104. *Studies on Specific Chemical Fission of Peptide Links. Part III.* Fission of Peptides containing One Glutamic Acid Residue.*

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Two α-glutamyl peptides (II) have been synthesised which differ in the bulk of the amino-acid residues adjacent to that of glutamic acid. A study is made of the cyclisation of the peptides by thionyl chloride to 1-acylpyrrolid-2-ones (III) and also of the subsequent alkaline hydrolysis of these products. The results obtained can be explained on the basis of steric effects.

It is shown, for four isomeric pairs, that methyl α- and γ-glutamate peptide esters can readily be distinguished by infrared measurements.

Part II * of this series described a procedure for the specific fission of peptide chains at those points where glutamic acid residues occur. The scheme involves the conversion of α-glutamyl peptides (as II) into 1-acylpyrrolid-2-ones (as III) which are then readily hydrolysed by dilute alkali; the same approach has been studied independently by Clayton, Kenner, and Sheppard.[1] Yields of the products of chain fission (at A in III) were sufficiently encouraging in our experiments to warrant a further study of this method.

* Part II, *J.*, 1956, 2076.

[1] Clayton, Kenner, and Sheppard, *J.*, 1956, 371.

The present renewed interest [2] in specific chemical fission of peptides prompts us to report the results, which were obtained in 1956—1957.

It seemed probable that cyclisation of α-glutamyl peptides (as II) to 1-acylpyrrolidones (as III) and the subsequent alkaline hydrolysis would both be affected by the size of closely situated groups. Accordingly, *N*-benzoylglycyl-α-DL-glutamyl(glycine hexylamide) (IIa) was prepared to serve as an example having the minimum bulk on each side of the glutamic acid residue; this is referred to below as the " glycine peptide." *N*-Benzoylglycyl-α-DL-glutamyl-(DL-phenylalanine amide) (IIb) was the selected case having a large group close to the α-carboxyl group of the glutamic acid; this we refer to as the " phenylalanine peptide."

The glycine peptide (IIa) was prepared by condensing *N*-benzoylglycyl-DL-glutamic acid with glycine hexylamide by the mixed-anhydride method [4] with the use of only one equivalent of ethyl chloroformate.[3] A good yield of the mixture of isomeric α- and γ-peptides (IIa and IVa respectively) was obtained which was fractionated by countercurrent distribution to give 65% of the required α-isomer (IIa). The more weakly acidic peptide was assigned the α-glutamyl structure (IIa) on the basis of previous experience [3,5] and this structure is confirmed below.

For the synthesis of the phenylalanine peptide, DL-phenylalanine amide [6] was made by condensing phthaloyl-DL-phenylalanine [7] with ammonia in a mixed anhydride reaction; the phthaloyl group was removed from the product in the usual way with hydrazine.[8] This route to the amide was used because a large amount of the starting material was available from other work. When DL-phenylalanine amide was condensed with *N*-benzoylglycyl-DL-glutamic acid by the method used for the peptide (IIa) above, a mixture of products was formed from which one diastereoisomer of the α-phenylalanine peptide (IIb) was isolated by a combination of countercurrent distribution and fractional crystallisation. The behaviour of this peptide during fractionation and the melting point of the isolated product give sound evidence for its homogeneity. One diastereoisomer of the γ-phenylalanine peptide (IVb) was isolated in the same way. As before, the weaker acid was assigned the α-glutamyl structure (IIb) and this assignment was later rigorously confirmed.

