76. *Peptides. Part V.* Condensation of the γ-Carboxyl Group of α-Glutamyl Peptides with the Peptide Chain.*

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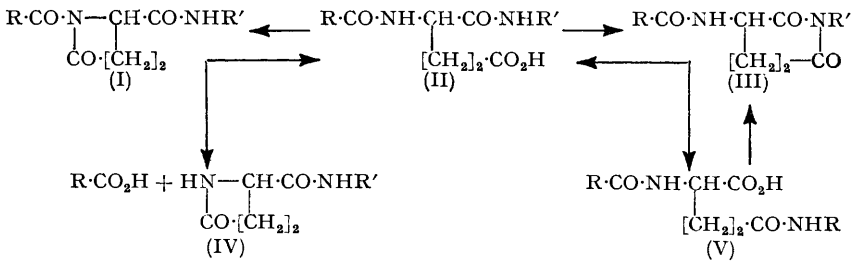
Condensation of α-glutamyl peptide derivatives (II) through the action of thionyl chloride and a tertiary amine may lead to either an oxopyrrolidine (I) or a dioxopiperidine (III), depending on the structure of the peptide. In the two cases studied, namely, (I*c*) and (VI), alkaline hydrolysis of the oxopyrrolidine largely regenerated the α-glutamyl compound. Alkaline hydrolysis of the dioxopiperidines, (III*a*) and (III*b*), regenerated a little of the starting material (II) but the main product was the γ-glutamyl isomer (V).

Methods for selective degradation of polypeptides into two or more smaller peptides would be valuable.[1,2] The aim of the present work was to cleave the acyl–glutamyl link in α-glutamyl peptides (II) by formation of a 1-acyl-2-oxopyrrolidine (I), followed by alkaline hydrolysis. It was expected that the hydrolysis would give the oxopyrrolidine (IV), since mixed anhydrides of α-acylamino-acids and simple carboxylic acids are attacked by amines at the more cationoid carbonyl group derived from the acylamino-acid.[3] Our results show, however, that the six-membered ring imide (III) may be produced in preference to the five-membered ring imide (I),[4] and that, even when the latter is produced, its hydrolysis may give predominantly the original peptide (II); therefore the method is unlikely to be useful. On the other hand the hydrolysis of the six-membered ring imides (III) follows the expected course and gives mainly the γ-glutamyl isomer (V) of the original α-glutamyl peptide (II) (cf. discussion by Battersby and Robinson [3]).

* Part IV, *J.*, 1953, 673.

[1] Elliott, *Biochem. J.*, 1952, **50**, 542; Sanger, *Adv. Protein Chem.*, 1952, **7**, 11.
[2] Battersby and Robinson, *J.*, 1955, 259.
[3] Wieland, Kern, and Sehring, *Annalen*, 1950, **569**, 117; Vaughan and Osato, *J. Amer. Chem. Soc.*, 1951, **73**, 5553; Wieland and Stimming, *Annalen*, 1953, **579**, 97.
[4] Clayton and Kenner, *Chem. and Ind.*, 1953, 1205.

As a substance of type (II) toluene-*p*-sulphonylglycyl-L-phenylalanyl-α-L-glutamylglycine *cyclo*hexylamide (II*a*) was selected; the preparation of this and other starting materials is discussed below. Treating the acid (II*a*) in dimethylformamide with **1** mol.



Series (*a*) : R = $C_7H_7{\cdot}SO_2{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH(CH_2Ph)$; R′ = $CH_2{\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$.
Series (*b*) : R = Ph, R′ = $CH_2{\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$.
Series (*c*) : R = $C_7H_7{\cdot}SO_2{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2$; R′ = $CH(CH_2Ph){\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$.

each of pyridine and thionyl chloride (to give the acid chloride [5]) and then with a second portion of pyridine gave a crystalline condensation product in **74%** yield, and 22% of (II*a*) was recovered. Hydrolysis of the neutral substance with dilute alkali furnished 9% of the original acid and 90% of the isomeric γ-glutamyl derivative (V*a*), identical with a sample produced by unambiguous synthesis. The structural relation of the two acids was confirmed by electrometric titration; compounds of type (V) are considerably stronger acids than those of type (II) owing to the proximity of the carboxyl and an amide group.[6]

The conversion of the α- (II*a*) into the γ-glutamyl derivative (V*a*) requires the dioxopiperidine structure (III*a*) for the neutral intermediate, so preparation of such imides was investigated by using the more accessible benzoyl-α-DL-glutamylglycine *cyclo*hexylamide (II*b*). The crystalline dioxopiperidine (III*b*) was obtained in good yield in the manner already described or by substituting ethyl chloroformate [7] for thionyl chloride. Simple dissolution of the acid (II*b*) in excess of acetic anhydride yielded **76%** (after these experiments had been completed the preparation of dioxopiperidines by means of hot acetic anhydride was reported [8]). In contrast with these successful preparations through mixed anhydrides with carboxylic acids, reaction between triethylamine and the mixed anhydride with lithium hydrogen sulphate [9] furnished only traces of the dioxopiperidine, and these may have arisen through disproportionation of the mixed into the symmetrical anhydride.

Alkaline hydrolysis of the dioxopiperidine (III*b*) gave a mixture of the α-glutamyl compound (II*b*) (**13%**) and the γ-isomer (V*b*) (85%), which was easily resolved by countercurrent distribution; the latter acid, being the stronger, was preferentially retained by a phosphate buffer. Battersby and Robinson [2] found that the action of alkali on the ethyl ester of an acid differing from (II*b*) merely in the substitution of an *n*-hexyl for the *cyclo*hexyl residue gave a **4 : 5** mixture of the acids corresponding to (II*b*) and (V*b*). It now appears likely that the route of this hydrolysis was only partly through the dioxopiperidine, as was envisaged by Battersby and Robinson as one possible explanation of their results.

Direct α ⟶ γ-isomerisation of (II*b*) into (V*b*) by a combined process of cyclisation and ring-opening was also attempted : tetraethyl pyrophosphate, a water-soluble anhydride which was moderately efficient in anhydrous dioxan, was used in aqueous alkaline solution, but at best only **32%** of the γ-acid was obtained. One cause of the low yield was doubtless the reversibility of the process, for the dioxopiperidine (III*b*) was formed in good yield from (V*b*) by the thionyl chloride method.

