found: C, 43·3; H, 6·2; N, 9·8. $C_{10}H_{16}O_7N_2$ requires C, 43·5; H, 5·8; N, 10·1%), $[\alpha]_D^{17}$ <1° (*c*, 1·6 in water), $[\alpha]_D^{17}$ +6° (*c*, 1·1 in water containing 1 mole of hydrochloric acid).

γ-L-Glutamyl-L-aspartic Acid.—The procedure used was as in the preparation of the above dipeptide, but neither diethyl carbobenzyloxy-γ-L-glutamyl-L-aspartate nor carbobenzyloxy-γ-L-glutamyl-L-aspartic acid could be obtained solid. The hydrogenation gave *γ-L-glutamyl-L-aspartic acid* which solidified when kept under acetone. It was dissolved in a little water and fractionally precipitated with acetone. Paper chromatography showed that the first fraction contained a little glutamic acid, but subsequent fractions gave only one spot and had m. p. 177—182°. The product was dried for 2 hours at 100°/18 mm. for analysis, but retained one molecule of water of crystallisation (Found: C, 38·4; H, 5·4; N, 9·5. $C_9H_{14}O_7N_2,H_2O$ requires C, 38·6; H, 5·8; N, 10·0%), $[\alpha]_D^{18}$ +19·5° (*c*, 2·0 in water containing 1 mole of hydrochloric acid).

γ-L-Glutamyl-L-tyrosine.—The procedure used was similar to that for the previous two dipeptides. Carbobenzyloxy-γ-L-glutamyl-L-tyrosine and its ethyl ester were obtained only as shellac-like masses, melting around 70° with loss of gas. *γ-L-Glutamyl-L-tyrosine* crystallised when kept under acetone; after reprecipitation from water with acetone, it had m. p. 219—221°. It was dried at 100°/18 mm. for analysis (Found: C, 53·9; H, 6·0; N, 8·7. $C_{14}H_{18}O_6N_2$ requires C, 54·2; H, 5·8; N, 9·0%), $[\alpha]_D^{15}$ +25·6° (*c*, 2·1 in water).

Carbobenzyloxy-γ-L-glutamylglycylglycine Ethyl Ester.—Carbobenzyloxy-γ-L-glutamylhydrazide (1·2 g.) was converted into the azide in chloroform (20 ml.). This solution was added at 0° to one of glycylglycine ethyl ester (from 4·0 g. of hydrochloride) in chloroform (20 ml.) and left for 2 hours at 0° and overnight at room temperature. The solution was then shaken with dilute hydrochloric acid. *Carbobenzyloxy-γ-L-glutamylglycylglycine ethyl ester* separated slowly from both phases. It was filtered off and recrystallised from hot ethyl acetate, the product (1·3 g., 76%) having m. p. 92—94°. Drying over phosphoric oxide in a vacuum-desiccator gave the *monohydrate* (Found: C, 52·0; H, 6·3; N, 9·3. $C_{19}H_{25}O_8N_3,H_2O$ requires C, 51·7; H, 6·2; N, 9·5%). When dried at 61°/18 mm., the substance liquefied; it solidified again on storage under light petroleum, and was re-dried at 61°/18 mm. (Found: C, 53·4; H, 5·7. $C_{19}H_{25}O_8N_3$ requires C, 53·9; H, 5·9%).

Carbobenzyloxy-γ-L-glutamylglycylglycine.—A solution of carbobenzyloxy-γ-L-glutamylglycylglycine ethyl ester (1·3 g.) in N-sodium hydroxide (7 ml.) was left for 2 hours at room temperature. It was then acidified with 5N-hydrochloric acid and cooled to 0°; after ½ hour *carbobenzyloxy-γ-L-glutamylglycylglycine* (1·0 g., 83%) was filtered off. It had m. p. 100—114°; after recrystallisation from water and washing of the dried product with a little boiling ethyl acetate, the m. p. was 124—127° (Found: N, 10·3. $C_{17}H_{21}O_8N_3$ requires N, 10·6%).