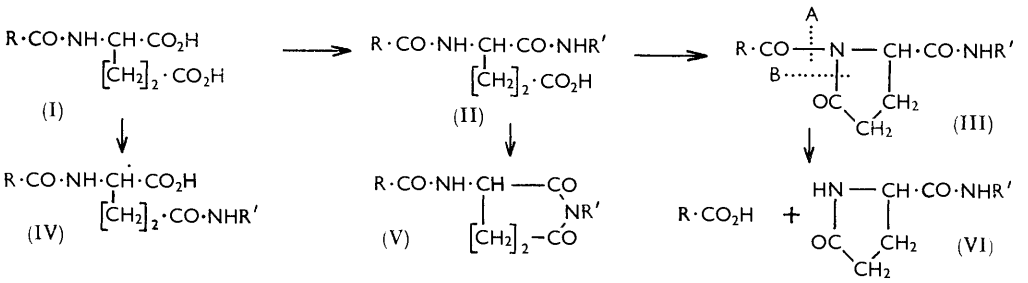
The glycine peptide (IIa) and the phenylalanine peptide (IIb) were both cyclised by thionyl chloride at −20° to give good yields of neutral products (89% and 63% respectively). However, fractional crystallisation of the neutral material in the glycine series showed it to be a mixture containing about 35% of the acylpyrrolidone (IIIa) together with the imide (Va). The imide (Va) was the only neutral product, formed in very low yield (5%), when the mixed carbonic anhydride (IIa; $CO{\cdot}O{\cdot}CO_2Et$ in place of CO_2H) derived from the glycine peptide (IIa) was allowed to cyclise at low temperature. Surprisingly, the γ-peptide in the glycine series (IVa) was quantitatively recovered after being treated under the same conditions as the α-isomer (IIa) in a mixed carbonic anhydride reaction.

In accord with earlier results,[1,3,5] alkaline hydrolysis of the imide (Va) gave a mixture consisting of 79% of the γ-peptide (IVa) and 21% of the α-peptide (IIa). These accounted for 96% of the imide used and a negligible amount of neutral material was formed.

The neutral product resulting from cyclisation of the phenylalanine peptide (IIb) with thionyl chloride was almost entirely the acylpyrrolidone (IIIb); a search for the imide

[2] Schmir, Cohen, and Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 2228; Corey and Haefele, *J. Amer. Chem. Soc.*, 1959, **81**, 2225; Ramachandran and Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 4028.
[3] Battersby and Robinson, *J.*, 1956, 2076.
[4] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547; Wieland and Bernhard, *Annalen*, 1951, **572**, 190.
[5] Battersby and Robinson, *J.*, 1955, 259.
[6] Koenigs and Mylo, *Ber.*, 1908, **41**, 4439.
[7] Sheehan and Frank, *J. Amer. Chem. Soc.*, 1949, **71**, 1856.
[8] Ing and Manske, *J.*, 1926, 2348.

yielded only a trace of material which could be this product and the amount was insufficient for characterisation. This result is in striking contrast to the predominant formation of imide from the glycine peptide (IIa) and its seems probable that this is due to the bulk of the phenylalanine residue. The electronic effect of the benzyl side chain on the nitrogen



Series (a): R = Ph·CO·NH·CH_2, R′ = CH_2·CO·NH·$[CH_2]_5$·Me
Series (b): R = Ph·CO·NH·CH_2, R′ = $CH(CH_2Ph)$·CO·NH_2
Series (c): R = C_7H_7·SO_2·NH·CH_2·CO·NH·CH_2, R′ = $CH(CH_2Ph)$·CO·NH·C_6H_{11}

atom involved in imide formation may also assist, but that effect is probably small; for example, one can compare the shift in acidic strength from acetic acid (pK_a 4·74) to β-phenylpropionic acid [9] (pK_a 4·66).

The above formation of acylpyrrolidones from the glycine and phenylalanine peptides confirms the structures (IIa) and (IIb) respectively assigned to them on the basis of acidic strengths.

Our main interest in studying the alkaline hydrolysis of the two acylpyrrolidones (IIIa) and (IIIb) was centred on the extent of the fission (at A in III); the acidic products, which by analogy with Battersby and Robinson's results [3] should contain the α- and γ-peptides (as II and IV respectively) in addition to *N*-benzoylglycine, were not examined. In the glycine series, hydrolysis of the acylpyrrolidone (IIIa) by 0·1N-sodium hydroxide in aqueous dioxan yielded approximately 50% of the 5-oxo-DL-pyrrolidone (VIa), so giving a measure of the chain fission. Under the same conditions, the acylpyrrolidone (IIIb) in the phenylalanine series gave approximately 40% of the corresponding product (VIb) of chain fission. Both pyrrolidones (VIa) and (VIb) showed carbonyl absorption in the infrared spectrum close to 1700 cm.$^{-1}$, in agreement with the value recorded by Barrass and Elmore [10] for a similar pyrrolidone.