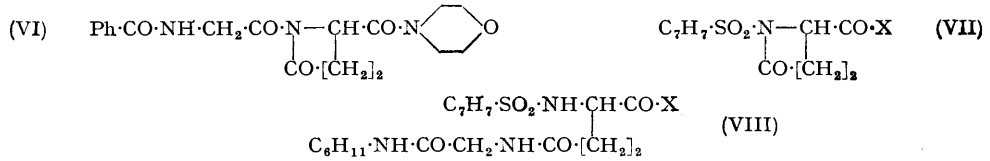
A third series of experiments was undertaken to test the possibility that the benzyl group of the phenylalanyl residue in (II*a*) had sterically hindered the formation of the

[5] Human and Mills, *Nature*, 1948, **158**, 877.
[6] Cohn and Edsall, " Proteins, Amino Acids and Peptides," Reinhold, New York, 1943, p. 134.
[7] Boissonas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547.
[8] Kovács, Medzihradszky, and Bruckner, *Naturwiss.*, 1954, **41**, 450.
[9] Kenner and Stedman, *J.*, 1952, 2069.

oxopyrrolidine (I*a*) [in (II*b*) the benzamido-group was not a suitable model for the peptide chain]. Cyclisation of toluene-*p*-sulphonylglycylglycyl-α-L-glutamyl-L-phenylalanine *cyclo*hexylamide (II*c*) by the thionyl chloride process gave an excellent yield of a non-crystalline neutral material, but alkaline hydrolysis of this regenerated the α-compound (II*c*) in 90% yield with no trace of the expected γ-isomer (V*c*). It was difficult to account for this result by invoking selective steric hindrance by the benzyl group of the hydrolysis of the dioxopiperidine (III*c*). It seemed more likely that the neutral intermediate was the oxopyrrolidine (I*c*) and that our initial assumption about the behaviour of such imides was incorrect. Indeed the potential lability of the oxopyrrolidine ring had been demonstrated in the ammonolysis of 1-acetyl-2-oxopyrrolidine-5-carboxylic acid to acetylglutamine.[10] A closer analogy which also supported our view was provided by the behaviour of a non-crystalline imide prepared from benzoylglycyl-α-L-glutamylmorpholine, which must have been 1-benzoylglycyl-5-morpholinocarbonyl-2-oxo-L-pyrrolidine (VI) in absence of the hydrogen necessary for dioxopiperidine formation. Alkaline hydrolysis of this oxopyrrolidine gave mainly the acid from which it had been prepared, together with some benzoylglycine. It would have been interesting to examine the cyclisation, which would not be sterically hindered in either direction, of toluene-*p*-sulphonyglycylglycyl-α-L-glutamylglycine *cyclo*hexylamide but work with this series of compounds was abandoned because of their excessive water-solubility, a property which they share with the morpholides mentioned above. However, we believe that our results establish the general courses, which will be controlled by structural influences in any instance, of the condensation and subsequent hydrolysis of α-glutamyl peptide derivatives.

Preparation of compounds of series (*b*) started from benzoyl-DL-glutamic anhydride, which was obtained from benzoyl-L-glutamic acid and acetic anhydride. Racemisation occurs likewise during anhydride formation from *p*-nitrobenzoyl- and phenylacetyl-L-glutamic acid,[11] but is absent in the dehydration of benzyloxycarbonyl- and phthaloyl-L-glutamic acid.[12] The racemic anhydrides must have been produced from the isomeric oxazolones, but, as has been shown in the other two cases,[13] the oxazolone structure cannot be assigned to the material obtained from benzoyl-L-glutamic acid. It shows negligible ultraviolet absorption at 250 mμ, and has an infrared doublet at 1770, 1818 cm.$^{-1}$ while lacking a band in the carbonyl region (1700—1750 cm.$^{-1}$). Moreover reaction between the anhydride and glycine *cyclo*hexylamide in chloroform solution gave a 6 : 7 mixture of benzoyl-α-DL-glutamylglycine *cyclo*hexylamide and the γ-isomer.

The other starting materials with the α-glutamyl structure were prepared from γ-benzyl hydrogen L-glutamate, preferably by acylating a suspension of the ester in aqueous dimethylformamide with the mixed sulphuric anhydride [9] of the appropriate acylamino-acid; despite the sparing solubility of γ-benzyl hydrogen L-glutamate the yields were about 50%. The resultant carboxylic acids were condensed with an amine by the sulphuric anhydride method, and finally the benzyl protecting group was removed by hydrogenolysis. The γ-glutamyl derivative (V*a*) was prepared by sulphuric anhydride acylation of γ-L-glutamylglycine *cyclo*hexylamide, which was obtained from 1-toluene-*p*-sulphonyl-2-oxo-



L-pyrrolidine-5-carboxylic acid (VII; X = OH) by interaction with glycine *cyclo*hexylamide followed by sodium–liquid ammonia treatment of the resultant amide (VIII; X = OH). We are greatly indebted to Dr. J. M. Swan, who informed us of this general

[10] J. A. King and McMillan, *J. Amer. Chem. Soc.*, 1952, **74**, 2859.
[11] F. E. King and Spensley, *J.*, 1950, 3159; Baker and Jones, *J.*, 1951, 1143.
[12] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192; F. E. King and Kidd, *J.*, 1949, 3315.
[13] Swan, *Australian. J. Sci. Res.*, 1952, **5**, *A*, 721; J. A. King, McMillan, and Genzer, *J. Amer. Chem. Soc.*, 1952, **74**, 5202.

method before its publication.[14,15] Application of the method to the synthesis of α-L-glutamylglycine *cyclo*hexylamide was less successful. Reaction between a chloroform solution of 1-toluene-*p*-sulphonyl-2-oxo-L-pyrrolidine-5-carbonyl chloride (VII; X = Cl) and a solution of glycine *cyclo*hexylamide in aqueous sodium hydrogen carbonate furnished a neutral oil in good yield, but alkaline hydrolysis of this gave, in addition to the expected toluene-*p*-sulphonyl-α-L-glutamylglycine *cyclo*hexylamide, small amounts of the γ-isomer (VIII; X = OH) and of toluene-*p*-sulphonyl-αγ-L-glutamyldi(glycine *cyclo*hexylamide) (VIII; X = $NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$). Formation of the last product signifies attack by the amine at the oxopyrrolidinecarbonyl group. If some of this attack occurred *before* the attack at the carbonyl of the acid chloride group, the resultant acid chloride (VIII; X = Cl) would cyclise easily to a dioxopiperidine, which would contaminate the desired product (VII; X = $NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$) in the neutral fraction and give rise to some (VIII; X = OH) on hydrolysis. Alternative explanations based on the assumption of side-reactions during the alkaline hydrolysis of (VII; X = $NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$) can be produced. Rudinger [15] likewise obtained a mixture from the reaction of the acid chloride (VII; X = Cl) with glycine in sodium hydrogen carbonate solution, but Swan and du Vigneaud [14] were able to couple the acid chloride satisfactorily with asparagine in presence of an aqueous suspension of magnesium oxide.

Experimental

M. p.s are corrected. pK_{MCS} is defined on p. 380.

Analytical samples were dried for 6 hr. at 80°/1 mm. over phosphoric oxide, unless otherwise stated. Evaporations were carried out under reduced pressure. Solutions in dimethylformamide of the lithium, potassium, and trimethylphenylammonium salts of acyl sulphates were prepared according to the general procedure of Kenner and Stedman.[9] When these solutions were used to acylate an amino-compound in aqueous solution, they were dropped slowly in an atmosphere of dry nitrogen into the aqueous solution of the amino-compound, which also contained an indicator; during this process N-sodium hydroxide was added simultaneously so as to maintain the colour of the indicator at that corresponding to the specified pH. *m*-Cresol-purple, thymol-blue, and phenolphthalein were used as indicators of pH 8·0, 8·7, and 9·0 respectively.

