*Syntheses from Phthalimido-acids. Part IV.** p-*Glycylaminobenzoic Acid and Derivatives.*

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p-(Phthalylglycylamino)benzoic acid has been prepared from *p*-aminobenzoic acid and phthalylglycyl chloride or its ethyl carbonic anhydride, and *p*-(formylglycylamino)benzoic acid by the azide or mixed anhydride method from formylglycine. By the action of hydrazine on ethyl *p*-(phthalylglycylamino)benzoate, and of ethanolic hydrogen chloride on *p*-(formylglycylamino)benzoic acid, ethyl *p*-glycylaminobenzoate was obtained, which was further hydrolysed to *p*-glycylaminobenzoic acid. *p*-Glycylaminobenzoyl-L-glutamic acid has been synthesised *via* its phthalyl derivative from *p*-aminobenzoyl-L-glutamic acid.

p-Glycylaminobenzoic acid was obtained by Tropp (*Ber.*, 1928, **61**, 1431) from *p*-chloroacetamidobenzoic acid and aqueous ammonia. In meeting the requirements of biochemical experiments we have investigated the synthesis of this compound by general methods developed since its description by Tropp. These include the use of phthalylated compounds, first applied to simple peptide synthesis by Sir Robert Robinson and one of us (see King and Kidd, *Nature*, 1948, **162**, 776; *J.*, 1949, 3315; cf. Sheehan and Frank, *J. Amer. Chem. Soc.*, 1949, **71**, 1856), and of the mixed (carbonic) anhydride coupling procedure (Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547; 1952, **74**, 6137; Wieland and Bernhard, *Annalen*, 1951, **572**, 190). It was also prepared *via* the *N*-formyl compound from *N*-formylglycine (D. A. A. Kidd, Thesis, Oxford, 1950), an example of a method of synthesis recently described by Waley for three other dipeptides (*Chem. and Ind.*, 1953, 107).

p-(Phthalylglycylamino)benzoic acid was prepared from phthalylglycyl chloride by direct condensation with *p*-aminobenzoic acid in aqueous solution (yield 77%) or through the ethyl carbonic anhydride (65%). The corresponding ethyl ester was obtained from phthalylglycyl chloride and ethyl *p*-aminobenzoate (yield 87%), or by treating *p*-(phthalylglycylamino)benzoic acid with 1·5% hydrogen chloride in alcohol; the use of boiling ethanol saturated with hydrogen chloride resulted in alcoholysis to phthalylglycine ethyl ester and ethyl *p*-aminobenzoate. Removal of the phthalyl group with ethanolic hydrazine gave ethyl *p*-glycylaminobenzoate, conveniently isolated as its hydrochloride (72%). *p*-Ethoxycarbonyl(hydroxyimino)acetanilide, obtained from *p*-aminobenzoic acid, chloral, and hydroxylamine, as in Sandmeyer's synthesis of hydroxyiminoacetanilide (*Helv. Chim. Acta*, 1919, **2**, 237; *Org. Synth.*, 1925, **5**, 71), was also used in a preparation of the *p*-glycylaminobenzoate, but catalytic hydrogenation of the hydroxyimino-compound yielded only 18% of the primary amine.

No product corresponding to the acid chloride (or oxazolone hydrochloride) was obtainable from formylglycine, *e.g.*, with thionyl chloride or with phosphorus pentachloride in acetyl chloride or other solvents. The formamido-acid also failed to give any recognisable compound with ethyl *p*-aminobenzoate when a mixture of the two substances was treated with phosphorus trichloride in boiling benzene, a coupling procedure devise by Süs (*Annalen*, 1951, **572**, 96). On the other hand, ethyl *p*-benzoyl- and *p*-acetyl-glycylaminobenzoate were successfully formed in this way (yields 73% and 64% respectively), the acetyl ester and related acid being also obtained (yields 52%) from ethyl *p*-aminobenzoate and from *p*-aminobenzoic acid by the action of acetylglycyl ethyl carbonic anhydride. The preparation of formylglycine derivatives was possible, however, when *N*-formylglycine was converted through the hydrazide into formylglycine azide, which, having given with aniline the anilide, was then used to yield *p*-(formylglycylamino)benzoic acid.