Clayton, Kenner, and Sheppard [1] have studied a close analogue (IIIc) of the acylpyrrolidone derived from the phenylalanine peptide (IIb); their material differs from ours only in having toluene-*p*-sulphonylglycyl in place of the benzoyl group and cyclohexyl in place of the n-hexyl residue. However, hydrolysis of their material (IIIc) under conditions which were similar to ours regenerated 90% of the starting material (IIc). A possible explanation of these differing results is that the highly polar toluene-*p*-sulphonyl group is interacting with the acyl-carbonyl group of the acylpyrrolidone (IIIc); a study of the analogue having benzoyl in place of the toluene-*p*-sulphonyl group would therefore be of interest.

Since a higher proportion of chain fission than that achieved above is very desirable, a study was made of the effect of different solvents and one other base on the hydrolysis. The results were not significantly different from those above when either aqueous tetrahydrofuran or dimethylformamide was used. This is in contrast to the appreciable effect that changes of solvent had on the hydrolyses of related compounds.[11] In addition, the hydrolysis of our acylpyrrolidones does not occur at room temperature at a significant rate in aqueous dioxan containing triethylamine.

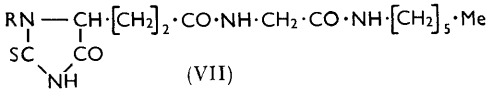
[9] Dippy, *Chem. Rev.*, 1939, **25**, 151.
[10] Barrass and Elmore, *J.*, 1957, 4830.
[11] Wieland and Weidenmuller, *Annalen*, 1955, **597**, 111.

Compound	Absorption bands in 1600—1800 cm.$^{-1}$ region (Nujol mull)	Ester >C=O absorption (cm.$^{-1}$) ($CHCl_3$ solution)
II; Me ester, R = Ph, R′ = CH_2·CO·NH·$[CH_2]_5$·Me	1617(sh)s, 1640vs, 1657vs, 1670(sh)s, 1751s	1728s
IV; Me ester, R = Ph, R′ = CH_2·CO·NH·$[CH_2]_5$·Me	1617(sh)m, 1650vs, 1690(sh)m, 1761s	1742s
II; Me ester, R = Ph·CO·NH·CH_2, NHR′ replaced by NEt_2	1615w, 1651vs, 1667(sh)s, 1681m, 1742s, 1751s	1733s
IV; Me ester, R = Ph·CO·NH·CH_2, NHR′ replaced by NEt_2	Amorphous	1757s
IIa; Me ester	1616(sh)m, 1652vs, 1680(sh)s, 1756s	1748s
IVa; Me ester	1615m, 1650vs, 1667vs, 1676(sh)s, 1701(sh)m, 1751s	1758s
IIb; Me ester	1619(sh)s, 1633vs, 1652(sh)s, 1669(sh)s, 1689s, 1745s	Insoluble
IVb; Me ester	1615(sh)s, 1649vs, 1660(sh)vs, 1683s, 1705s, 1760s	Insoluble
IIIa	1617w, 1659vs, 1702s, 1718(sh)m, 1767s	
Va	1617(sh)m, 1638(sh)s, 1658vs, 1677vs, 1704s, 1751w	
VIa	1640(sh)s, 1660vs, 1676(sh)s, 1705vs	
IIIb	1614w, 1651vs, 1690vs, 1701(sh)s, 1744s, 1761s	
VIb	1655s, 1678(sh)s, 1688s	
VII; R = Ph·CO	1648vs, 1683s, 1754s	
VII; R = H	1650vs, 1711(sh)s, 1731vs	

The foregoing work supports the conclusions[3] drawn from earlier experiments. However, it is now clear that the results are markedly affected by changes in the environment of the glutamic acid residue. Probably, therefore, the acylpyrrolidone method should only be considered for peptides containing one glutamic acid group. The resultant mixture would then be sufficiently simple to allow the peptides that had not undergone fission to be recycled through the process to increase the yield.

The infrared spectra of some of our peptides are of interest. It has been found that the ester-carbonyl absorptions of α-glutamyl peptide methyl esters (esters of II) lie 10—20 cm.$^{-1}$ lower than those of the γ-glutamyl isomers (esters of IV); the esters shown in the Table were prepared from the corresponding acids by using diazomethane. The lower frequency of the carboxylic acid band for α-glutamyl peptides than for the γ-isomers has been pointed out previously by Clayton, Kenner, and Sheppard,[1] but in the case of the free acids there is considerable variation with structure which the authors reasonably ascribe to differences in the hydrogen bonding of the carboxyl group. The esters studied so far give sharp absorption peaks with somewhat steadier values and this approach promises to be a simple one for orienting α- and γ-glutamyl peptides when both isomers are available.