γ-Benzyl Hydrogen L-*Glutamate* (cf. ref. 16).—A solution of L-glutamic acid (44·2 g.) and toluene-*p*-sulphonic acid (54·7 g.) in benzyl alcohol (217 c.c.) was distilled at 120° (bath-temp.)/2 mm. during 1½ hr. The concentrated residue crystallised when triturated with ether in a mortar. The solid was dried, and dissolved in benzyl alcohol (217 c.c.) together with toluene-*p*-sulphonic acid (2 g.). The processes of distillation and crystallisation were then repeated. Addition of diethylamine (34 c.c.) to a solution of the resultant salt in ethanol (600 c.c.) precipitated γ-benzyl hydrogen L-glutamate, which recrystallised from water (1 l.) in plates (30·7 g., 43%), m. p. 176—176·5°, $[\alpha]_D^{18}$ +19·3° (*c*, 1·14 in acetic acid), pK_1 2·7, pK_2 9·1 (in water) (Found: C, 61·0; H, 6·6; N, 6·3. Calc. for $C_{12}H_{15}O_4N$: C, 60·8; H, 6·4; N, 5·9%).

*Benzoyl-*DL-*glutamic Anhydride.*—Benzoyl-L-glutamic acid (1 g.) and acetic anhydride (8 c.c.) were shaken together until dissolution had taken place (½—1 hr.) and for 2 hr. further. Benzoyl-DL-glutamic anhydride (0·65 g.) was precipitated by addition of ether (15 c.c.) and light petroleum (25 c.c.; b. p. 40—60°); in some preparations the anhydride crystallised directly from the reaction mixture. Battersby and Robinson [2] record m. p. 154—155° (uncorr.). After recrystallisation from acetic anhydride, our material had m. p. 153°, $[\alpha]_D^{16}$ 0° (*c*, 2·14 in dioxan) (Found: C, 62·0; H, 4·8; N, 5·8. Calc. for $C_{12}H_{11}O_4N$: C, 61·8; H, 4·8; N, 6·0%), and had $\varepsilon_{max.}$ 9470 at 226 mμ in dioxan. Benzoyl-L-glutamic acid had ε_{max} 9680 at 225 mμ in dioxan.

Benzyloxycarbonylglycine cyclo*Hexylamide.*—*cyclo*Hexylamine (45 c.c.) was added to a solution of trimethylphenylammonium benzyloxycarbonylglycyl sulphate (30 mmoles) in dimethylformamide (75 c.c.) and the mixture was kept for 1 hr. at 20°. The ethyl acetate solution, obtained by evaporation of the solvent and partition of the residue between ethyl acetate and 3N-hydrochloric acid, was washed with water and saturated sodium hydrogen

[14] Swan and du Vigneaud, *ibid.*, 1954, **76**, 3110.
[15] Rudinger, *Coll. Czech. Chem. Comm.*, 1954, **19**, 375.
[16] Miller and Waelsch, *J. Amer. Chem. Soc.*, 1952, **74**, 1092.

carbonate solution before being dried and evaporated. The cyclo*hexylamide* (7·8 g., 90%) crystallised from ethyl acetate–light petroleum (b. p. 40—60°) in needles, m. p. 110° (Found: C, 66·1; H, 7·9; N, 9·8. $C_{16}H_{22}O_3N_2$ requires C, 66·2; H, 7·6; N, 9·7%).

Glycine cyclo*Hexylamide Hydrobromide.*—A solution of benzyloxycarbonylglycine *cyclo*hexylamide (2·22 g.) in N-hydrogen bromide in acetic acid (23·1 c.c.) was heated on a steam-bath until evolution of carbon dioxide ceased (10 min.). Addition of ether (200 c.c.) to the cold solution caused crystallisation of the *hydrobromide* (1·76 g., 96%), m. p. 186·5° unaltered by recrystallisation from ethanol–ether (Found: C, 40·8; H, 7·5; N, 11·5. $C_8H_{17}ON_2Br$ requires C, 40·5; H, 7·2; N, 11·8%).

Benzoyl-DL-(α *and* γ)-*glutamylglycine* cyclo*Hexylamides* (II*b*) *and* (V*b*).—Benzoyl-DL-glutamic anhydride (3·49 g., 15 mmoles) was added in several portions during 30 min. to a swirled and cooled solution of glycine *cyclo*hexylamide hydrobromide (3·55 g., 15 mmoles) and triethylamine (2·1 c.c., 15 mmoles) in chloroform (40 c.c.). Next day 3N-hydrochloric acid (20 c.c.) and more chloroform (40 c.c.) were added. The layers were separated and the aqueous layer was re-extracted with chloroform (20 c.c.). The material in the chloroform extracts was distributed (counter-current) between ethyl acetate and a phosphate buffer (0·8M-KH_2PO_4; 0·2M-K_2HPO_4) (10 transfers; 100 c.c. phases). Tubes 3—8 contained *benzoyl*-DL-α-*glutamylglycine* cyclo*hexylamide* (*K* 1·5; 2·60 g., 45%), which was recrystallised from aqueous ethanol and had m. p. 193°, pK_{MCS} 6·1 (Found: C, 61·8; H, 6·7; N, 10·9. $C_{20}H_{27}O_5N_3$ requires C, 61·7; H, 7·0; N, 10·8%). Tubes 0—3 contained *benzoyl*-DL-γ-*glutamylglycine* cyclo*hexylamide* (*K* 0·12; 3·06 g., 53%), which was recrystallised several times from aqueous ethanol and had m. p. 175·5°, pK_{MCS} 5·4 (Found: C, 61·9; H, 6·6; N, 11·1%).

Benzoyl-DL-*glutamoylglycine* cyclo*Hexylamide* (III*b*).—A solution of benzoyl-DL-α-glutamylglycine *cyclo*hexylamide (0·389 g., 1 mmole) in dioxan (100 c.c.) was concentrated to 40 c.c., a 20 cm. Fenske fractionating column being used. Thionyl chloride (0·08 c.c., 1 mmole), pyridine (0·09 c.c., 1 mmole), and after 40 min. further pyridine (0·09 c.c., 1 mmole), were added to the cooled solution. At the second stage pyridine hydrochloride was precipitated. The mixture was kept for 1 hr. before addition of water (2 c.c.) and evaporation of the solvents. The residue was dissolved in a mixture of butan-1-ol (50 c.c.) and ethyl acetate (50 c.c.), which was extracted with saturated sodium hydrogen carbonate solution (2 × 25 c.c.) and water (25 c.c.). Evaporation of the dried (Na_2SO_4) extract gave the crystalline *dioxopiperidine* (0·369 g., 99%), which, recrystallised from aqueous ethanol, had m. p. 261·5—262·5° (decomp.) (Found: C, 64·9; H, 6·9; N, 11·1. $C_{20}H_{25}O_4N_3$ requires C, 64·7; H, 6·8; N, 11·3%).