Removal of the protecting formyl group without disturbance of the peptide link depends on the sensitivity of formamides to ethanolic hydrogen chloride, a property

* Part III, *J.*, 1951, 2976.

utilised in the preparation of ββ-diethoxyalanine and its derivatives (cf. Brown, "The Chemistry of Penicillin," Oxford Univ. Press, 1949, p. 492). After treatment with this reagent, *p*-(formylglycylamino)benzoic acid gave the dipeptide ester, identical with the material obtained from ethyl *p*-(phthalylglycylamino)benzoate, and hydrolysed with cold aqueous alkali to the *p*-(glycylamino)benzoic acid (Tropp, *loc. cit.*) (yield from the formyl acid, 38%).

p-(Formylglycylamino)benzoic acid (47%) was also prepared by the mixed carbonic anhydride method from *p*-aminobenzoic acid, but with ethyl *p*-aminobenzoate, only ethyl *p*-ethoxycarbonylaminobenzoate (55%) was obtained. As there appeared to be no reason to suspect the presence of unchanged ethyl chloroformate in the mixture before the addition of the aminobenzoate, this result is attributed to the abnormal behaviour of the mixed anhydride in yielding the cation EtO_2C^+ instead of the expected species $H{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO^+$.

The *N*-acyl derivatives of *p*-glycylaminobenzoic acid proved unsuitable for the synthesis of a typical derivative of the dipeptide, *i.e.*, *p*-glycylaminobenzoyl-L-glutamic acid. Thus ethyl *p*-(formylglycylamino)benzoate failed to react with hydrazine, thus excluding the azide method of condensation, and its triethylamine salt was too insoluble to allow of the preparation of the mixed carbonic anhydride. The phthalyldipeptide, on the other hand, appears to form anhydrides with both ethyl and *iso*butyl chlorocarbonate, but it was recovered unchanged after treatment of the supposed anhydrides both with L-glutamic acid and with glycine (experiments by Mr. R. Wade). The desired tripeptide was therefore prepared from phthalylglycyl chloride and *p*-aminobenzoyl-L-glutamic acid, the intermediate phthalyl compound being hydrolysed with hydrazine.

Experimental

p-(*Phthalylglycylamino*)*benzoic Acid.*—(i) A solution of *p*-aminobenzoic acid (2·74 g.) in water (50 c.c.) containing sodium carbonate (1·1 g., 1 equiv.) was cooled in an ice-bath and stirred during the simultaneous addition in 1 hr. of solutions of phthalylglycyl chloride (4·46 g.) in dioxan (50 c.c.), and sodium carbonate (1·1 g.) in water (50 c.c.). After a further hour the solution was acidified (Congo-red) with 2N-hydrochloric acid, and the white precipitate collected and crystallised from aqueous 2-ethoxyethanol or aqueous ethanol. p-(*Phthalylglycylamino*)-*benzoic acid* separated in matted hair-like needles (5 g., 77%), m. p. 331—332° (decomp.), raised by recrystallisation to 332—333° (decomp.) (Found: C, 62·8; H, 4·0; N, 8·9. $C_{17}H_{12}O_5N_2$ requires C, 63·0; H, 3·7; N, 8·6%).

(ii) A solution of phthalylglycine (2·05 g.) in dry dioxan (20 c.c.) containing triethylamine (1·4 c.c., 1 equiv.) was cooled to 10° and stirred during the addition of ethyl chloroformate (0·96 c.c., 1 equiv.). Triethylamine hydrochloride was precipitated and 10 min. later the mixture was treated with a solution of *p*-aminobenzoic acid (1·37 g.) in aqueous N-sodium hydroxide (10 c.c., 1 equiv.). After effervescence had ceased, a small volume of N-sodium hydroxide was added and the solution thrice extracted with ether. The aqueous phase was acidified (Congo-red) with 5N-hydrochloric acid, and the precipitated *p*-(phthalylglycylamino)benzoic acid (2·12 g., 65%), m. p. 330° (decomp.), was collected and crystallised from aqueous ethanol from which it separated in matted needles.

The action of aqueous hydrazine solution on *p*-(phthalylglycylamino)benzoic acid dissolved in boiling ethanol or in cold aqueous sodium carbonate solution appeared to remove the protecting group, but owing to the low solubility of *p*-glycylaminobenzoic acid in hydrochloric acid it could not be separated from the accompanying phthalhydrazide.