Experiments have been carried out on the reactivity of two of the γ-glutamyl peptides (IV) prepared in this work towards diphenyl phosphorisothiocyanatidate.[12] *N*-Benzoyl-γ-DL-glutamyl(glycine hexylamide)[5] (IV; R = Ph, R′ = CH_2·CO·NH·$[CH_2]_5$·Me) was smoothly converted by this reagent into 65% of the thiohydantoin (VII; R = Ph·CO);



(VII)

hydrolysis of this product with aqueous alkali gave 77% of the theoretical yield of benzoic acid together with the hydantoin (VII; R = H). However, the peptide (IVa) gave little or none of the expected acylthiohydantoin when treated with the same reagent under the same or similar conditions.

[12] Kenner, Khorana, and Stedman, *J.*, 1953, 673.

Experimental

For general directions see Part II.[3]

*N-Benzoylglycyl-α- and -γ-*DL*-glutamyl(glycine Hexylamides)* (IIa) *and* (IVa).—Ethyl chloroformate (2·4 ml., 1 equiv.) was added to a stirred solution of *N*-benzoylglycyl-DL-glutamic acid [3] (7·72 g.) and triethylamine (3·5 ml., 1 equiv.) in chloroform (50 ml.) and dimethylformamide (50 ml.) at 0°. After 20 min., a solution of glycine hexylamide hydrochloride [5] (4·87 g., 1 equiv.) and triethylamine (3·5 ml.) in chloroform (20 ml.), dimethylformamide (5 ml.), and water (5 ml.) was added and stirring was continued for 3 hr. at 0°. The solvents were evaporated and the residue was partitioned between water (50 ml.) and ethyl acetate (100 ml., and, after filtration and separation, with 200 ml.). The aqueous phase was then adjusted to pH 2 with concentrated hydrochloric acid, and the precipitated solid (4·71 g.) was collected and dried. The combined ethyl acetate extracts were shaken with 0·1N-sodium hydroxide (2 × 150 ml.), and the alkaline extract was acidified to precipitate crystals (0·78 g.). The filtrate was extracted with ethyl acetate (2 × 150 ml.), and this extract was dried and evaporated to give more solid (1·42 g.).

The combined solids (6·81 g.) were fractionated by countercurrent distribution (scattered in the first 11 tubes; 124 transfers) between ethyl acetate (4 vol.), butan-1-ol (1 vol.), and aqueous phosphate buffer (5·6 vol.); [from 0·5M-KH_2PO_4 (4 vol.) and 0·5M-K_2HPO_4 (2·8 vol.)]. Two completely separated peaks were obtained (*K* 0·22, 34%; *K* 0·94, 46%). The organic layer from tubes 13—42 (*K* 0·22) was extracted with an excess of N-sodium hydroxide, and this extract was combined with the aqueous layer from these tubes. This solution was adjusted to pH 10 with N-sodium hydroxide and washed with ethyl acetate. The aqueous solution was acidified and left overnight; *N-benzoylglycyl-γ-*DL*-glutamyl(glycine hexylamide)* (IVa) was precipitated as prisms (2·03 g.), m. p. 200—201°, unchanged by further recrystallisation from ethanol (Found: C, 58·9; H, 7·0; N, 12·3%; equiv., 440. $C_{22}H_{32}N_4O_6$ requires C, 58·9; H, 7·2; N, 12·5%; equiv., 448). A further quantity of the same peptide (0·28 g.), m. p. 200°, was obtained by extraction of the above filtrate with ethyl acetate.

*N-Benzoylglycyl-α-*DL*-glutamyl(glycine hexylamide)* (IIa), recovered in the same way from tubes 45—95 (*K* 0·94), formed needles (3·14 g.), m. p. 162—163°, unchanged by further recrystallisation from ethanol (Found: C, 59·0; H, 7·4; N, 12·5%; equiv., 437).