The dioxopiperidine was also obtained when the thionyl chloride was replaced by ethyl chloroformate (95% yield) or by tetraethyl pyrophosphate (1 equiv. giving 67%, 2 equivs. giving 89%). In the last case recovery was complicated by the presence of excess of neutral pyrophosphate. Solution of the α-glutamyl peptide in excess of cold acetic anhydride also yielded the dioxopiperidine (76%). Treatment of the lithium salt of the α-glutamyl peptide with 1 equiv. of the sulphur trioxide–dimethylformamide complex followed by triethylamine (1 equiv.) gave only 6% of the dioxopiperidine, 83% of the starting material being recovered.

Treatment of benzoyl-DL-γ-glutamylglycine *cyclo*hexylamide with thionyl chloride and pyridine also gave the dioxopiperidine (93%).

Hydrolysis of Benzoyl-DL-*glutamoylglycine* cyclo*Hexylamide* (III*b*).—A solution of the dioxopiperidine (0·346 g.) in dioxan (25 c.c.) and 4N-sodium hydroxide (5 c.c.) was kept for 15 min. at 20° and then acidified and evaporated. The residue was distributed between ethyl acetate (25 c.c.) and 3N-hydrochloric acid (25 c.c.), which was then extracted with ethyl acetate (6 × 50 c.c.). The mixture of the benzoyl-DL-(α and γ)-glutamylglycine *cyclo*hexylamides obtained by evaporating the dried ethyl acetate extracts was distributed (counter-current) in the same way as during their preparation. This gave the α-isomer (0·048 g., 13%) and the γ-isomer (0·308 g., 85%) with the correct m. p.s. Their identity was also established by chromatography in ethyl acetate on paper which had been sprayed with phosphate buffer (0·4M-KH_2PO_4; 0·6M-K_2HPO_4) [ascending R_F 0·50 (α), 0·08 (γ)].

Isomerisation of Benzoyl-DL-α-*glutamylglycine* cyclo*Hexylamide in Aqueous Solution by Means of Tetraethyl Pyrophosphate.*—The α-glutamyl peptide (0·389 g., 1 mmole) was dissolved in dimethylformamide (2·5 c.c.) and tetraethyl pyrophosphate (2·5 c.c., 10 mmoles). To this was added a solution of potassium hydrogen carbonate (1·17 g., 11 mmoles) in water (5 c.c.), and the mixture was left for 14 days with intermittent shaking. It was then made alkaline (phenolphthalein) with sodium hydroxide solution and, after 1 hr. further, worked up as in the foregoing experiment. The counter-current distribution separated the recovered starting material (0·235 g., 63%) from its γ-isomer (0·094 g., 25%).

When more pyrophosphate (50 mmoles) was used, only 36% of the starting material was recovered together with 32% of the γ-isomer. Substitution of zinc oxide (0·36 g., 11 mmoles) for the potassium hydrogen carbonate afforded 0·078 g. (20%) of the γ-peptide. When excess of pyridine was used instead, no isomerisation was detected.

Benzyloxycarbonyl-α-L-glutamylglycine cyclo*Hexylamide γ-Benzyl Ester.*—Ethyl chloroformate (0·68 c.c., 7·15 mmoles) was added to a solution of γ-benzyl hydrogen benzyloxycarbonyl-L-glutamate [17] (2·65 g., 7·15 mmoles) and triethylamine (0·99 c.c., 7·15 mmoles) in dioxan (15 c.c.), which was kept at 10°. A precipitate of triethylamine hydrochloride appeared immediately. After 10 min. a mixture of glycine *cyclo*hexylamide hydrobromide (1·7 g., 7·15 mmoles), triethylamine (7·15 mmoles), and dioxan (15 c.c.) was added. Next day the solvents were evaporated and the residue was treated with ethyl acetate (40 c.c.) and 3N-hydrochloric acid (10 c.c.). The majority of the product crystallised and was collected after having been washed in suspension with hydrochloric acid, water, and sodium hydrogen carbonate solution. The remainder was recovered from the ethyl acetate. The *dipeptide derivative* (3·2 g., 88%) was recrystallised from ethanol and had m. p. 173·5—174·5°, $[\alpha]_D^{15\cdot5}$ +2·5° (*c*, 1·7 in dimethylformamide) (Found : C, 65·8; H, 6·5; N, 8·4. $C_{28}H_{35}O_6N_3$ requires C, 66·0; H, 6·9; N, 8·3%).

α-L-*Glutamylglycine* cyclo*Hexylamide.*—Hydrogen was bubbled for 4 hr. through a stirred suspension of palladium black in dioxan (15 c.c.) and 95% ethanol (20 c.c.) containing the foregoing dipeptide derivative (3·38 g., 6 mmoles). The catalyst was removed and washed with warm water. Evaporation of the filtrate and washings followed by addition of ethanol furnished α-L-*glutamylglycine* cyclo*hexylamide* (1·3 g., 62%), m. p. 158° (decomp.), $[\alpha]_D^{16\cdot5}$ +50·4° (*c*, 1·15 in N-HCl) (Found : C, 54·7; H, 8·1; N, 14·5. $C_{13}H_{23}O_4N_3$ requires C, 54·7; H, 8·1; N, 14·7%).

Toluene-p-*sulphonylglycyl-L-phenylalanyl-α-L-glutamylglycine* cyclo*Hexylamide* (II*a*).—α-L-glutamylglycine *cyclo*hexylamide (5 mmoles) was acylated at pH 8·7 by potassium toluene-*p*-sulphonylglycyl-L-phenylalanyl sulphate (5 mmoles), prepared from toluene-*p*-sulphonylglycyl-L-phenylalanine [18] (m. p. 160·5°). The reaction mixture was brought to pH 6 and evaporated at 50°/2 mm. Almost all the product crystallised directly when ethyl acetate (40 c.c.) and 3N-hydrochloric acid (5 c.c.) were added to the residue, and the remainder was recovered by ethyl acetate extraction (4 × 60 c.c.) of the aqueous layer, evaporation of the combined ethyl acetate solutions, and trituration with light petroleum. The *tetrapeptide derivative* (2·26 g., 71%), recrystallised from ethanol, had m. p. 219·5—220·5° (decomp.), $[\alpha]_D^{16}$ −9·1° (*c*, 1·25 in EtOH), pK_{MCS} 6·2 (Found : C, 57·6; H, 6·1; N, 10·8. $C_{31}H_{41}O_8N_5S$ requires C, 57·8; H, 6·4; N, 10·9%). Repetition of the acylation process with the aqueous liquor of the above extraction and a fresh portion (1·5 mmoles) of the sulphate anhydride afforded a further 0·36 g. (11%) of the tetrapeptide derivative.