γ-L-Glutamylglycylglycine.—Carbobenzyloxy-γ-L-glutamylglycylglycine (1·0 g.) was hydrogenated in the usual manner in aqueous methanol. After evaporation of the filtrate to small volume under reduced pressure, ethanol was added. After 1 hour at 0°, the *γ-L-glutamylglycylglycine* (0·5 g.) was filtered off. It had m. p. 176—178°, raised by reprecipitation from water by ethanol to 179—180°. It was dried at 100°/18 mm. for analysis (Found: C, 41·3; H, 5·6; N, 15·5. $C_9H_{15}O_6N_3$ requires C, 41·4; H, 6·0; N, 16·1%) and had $[\alpha]_D^{12}$ +9·4° (*c*, 2·75 in water).

Carbobenzyloxy-γ-L-glutamylglycine (Alternative Technique).—Carbobenzyloxy-γ-L-glutamylhydrazide (1·0 g.) was converted into the azide in ethyl acetate (15 ml.) and then added slowly at 0° to a solution of glycine (0·4 g.) and potassium hydrogen carbonate (1·5 g.) in water (10 ml.), stirred mechanically. Stirring was continued for 15 minutes, and the solution left for 2 hours at room temperature. The aqueous layer was acidified and extracted 3 times with an equal volume of ethyl acetate. The combined extracts were washed with a little water, dried (Na_2SO_4), and evaporated under reduced pressure. The remaining carbobenzyloxy-γ-L-glutamylglycine was washed with a little ether and ethyl acetate and collected. Recrystallisation from hot water raised the m. p. from 147—155° to 158—161° (0·45 g., 37%).

R_F *Values of Some γ-L-Glutamyl-peptides.*—Paper partition chromatography, with phenol saturated

with water as the mobile phase in an atmosphere containing ammonia, confirmed the purity of the above γ-glutamyl-peptides and gave the following R_F values: γ-L-glutamyl-glycine 0·20, -L-glutamic acid 0·08, -L-aspartic acid 0·04, -L-tyrosine 0·39, and -glycylglycine 0·24.

Hydrolysis of L-*Glutamyl-peptides.*—Solutions (*ca.* 1%) of L-glutamyl-peptides were heated in sealed tubes by means of a steam-jacket. Samples were removed at intervals and examined by paper partition chromatography, using phenol saturated with water as the mobile phase in an atmosphere containing ammonia; after being dried, the paper was sprayed with a solution of ninhydrin in moist butanol and the colour developed by heat. With solutions in water and in 0·5N-hydrochloric acid, the following results were observed after 24 hours at 100°. The symbols indicate the approximate intensity of the ninhydrin coloration: −, none detected; ±, trace; +, weak; ++, fairly strong; +++, strong; ++++, very strong.

	Aqueous solution.			0·5N-Acid solution.		
Dipeptide.	Dipeptide.	Glutamic acid.	Second amino-acid.	Dipeptide.	Glutamic acid.	Second amino-acid.
γ-L-Glutamylglycine	+	—	++++	—	++++	++++
γ-L-Glutamyl-L-tyrosine	+	—	+++	—	++++	+++
γ-L-Glutamyl-L-aspartic acid ...	—	—	++++	—	++++	++++
γ-L-Glutamyl-L-glutamic acid...	+	+++		—	++++	
α-L-Glutamylglycine	+	—	+++	—	+++	++++
α-L-Glutamyl-L-aspartic acid ...	±	—	++++	—	++++	++++
α-L-Glutamyl-L-valine	—	—	—	+	+++	+++
α-L-Glutamyl-L-leucine	±	—	—	+++	+++	+++
α-L-Glutamyl-L-tyrosine	—	—	—	++	+++	+++
α-L-Glutamyl-L-glutamic acid...	—	±		—	++++	

An aqueous solution of glutamic acid, heated for 24 hours at 100°, gave a strong colour in the normal position. Under the same conditions, an aqueous solution of γ-L-glutamylglycylglycine gave: tripeptide, +; glutamic acid, −; glycylglycine, ++; glycine, +. Glycylglycine in 2% acetic acid similarly gave: glycylglycine, ++++; glycine, +.

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