Cyclisation of N*-Benzoylglycyl-α-*DL*-glutamyl(glycine Hexylamide)* (IIa).—(a) *By thionyl chloride.* This peptide (1·86 g.) was treated with purified thionyl chloride (50 ml.) at 0° for 125 hr. Evaporation of the thionyl chloride at 0° left a gum which was partitioned between 5% aqueous sodium hydrogen carbonate (150 ml.) and ethyl acetate (400 ml., and, after separation, with 250 ml.). The organic extract was washed with water, dried, and evaporated to leave a brown crystalline residue (1·60 g., 89%) which was recrystallised from ethyl acetate to give *N-benzoylglycyl-5-oxo-*DL*-pyrrolidine-2-carbonyl(glycine hexylamide)* (IIIa) (0·58 g.) as needles, m. p. 193—194° raised by recrystallisation from the same solvent to 195—196° (Found: C, 61·4; H, 6·9; N, 13·6. $C_{22}H_{30}N_4O_5$ requires C, 61·4; H, 7·0; N, 13·0%).

Evaporation of the ethyl acetate mother-liquors above left the imide, DL-α-N*-benzoylglycyl-aminoglutaryl(glycine hexylamide)* (Va), as nodules (0·90 g.), m. p. 150—153°, changed by recrystallisation from ethyl acetate to 150° (Found, in material dried at 130°: C, 61·2; H, 6·8; N, 13·3. $C_{22}H_{30}N_4O_5$ requires C, 61·4; H, 7·0; N, 13·0%).

(b) *By the mixed carbonic anhydride method.* A stirred solution of the peptide (IIa) (0·15 g.) and triethylamine (0·047 ml., 1 equiv.) in dioxan (15 ml.) and dimethylformamide (5 ml.) was treated at 0° with ethyl chloroformate (0·032 ml., 1 equiv.). After 20 min. at 0°, the solution was stirred for 2 hr. as the cooling-bath warmed to room temperature, and was then kept overnight. The organic solvents were evaporated and the residue was partitioned between 0·1N-hydrochloric acid (30 ml.) and ethyl acetate (2 × 50 ml.). The combined ethyl acetate solutions were extracted with an excess of 5% aqueous sodium hydrogen carbonate (40 ml.), washed with water, dried, and evaporated to give a neutral fraction (7·5 mg., 5%). This crystallised from ethyl acetate to give the imide (Va), m. p. 151°; its infrared spectrum was identical with that of the sample prepared as under (a).

Alkaline Hydrolysis of N*-Benzoylglycyl-5-oxo-*DL*-pyrrolidine-2-carbonyl(glycine Hexylamide)* (IIIa).—The acylpyrrolidone (95·2 mg.) in dioxan (10 ml.) and water (3·5 ml.) was titrated with 0·1N-sodium hydroxide (2·25 ml., 1 equiv.); all the alkali was consumed in 5 min. The dioxan was evaporated and the residue partitioned between water (10 ml.) and ethyl acetate

(2 × 50 ml.). The organic extract was washed with an excess of 10% aqueous potassium carbonate solution (40 ml.), with water, and then dried and evaporated to give a crystalline residue (32·2 mg., 54%), m. p. 95—96°. Recrystallisation twice from ethyl acetate gave 5-oxo-DL-pyrrolidine-2-carbonyl(glycine hexylamide), m. p. 100° (Found in material dried at 110°: C, 57·5; H, 8·4; N, 15·3. Calc. for $C_{13}H_{23}N_3O_3$: C, 57·9; H, 8·6; N, 15·6%). This material gave an infrared spectrum identical with that of the slightly impure material obtained by Battersby and Robinson.[3]

When the acylpyrrolidone was similarly hydrolysed in 1 : 15 aqueous tetrahydrofuran or in dimethylformamide, 53% and 54% respectively of neutral material was obtained.

Alkaline Hydrolysis of DL-α-N-*Benzoylglycylaminoglutaryl(glycine Hexylamide)* (Va).—A solution of the imide (Va) (0·22 g.) in dioxan (12 ml.) and water (6 ml.) was titrated with 0·1N-sodium hydroxide (5·14 ml., 1 equiv.) during 5 min. The products were isolated as above to give neutral material (7·1 mg.) and an acidic fraction (0·223 g., 96%). The latter was fractionated by countercurrent distribution (75 transfers) in the system used in the first experiment described above; two separate peaks (*K* 0·30, 79%; *K* 1·2, 21%) were obtained. The solute from the former (tubes 9—30) was recovered as before and crystallised from ethanol, to give the γ-glutamyl peptide (IVa), m. p. and mixed m. p. 200—201°. The second peak (tubes 31—51) similarly yielded the slightly impure α-glutamyl peptide (IIa), m. p. 145°, mixed m. p. raised to 151—152°.