Toluene-p-*sulphonylglycyl-L-phenylalanyl-L-glutamoylglycine* cyclo*Hexylamide* (III*a*).—A solution of toluene-*p*-sulphonylglycyl-L-phenylalanyl-α-L-glutamylglycine *cyclo*hexylamide (II*a*) (0·322 g., 0·5 mmole) in dimethylformamide (40 c.c.) was concentrated to 7 c.c., a 20 cm. Fenske fractionating column at 50°/14 mm. being used. Pyridine (0·05 c.c., 0·62 mmole), thionyl chloride (0·044 c.c., 0·62 mmole), and, 1 hr. later, more pyridine (0·05 c.c.) were added to the solution, which was kept at 20° for 2 hr. in all before the addition of water (2 c.c.). Next day the solution was evaporated and the residue was distributed between saturated sodium hydrogen carbonate solution and ethyl acetate, which was extracted with a second portion of hydrogen carbonate solution. The *dioxopiperidine* (0·231 g., 74%) was obtained by evaporation of the ethyl acetate and was recrystallised from ethanol, m. p. 214·5° (Found : C, 59·75; H, 6·55; N, 11·1. $C_{31}H_{39}O_7N_5S$ requires C, 59·5; H, 6·3; N, 11·2%). Some starting material (0·072 g., 22%) was recovered by acidification of the sodium hydrogen carbonate solutions.

Treatment of the potassium salt of the amide (II*a*) with 1 equiv. of the sulphur trioxide–dimethylformamide complex followed by 1 equiv. of triethylamine afforded only 10% of neutral material, m. p. 170—178°, which was shown by recrystallisation to be impure (III*a*); 82% of the original acid was recovered.

A solution of (II*a*) (0·286 g.) in thionyl chloride (0·5 c.c.) was kept at 19° for 5 min. before evaporation at 50°. Benzene was thrice distilled from the yellow residue, which was resolved into an acidic fraction (0·160 g.) and a neutral fraction (0·120 g.). Recrystallisation of the latter from ethanol gave a 30% yield of the product (III*a*).

Toluene-p-*sulphonylglycyl-L-phenylalanyl-γ-L-glutamylglycine* cyclo*Hexylamide* (V*a*) *from*

[17] Hanby, Waley, and Watson, *J.*, 1950, 3245.
[18] Clayton, Kenner, *et al.*, unpublished work.

(III*a*).—A solution of the dioxopiperidine (III*a*) (0·022 g.) in 0·5N-sodium hydroxide (1 c.c.) was kept for 10 min. at 18° and then acidified. The crystalline precipitate was taken up in ethyl acetate, which was washed with water before extraction with sodium hydrogen carbonate solution. The alkaline solution was acidified and extracted with ethyl acetate, which was evaporated to a crystalline residue of the *γ-glutamyl derivative* (0·020 g., 90%), m. p. 173° after recrystallisation from ethyl acetate (Found: C, 57·9; H, 6·4; N, 10·9. $C_{31}H_{41}O_8N_5S$ requires C, 57·8; H, 6·4; N, 10·9%). In an experiment on a larger scale, 9% of the isomeric α-glutamyl derivative separated when ethanol was used for the initial crystallisation. Ascending paper chromatography in butan-1-ol saturated with 2N-ammonia separated the dioxopiperidine (III*a*) (R_F 0·80), the γ-glutamyl derivative (V*a*) (R_F 0·53), and the α-isomer (II*a*) (R_F 0·61).

Toluene-p-*sulphonyl-γ-*L-*glutamylglycine* cyclo*Hexylamide* (VIII; X = OH).—A solution of 1-toluene-*p*-sulphonyl-2-oxo-L-pyrrolidine-5-carboxylic acid (VII; X = OH) [19] (2·80 g., 10 mmoles), glycine *cyclo*hexylamide hydrobromide (2·50 g., 10·5 mmoles), and triethylamine (3·08 c.c., 22 mmoles) in acetonitrile (20 c.c.) was boiled under reflux for 3·5 hr. and then evaporated. The residue was dissolved in N-sodium hydroxide (40 c.c.), which was washed with ethyl acetate (2 × 50 c.c.). Acidification of the ice-cold aqueous layer yielded the crystalline *dipeptide derivative* (3·53 g., 81%), m. p. 199—200° after recrystallisation from aqueous ethanol, $[\alpha]_D^{20}$ −5·8° (*c*, 2 in 0·1N-NaOH), pK_{MCS} 5·2 (Found: C, 54·4; H, 6·6; N, 9·8. $C_{20}H_{29}O_6N_3S$ requires C, 54·7; H, 6·6; N, 9·6%).

*γ-*L-*Glutamylglycine* cyclo*Hexylamide*.—Sodium was added to a solution of the foregoing toluene-*p*-sulphonyl derivative (VIII; X = OH) (2·95 g.) in liquid ammonia (300 c.c.) until the blue colour persisted for 10 min. Dowex-50 ion-exchange resin (18 g.) in the ammonium salt form was added and the mixture was left in an open flask. Next day the last traces of ammonia were removed by vacuum-evaporation and water (25 c.c.) was added to the residue. The resin was removed by filtration and washed with water (35 c.c.). The combined aqueous solutions were clarified by filtration through Hyflo Supercel, were brought to pH 6 by addition of a few drops of acetic acid and were diluted with ethanol (200 c.c.). The small quantity of inorganic material, which separated when the solution was kept overnight at 0°, was removed and the product (1·98 g.), m. p. 171—176° with preliminary softening, was precipitated by addition of ether (1 l.). It could neither be crystallised nor obtained analytically pure, although it gave a single spot of R_F 0·32, detected by ninhydrin, on paper chromatography in butan-1-ol saturated with 3N-ammonia.

Toluene-p-*sulphonylglycyl-*L-*phenylalanyl-γ-*L-*glutamylglycine* cyclo*Hexylamide* (V*a*).—Lithium toluene-*p*-sulphonylglycyl-L-phenylalanyl sulphate (1 mmole) was brought into reaction with γ-L-glutamylglycine *cyclo*hexylamide (1 mmole) at pH 8. The resulting solution was brought to pH 6 by addition of 3N-hydrochloric acid and evaporated. The solution of the residue in 3N-hydrochloric acid (10 c.c.) was extracted with ethyl acetate (6 × 50 c.c.), which was dried and evaporated. Crystallisation of the glassy residue from ethyl acetate furnished the tetrapeptide derivative (0·430 g., 67%), m. p. 169—170°, pK_{MCS} 5·5 (Found: N, 10·6. Calc. for $C_{31}H_{41}O_8N_5S$: N, 10·9%). This material was indistinguishable by infrared spectrography or paper chromatography from that prepared by hydrolysis of the dioxopiperidine (III*a*) (see above).