DL-*Phenylalanine Amide.*—A stirred solution of phthaloyl-DL-phenylalanine [7] (25 g.) and triethylamine (11·83 ml.) in dioxan (200 ml.) and chloroform (100 ml.) was treated with ethyl chloroformate (8·1 ml., 1 equiv.) at 0°. After 20 min., ammonia (30 ml.; *d* 0·880) was added, and the stirring was continued for 4 hr. at 0°. The mixture was then allowed to warm to room temperature overnight. The precipitated solid (21·48 g.), m. p. 230°, was recrystallised from ethyl acetate to give *phthaloyl-DL-phenylalanine amide* as needles, m. p. 231° (Found: C, 69·8; H, 4·8; N, 9·6. $C_{17}H_{14}N_2O_3$ requires C, 69·4; H, 4·8; N, 9·5%). The aqueous mother-liquor (above) was evaporated and the residue partitioned between water (100 ml.) and ethyl acetate (400 ml.). The organic extract was washed with 0·5N-hydrochloric acid (100 ml.), water (100 ml.), 5% aqueous sodium hydrogen carbonate (100 ml.) and water (100 ml.) and then dried and evaporated to give more phthaloyl-DL-phenylalanine amide (1·78 g.), m. p. 230°.

A solution of this product (11·87 g.) in ethanol (280 ml.) was heated under reflux with hydrazine hydrate (2·08 ml., 1 equiv.) for 1 hr., then cooled, filtered, and evaporated to dryness. The resultant solids were combined and treated with water (40 ml.) and N-hydrochloric acid (60 ml., 1·5 equiv.) at 50° for 5 min. After being filtered, the solution was evaporated until crystallisation started; then an excess of saturated aqueous potassium carbonate solution was added; the precipitated crystals of DL-phenylalanine amide were collected (3·83 g., 58%), m. p. 136—137·5° raised by recrystallisation from chloroform to 137—138° [Koenigs and Mylo [6] record m. p. 138—140° (corr.)].

N-*Benzoylglycyl-α- and -γ-*DL-*glutamyl(phenylalanine Amides)* (IIb) *and* (IVb).—Ethyl chloroformate (2·74 ml., 1 equiv.) was added to a stirred solution of *N*-benzoylglycyl-DL-glutamic acid [3] (8·76 g.) and triethylamine (3·94 ml., 1 equiv.) in chloroform (250 ml.) and dimethylformamide (250 ml.) at 0°. After 20 min., a solution of DL-phenylalanine amide (4·61 g., 1 equiv.) and triethylamine (3·94 ml.) in chloroform (190 ml.), dimethylformamide (25 ml.), and water (30 ml.) was added and stirring continued for 3 hr. at 0°. The solvents were evaporated and the residue was partitioned between water (170 ml.) and ethyl acetate (230 ml.), the aqueous layer being adjusted to pH 2. Crystals slowly separated which were collected (3·51 g.), m. p. 205—208°. The aqueous layer was extracted with more ethyl acetate (2 × 150 ml.), and the combined organic solutions were then shaken with an excess of 0·5N-sodium hydroxide (3 × 200 ml.). Acidification of the alkaline solution gave crystals (2·61 g.), m. p. 170—180°, and the mother-liquor from them was extracted with ethyl acetate (3 × 500 ml.), to give an amorphous acidic fraction (5·17 g.).

A portion of the combined crystalline material (4·75 g.) was fractionated by countercurrent distribution (scattered in the first ten tubes) between ethyl acetate (4 vol.), butan-1-ol (1 vol.), and aqueous phosphate buffer (5·33 vol.) [from 0·5M-K_2HPO_4 (1·33 vol.) and 0·5M-KH_2PO_4 (4 vol.)]. After 18 transfers, solid *A* (0·96 g.) separated in several tubes; this was collected and the solutions were returned to the correct tubes in the machine before the fractionation was continued. After 90 transfers, two completely separated peaks were obtained (*K* 0·20, 16%; *K* 1·20, 62%) together with a coloured forerun which was rejected.

The solute from the former peak (tubes 4—26) was recovered as above and crystallised from ethanol, to give the *γ-phenylalanine peptide* (IVb) (0·22 g.), m. p. 177—178°, unchanged by further recrystallisation (Found: C, 60·6; H, 6·2; N, 12·45. $C_{23}H_{26}N_4O_6$ requires C, 60·8; H, 5·8; N, 12·3%).