*Benzyloxycarbonyl-*L-*phenylalanine* cyclo*Hexylamide*.—This *compound* was prepared in 65% yield in a manner similar to that previously described for the glycine analogue. It had m. p. 165°, $[\alpha]_D^{20}$ −1·2° (*c*, 1·6 in dimethylformamide) (Found: C, 72·3; H, 7·5; N, 7·6. $C_{23}H_{28}O_3N_2$ requires C, 72·6; H, 7·4; N, 7·4%).

L-*Phenylalanine* cyclo*Hexylamide*.—A solution of the foregoing benzyloxycarbonyl derivative (4·9 g., 13 mmoles) in acetic acid (32 c.c.) containing hydrogen bromide (40 mmoles) was heated on a steam-bath until the evolution of carbon dioxide ceased (15 min.) and was then evaporated. The acidic solution of the residue in water (150 c.c.) was washed with ethyl acetate (2 × 50 c.c.) and then brought to pH 9 by addition of dilute aqueous sodium hydroxide. The precipitated cyclo*hexylamide* was extracted into ethyl acetate (3 × 100 c.c.) and then crystallised in needles (2·5 g., 82%), m. p. 101—102°, $[\alpha]_D^{22}$ +19·7° (*c*, 3·4 in EtOH) (Found, in material dried at 50°: C, 73·2; H, 9·0; N, 11·6. $C_{15}H_{22}ON_2$ requires C, 73·1; H, 9·0; N, 11·4%).

γ-Benzyl Toluene-p-*sulphonylglycylglycyl-*L-*glutamate*.—A suspension of γ-benzyl hydrogen L-glutamate (2 mmoles) in 50% aqueous dimethylformamide was acylated at pH 8·5 by lithium toluene-*p*-sulphonylglycylglycyl sulphate (2 mmoles). The resultant clear solution was kept for 30 min. before acidification (pH 6) and evaporation. The product was separated from the

[19] Rudinger, *Coll. Czech. Chem. Comm.*, 1954, **19**, 369.

residue by dissolution in 3N-hydrochloric acid and extraction with ethyl acetate (3 × 25 c.c.). Counter-current distribution (20 transfers; 20 c.c. phases) between ethyl acetate and a phosphate buffer (0·8M-KH_2PO_4; 0·2M-K_2HPO_4) afforded the *γ-benzyl ester* (0·512 g., 52%), K 1·8 (tubes 8—18), which crystallised from ethyl acetate–light petroleum (b. p. 40—60°) in platelets, m. p. 101—103°, pK_{MCS} 5·4, $[\alpha]_D^{22}$ +9·4° (*c*, 3·7 in EtOH) (Found: C, 54·4; H, 5·1; N, 8·4. $C_{23}H_{27}O_8N_3S$ requires C, 54·6; H, 5·4; N, 8·3%). Toluene-*p*-sulphonylglycylglycine (0·172 g., 32%), m. p. 175°, K 0·065, was recovered from tubes 0—3.

Toluene-p-*sulphonylglycylglycyl*-α-L-*glutamyl*-L-*phenylalanine* cyclo*Hexylamide* γ-*Benzyl Ester*.—A solution of L-phenylalanine *cyclo*hexylamide (0·246 g., 1 mmole) and triethylamine (0·14 c.c., 1 mmole) in dimethylformamide (5 c.c.) was added to a dimethylformamide solution (10 c.c.) of lithium toluene-*p*-sulphonylglycylglycyl-α-L-glutamyl sulphate γ-benzyl ester (1 mmole). Next day the solution was evaporated and the residue was distributed between ethyl acetate (200 c.c.) and N-hydrochloric acid (20 c.c.). Evaporation of the ethyl acetate, after it had been washed with more N-hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, yielded the *γ-benzyl ester* (0·599 g., 82%), m. p. 195—200° (Found: C, 62·0; H, 6·3; N, 9·8. $C_{38}H_{47}O_8N_5S$ requires C, 62·2; H, 6·4; N, 9·5%).

Toluene-p-*sulphonylglycylglycyl*-α-L-*glutamyl*-L-*phenylalanine* cyclo*Hexylamide* (II*c*).—The foregoing γ-benzyl ester (1·5 g.) was hydrogenated at atmospheric pressure and 20° with palladium black catalyst in dimethylformamide (50 c.c.) and water (5 c.c.). The calculated quantity of hydrogen was absorbed in 2·5 hr. The catalyst was removed and the solvent evaporated. The residue was taken up in saturated sodium hydrogen carbonate solution (75 c.c.), which was washed with ethyl acetate (50 c.c.). The *carboxylic acid* (1·15 g., 82%) was precipitated by acidification of the carbonate solution and, after recrystallisation from aqueous ethanol, had m. p. 188·5—190°, pK_{MCS} 6·1, $[\alpha]_D^{21}$ −22·0° (*c*, 3 in dimethylformamide) (Found: C, 57·5; H, 6·1; N, 11·1. $C_{31}H_{41}O_8N_5S$ requires C, 57·8; H, 6·4; N, 10·9%).

Dehydration of the cyclo*Hexylamide* (II*c*) *and its Regeneration from the Condensation Product*.—A solution of the tetrapeptide derivative (II*c*) (0·645 g., 1 mmole) in dioxan (300 c.c.) was concentrated under reduced pressure to about 200 c.c. and then cooled to 10°. When triethylamine (0·17 c.c., 1·2 mmoles) and thionyl chloride (0·087 c.c., 1·2 mmoles) were added, a precipitate of triethylamine hydrochloride appeared. After 20 min. a second similar portion of triethylamine was added and, 1 hr. later, water (2 c.c.) also. The solution was evaporated and the residue distributed between butan-1-ol (100 c.c.), ethyl acetate (100 c.c.), and water (25 c.c.). The organic layer was washed with sodium hydrogen carbonate solution (2 × 50 c.c.) and water (2 × 50 c.c.) before being evaporated. The resulting neutral material (0·626 g., 100%), m. p. 177—179° (decomp.), was an amorphous powder; its infrared spectrum is recorded below.

This neutral material (0·308 g., 0·5 mmole) was dissolved in dimethylformamide (10 c.c.) and water (5 c.c.), and treated with N-sodium hydroxide (0·6 c.c.). After 1 hr. the solution was acidified (pH 6) and evaporated. The residue crystallised easily when ethyl acetate (40 c.c.) and M-potassium phosphate buffer (40 c.c.) were added. The mixed solvents were acidified with phosphoric acid (3 c.c.) and removed, leaving the product (II*c*) (0·245 g.), m. p. 184°, and more (0·038 g., 90% in all) was recovered from the ethyl acetate. The substance was identified by m. p. (raised to 187—189° by recrystallisation from aqueous ethanol), mixed m. p., and paper chromatography in butan-1-ol saturated with 3N-ammonia (R_F 0·78), which failed to disclose the presence of any other acidic substance even in the crystallisation liquors.

γ-Benzyl Hydrogen Toluene-p-*sulphonylglycyl*-L-*phenylalanyl*-L-*glutamate*.—A solution of γ-benzyl hydrogen L-glutamate (2·4 mmoles) in formamide (14 c.c.) and water (10 c.c.) was acylated at pH 9 by potassium toluene-*p*-sulphonylglycyl-L-phenylalanyl sulphate (2 mmoles). The resulting solution was neutralised with dilute sulphuric acid and evaporated. The product was extracted with ethyl acetate (6 × 50 c.c.) from 3N-hydrochloric acid (30 c.c.). Evaporation yielded a formamide-containing syrup, which crystallised on addition of water. The *tripeptide ester* (0·54 g., 45%) was recrystallised from ethyl acetate–light petroleum (b. p. 40—60°) and had m. p. 146—147°, pK_{MCS} 5·5, $[\alpha]_D^{16}$ −20·9° (*c*, 2 in EtOH) (Found: C, 60·7; H, 5·7; N, 7·2. $C_{30}H_{33}O_8N_3S$ requires C, 60·5; H, 5·6; N, 7·1%).

γ-Benzyl Hydrogen Benzoylglycyl-L-*glutamate*.—A suspension of γ-benzyl hydrogen L-glutamate (22 mmoles) in 50% aqueous dimethylformamide was acylated with lithium benzoylglycyl sulphate (20 mmoles) at pH 9. The resulting clear solution was kept for 30 min. before being acidified (pH 6) with dilute sulphuric acid and evaporated. The residue was distributed between ethyl acetate (100 c.c.) and 3N-sulphuric acid (50 c.c.) which was further extracted with ethyl acetate (50 c.c.). The material in the ethyl acetate layers was distributed (14 transfers; 100 c.c. phases) between ethyl acetate and phosphate buffer (0·8M-KH_2PO_4; 0·2M-K_2HPO_4).

γ-Benzyl hydrogen benzoylglycyl-L-glutamate (5·21 g., 65%), m. p. 136—137·5° after recrystallisation from ethyl acetate, pK_{MCS} 5·3, $[\alpha]_D^{21}$ −6·85° (*c*, 1·6 in dimethylformamide) (Found: C, 63·6; H, 5·8; N, 7·35. $C_{21}H_{22}O_6N_2$ requires C, 63·3; H, 5·6; N, 7·0%), was recovered from tubes 4—12 (*K* 1·50). Tubes 0—3 contained benzoylglycine (0·94 g., 25%).

Benzoylglycyl-α-L-glutamylmorpholine.—Morpholine (5 c.c.) was added to a solution of lithium benzoylglycyl-α-L-glutamyl sulphate γ-benzyl ester (8 mmoles) in dimethylformamide (75 c.c.), which was then kept for 1 hr. at 20° before being evaporated. The residue was distributed between N-sulphuric acid (50 c.c.) and ethyl acetate (200 c.c.), which was washed successively with N-sulphuric acid (50 c.c.), water (50 c.c.), sodium hydrogen carbonate solution (3 × 50 c.c.), and water (50 c.c.). Evaporation of the dried (Na_2SO_4) ethyl acetate solution furnished a syrupy benzyl ester (2·53 g., 68%), which was shaken in 95% ethanol (200 c.c.) with palladium black catalyst and hydrogen until uptake of the latter reached that theoretically required (7 hr.). The material obtained by filtration and evaporation was distributed between ethyl acetate (75 c.c.) and N-sodium carbonate which was then acidified and repeatedly extracted with ethyl acetate (500 c.c. in all). The *morpholide* (1·345 g., 67%) crystallised slowly when very dilute hydrochloric acid was added to the residue from evaporation of the ethyl acetate. It was recrystallised from water and had m. p. 117—118°, pK_{MCS} 6·1 (Found: C, 57·6; H, 6·4; N, 10·9. $C_{18}H_{23}O_6N_3$ requires C, 57·3; H, 6·1; N, 11·1%).

Formation and Hydrolysis of 1-*Benzoylglycyl-5-morpholinocarbonyl-2-oxo-L-pyrrolidine* (VI).—Triethylamine (0·21 c.c., 1·5 mmoles) and thionyl chloride (0·11 c.c., 1·5 mmoles) were added to a solution of benzoylglycyl-α-L-glutamylmorpholine (0·511 g., 1·35 mmoles) in dioxan (50 c.c.), which had been dried by concentration from 120 c.c. A second equal portion of triethylamine was added after 30 min. and this was followed after 1·5 hr. by water (2 c.c.). The solvents were evaporated and the residue was dissolved in butan-1-ol, which was then washed with dilute hydrochloric acid and aqueous sodium hydrogen carbonate; the aqueous washings were back-extracted with butanol. Evaporation of the combined butanol extracts left the neutral fraction as a pale yellow glass (0·379 g., 78%), which was hydrolysed by N-sodium hydroxide (1·6 c.c.) and water (10 c.c.). After 1 hr. the solution was acidified and evaporated to dryness. Paper chromatography of the residue in butanol saturated with 3N-ammonia showed that the predominating product was the original benzoylglycyl-α-L-glutamylmorpholine (R_F 0·26); a smaller quantity of benzoylglycine (R_F 0·33) was also detected.

*Toluene-*p*-sulphonyl-α-L-glutamylglycine* cyclo*Hexylamide and Toluene-*p*-sulphonyl-αγ-L-glutamyldi(glycine* cyclo*Hexylamide*).—Glycine *cyclo*hexylamide hydrobromide (3·076 g., 13 mmoles), sodium hydrogen carbonate (2·18 g., 26 mmoles), and water (25 c.c.) were added successively to a solution of 1-toluene-*p*-sulphonyl-2-oxo-L-pyrrolidine-5-carbonyl chloride [14] (3·70 g., 12·2 mmoles) in chloroform (50 c.c.) at 0°. The flask was shaken vigorously until the evolution of carbon dioxide had ceased (5 min.) and was then left for 30 min. further at 0°. The chloroform layer was then washed with sodium hydrogen carbonate solution (2 × 25 c.c.) and N-hydrochloric acid (2 × 25 c.c.) before being evaporated. N-Sodium hydroxide (10 c.c.) was added to the cold (0°) solution of the resulting neutral oil (4·04 g., 80%) in dimethylformamide (40 c.c.), which was kept for 30 min. before being acidified (pH 6) and evaporated. The residue was taken up in water (50 c.c.) and the minimum quantity of N-sodium hydroxide. This solution was washed with ethyl acetate (50 c.c.) and then covered with ethyl acetate (100 c.c.). When the aqueous layer was acidified to Congo-red, there was immediate precipitation of the very insoluble *toluene-*p*-sulphonyl-αγ-L-glutamyldi(glycine* cyclo*hexylamide*) (0·378 g.), which was purified by repeated washing in suspension in sodium hydrogen carbonate solution, water, and ethanol and had m. p. 211—214° (Found: C, 58·0; H, 7·3; N, 12·2. $C_{28}H_{43}O_6N_5S$ requires C, 58·2; H, 7·5; N, 12·1%). The ethyl acetate layer was separated from the acidic aqueous layer and was kept at 0° overnight, during which time toluene-*p*-sulphonyl-γ-L-glutamylglycine *cyclo*hexylamide (0·401 g.), m. p. 197—199° raised to 198—199° on admixture of authentic material (m. p. 199—200°), crystallised. Evaporation of the ethyl acetate liquor afforded *toluene-*p*-sulphonyl-α-L-glutamylglycine* cyclo*hexylamide* (2·77 g., 52%), which was recrystallised from aqueous ethanol and had m. p. 157—160°, $[\alpha]_D^{16}$ −7·7° (*c*, 3·6 in dimethylformamide), pK_{MCS} 6·1 (Found: C, 54·8; H, 6·8; N, 9·5. $C_{20}H_{29}O_6N_3S$ requires C, 54·7; H, 6·6; N, 9·6%).