The solute from the major peak (tubes 29—64) crystallised from ethanol, to give the *α-phenylalanine peptide* (IIb) (1·94 g.), m. p. 219°, raised by recrystallisation from ethanol to 224—225·5° (Found: C, 60·5; H, 5·7; N, 12·5%).

Solid *A* was shown to be the α-phenylalanine peptide (IIb) by m. p. and infrared spectrum.

N-*Benzoylglycyl*-5-*oxo*-DL-*pyrrolidine*-2-*carbonyl*-(DL-*phenylalanine Amide*) (IIIb).—A solution of the phenylalanine peptide (IIb) (1·56 g.) in thionyl chloride (16 ml.) was kept for 72 hr. at 0° and then evaporated at 0°. The residual gum was partitioned between 5% aqueous sodium hydrogen carbonate (300 ml.) and ethyl acetate (1 l. and after separation, with 300 ml.); evaporation of the combined ethyl acetate extracts gave a neutral gum (0·94 g., 63%). This crystallised from ethyl acetate to give the *oxopyrrolidine* (IIIb) as prisms (0·83 g.), m. p. 241—242° (Found: C, 63·3; H, 5·5; N, 13·35. $C_{23}H_{24}N_4O_5$ requires C, 63·3; H, 5·5; N, 12·8%).

Evaporation of the mother-liquors gave a solid (80 mg.) which after repeated crystallisation from ethyl acetate had m. p. 190—191°.

Hydrolysis of N-*Benzoylglycyl*-5-*oxo*-DL-*pyrrolidine*-2-*carbonyl*-(DL-*phenylalanine Amide*) (IIIb).—This material (0·44 g.) in dioxan (22 ml.) was titrated with 0·1N-sodium hydroxide (10·31 ml., 1·03 equiv.); all the alkali was consumed in 10 min. The dioxan was evaporated and the residue was fractionated as for the glycine analogue above. The neutral fraction (120 mg., 43%) crystallised from ethanol to give 5-*oxo*-DL-*pyrrolidine*-2-*carbonyl*-(DL-*phenylalanine amide*), m. p. 238° (Found: C, 61·9; H, 6·3. $C_{14}H_{17}N_3O_3$ requires C, 61·1; H, 6·2%).

2-(N-*Benzoyl*-5-*thiohydantoinyl*)*propionyl*(*glycine Hexylamide*) (VII; R = Ph·CO).—A solution of *N*-benzoyl-γ-DL-glutamyl(glycine hexylamide) (IV; R = Ph, R′ = CH_2·CO·NH·[CH_2]$_5$·Me) (275 mg.) in acetonitrile (4 ml.) and triethylamine (0·11 ml., 1 equiv.) was shaken with diphenyl phosphorisothiocyanatidate[12] (0·23 g., 1 equiv.) for 48 hr. at room temperature. The precipitate (198 mg., 65%) was collected and recrystallised from 30% aqueous ethanol, to give the *thiohydantoin* (VII; R = Ph·CO), m. p. 197° (Found: C, 58·4; H, 6·8; N, 12·0; S, 7·0. $C_{21}H_{28}N_4O_4S$ requires C, 58·3; H, 6·5; N, 12·9; S, 7·4%).

Alkaline Hydrolysis of the Thiohydantoin (VII; R = Ph·CO).—This material (176 mg.) was shaken with 0·02N-sodium hydroxide (40·8 ml., 2 equiv.), and the resultant solution was kept at 20° for 30 min. Addition of dilute hydrochloric acid to pH 6 precipitated a solid (102 mg.) which was recrystallised from ethanol, to give 2-(5-*thiohydantoinyl*)*propionyl*(*glycine hexylamide*) (VII; R = H), m. p. 177° (Found: C, 50·8; H, 7·3. $C_{14}H_{24}N_4O_3S$ requires C, 51·2; H, 7·4%).

After the aqueous solution has been extracted with ethyl acetate which removed more thiohydantoin (VII; R = H) (46 mg.), it was acidified with dilute hydrochloric acid and extracted with light petroleum (b. p. 40—60°; 1·5 l.). This extract was washed with water, dried, and evaporated to give benzoic acid (38·5 mg., 77%), m. p. 116—118°, mixed m. p. 118—120°.

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