*Toluene-*p*-sulphonyl-γ-L-glutamyl-L-phenylalanine* cyclo*Hexylamide.*—A solution of 1-toluene-*p*-sulphonyl-2-oxo-L-pyrrolidine-5-carboxylic acid [15] (VII; X = OH) (0·283 g., 1 mmole), L-phenylalanine *cyclo*hexylamide (0·266 g., 1·1 mmole), and triethylamine (0·14 c.c., 1 mmole) in acetonitrile (2·5 c.c.) was boiled under reflux for 4·5 hr. Next day the solvents were evaporated

[20] Mizutani, *Z. phys. Chem.*, 1925, **116**, 350; Simon and Heilbronner, *Helv. Chim. Acta*, 1955, **38**, 508.

and the residue was dissolved in water (10 c.c.) and 3N-sodium hydroxide (2 c.c.). The solution was washed with ether (2 × 10 c.c.) and then acidified. The precipitated gel was extracted into ethyl acetate (2 × 50 c.c.), which was dried and evaporated. Crystallisation of the residue (0·400 g.) from aqueous ethanol furnished the *dipeptide derivative* (0·241 g., 48%), m. p. 211—212°, pK_{MCS} 5·2 (Found : C, 61·5; H, 6·6; N, 8·1. $C_{27}H_{35}O_6N_3S$ requires C, 61·2; H, 6·7; N, 7·9%).

Acidic Dissociation Constants.—pK_{MCS} refers to the pH recorded at 20° by a Pye glass-electrode and meter of a solution in 75 vol. % aqueous 2-methoxyethanol at the half-neutralisation point. The meter was standardised with an aqueous buffer at pH 4. The values given previously [4] for compounds (II*a*) and (V*a*) were low, owing to an error in standardisation. The present values [6·1—6·2 for the α-glutamyl series (II), 5·3—5·5 for the γ-series (V)] are in the expected region when allowance is made for the acid-weakening effect of the organic solvent.[20]

Infrared Spectra.—These were determined with a Perkin-Elmer model 21 instrument (sodium chloride prism), Nujol mulls being used, unless otherwise stated. Attention was drawn previously [4] to the fact that in a pair of isomeric glutamyl derivatives the carboxylic acid band is, as expected, at a higher frequency in the γ-isomer, which has the more strongly acidic α-carboxylic acid group. The two further pairs of isomers prepared since then conform with this rule, but the correlation is of little value because the difference between a pair of isomers (15—21 cm.$^{-1}$) is less than the variation in frequency with different structures; this variation is doubtless partly due to differences in the hydrogen-bonding of the carboxyl group. Peptide derivatives with a free α-carboxylic acid group had absorption maxima as follows : toluene-*p*-sulphonylglycine (pK_{MCS} 5·3) 1727, toluene-*p*-sulphonylglycylglycine (pK_{MCS} 5·4) 1733, toluene-*p*-sulphonyl-γ-L-glutamylglycine *cyclo*hexylamide 1704, toluene-*p*-sulphonyl-γ-L-glutamyl-L-phenylalanine *cyclo*hexylamide 1739 (and a weak absorption at 1704), benzoyl-γ-DL-glutamylglycine *cyclo*hexylamide 1745, γ-benzyl hydrogen benzoylglycyl-L-glutamate 1733 and 1745, γ-benzyl hydrogen toluene-*p*-sulphonylglycylglycyl-L-glutamate 1736 and 1754, γ-benzyl hydrogen toluene-*p*-sulphonylglycyl-L-phenylalanyl-L-glutamate 1730 (broad band), toluene-*p*-sulphonylglycyl-L-phenylalanyl-γ-L-glutamylglycine *cyclo*hexylamide 1733 cm.$^{-1}$. Peptide derivatives with a free γ-carboxylic acid group had the following absorption maxima : toluene-*p*-sulphonyl-α-L-glutamylglycine *cyclo*hexylamide 1686, benzoyl-α-DL-glutamylglycine *cyclo*hexylamide 1730, benzoylglycyl-α-L-glutamylmorpholine 1739, toluene-*p*-sulphonylglycylglycyl-α-L-glutamyl-L-phenylalanine *cyclo*hexylamide 1739, toluene-*p*-sulphonylglycyl-L-phenylalanyl-α-L-glutamylglycine *cyclo*hexylamide 1712 (and a weak absorption at 1692) cm.$^{-1}$.

Benzoyl-DL-glutamoylglycine *cyclo*hexylamide had maxima at 654, 677, 693, 717, 745, 775, 798, 862, 896, 905, 924, 928, 948, 962, 1007, 1028, 1060, 1079, 1100, 1156, 1172, 1229, 1250, 1277, 1332, 1359, 1416, 1495, 1550, 1587, 1613, 1642, 1681, 1742 cm.$^{-1}$. Toluene-*p*-sulphonylglycyl-L-phenylalanyl-L-glutamoylglycine *cyclo*hexylamide had maxima at 701, 732 (very broad band), 785, 813, 818, 860, 895, 918, 961, 984, 1008, 1056, 1091, 1105, 1163, 1172, 1242, 1252, 1332, 1359, 1399, 1553, 1647, 1704, 1742 cm.$^{-1}$. 1-Methyl-2 : 6-dioxopiperidine in liquid film had maxima at 658, 694, 883, 925, 1044, 1111, 1156, 1199, 1292, 1328, 1357, 1414, 1464, 1675, 1730 cm.$^{-1}$. The neutral product from cyclisation of toluene-*p*-sulphonylglycylglycyl-α-L-glutamyl-L-phenylalanine *cyclo*hexylamide (probably I*c*) in a potassium bromide disc gave a poorly resolved spectrum with maxima at 662, 699, 744, 815, 842, 890, 1030, 1094, 1159, 1250, 1328, 1403, 1453, 1546, 1647 cm.$^{-1}$.

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