Ethyl p-(*Phthalylglycylamino*)*benzoate.*—(i) A solution of ethyl *p*-aminobenzoate (13·2 g., 2 equivs.) in dry chloroform (50 c.c.) was stirred during the dropwise addition in 2 hr. of phthalylglycyl chloride (8·92 g.) likewise dissolved in chloroform (50 c.c.). Additional solvent was introduced during the reaction to allow efficient stirring to be maintained. After a further hour, the chloroform was evaporated under reduced pressure, and the residue washed with 2N-hydrochloric acid and with water, the *ethyl* p-(*phthalylglycylamino*)*benzoate* crystallising from ethanol as glistening plates or rods (12·3 g., 87%) (Found: C, 64·8; H, 4·2; N, 8·4. $C_{19}H_{16}O_5N_2$ requires C, 64·8; H, 4·6; N, 8·0%).

(ii) A suspension of *p*-(phthalylglycylamino)benzoic acid (1·0 g.) in anhydrous ethanol (50 c.c.) containing hydrogen chloride (1·5%) was heated under reflux until dissolution was almost complete. The liquid was filtered and then cooled, whereupon ethyl *p*-(phthalylglycylamino)benzoate (0·44 g.) separated in plates, m. p. 207—208°, raised by recrystallisation from

ethanol to 212° (mixed m. p. 212°). When the filtrate was evaporated and the residue crystallised from ethanol a further quantity (0·20 g.) of the ester, m. p. 210°, was obtained (total 59%).

A suspension of *p*-(phthalylglycylamino)benzoic acid (1·0 g.) in absolute ethanol (50 c.c.) saturated with hydrogen chloride was heated under reflux for 1 hr. The solvent was then removed and the residue washed with dilute hydrochloric acid and with water. The undissolved solid consisted of phthalylglycine ethyl ester and crystallised from aqueous ethanol in rods (0·63 g., 88%), m. p. 108°, raised by recrystallisation to 110°, mixed m. p. with an authentic specimen 111°. The hydrochloric acid washings gave, when basified, ethyl *p*-aminobenzoate which after crystallisation from aqueous ethanol had m. p. and mixed m. p. 88°.

Ethyl p-*Glycylaminobenzoate*.—(i) A solution of ethyl *p*-(phthalylglycylamino)benzoate (14·1 g.) in ethanol (560 c.c.) was treated with aqueous hydrazine (4 c.c., 33·2%, 1·04 equivs.) and heated under reflux for 1 hr. The pale yellow solution was evaporated to dryness and after treatment with 2N-hydrochloric acid the mixture was set aside at 0° for several hours. The precipitate was collected and repeatedly triturated with water containing a little hydrochloric acid. The filtrates were combined and evaporated under reduced pressure at 45°, long needles of *ethyl* p-*glycylaminobenzoate hydrochloride* (7·5 g., 72%), m. p. 238—240° (decomp.), separating during the evaporation. Precipitation of the hydrochloride from ethanol with anhydrous ether gave needles, m. p. 244—246° (decomp.) (Found: C, 51·2; H, 5·8; N, 10·7. $C_{11}H_{14}O_3N_2$,HCl requires C, 51·0; H, 5·8; N, 10·8%).

The hydrochloride was dissolved in a little water and treated with aqueous sodium carbonate. Crystallisation of the heavy white precipitate from water afforded *ethyl* p-*glycylaminobenzoate monohydrate* as long rods, m. p. 96—97° (Found: C, 55·0; H, 7·0; N, 11·8. $C_{11}H_{14}O_3N_2,H_2O$ requires C, 55·0; H, 6·7; N, 11·7%). The anhydrous *ester*, m. p. 88·5—89°, was obtained when the monohydrate was dried in a vacuum at 60° (Found: C, 59·7; H, 6·4; N, 12·5. $C_{11}H_{14}O_3N_2$ requires C, 59·5; H, 6·3; N, 12·6%).

(ii) A mixture of *p*-ethoxycarbonyl(hydroxyimino)acetanilide (below) (2·64 g.), palladium–charcoal (0·7 g.), palladium chloride (0·3 g.), hydrochloric acid (35%, 2·5 c.c.), and ethanol (50 c.c.) was shaken in hydrogen (20 atm.). The reddish-brown product obtained by evaporation was extracted with 2N-hydrochloric acid, and the extract concentrated. The crystalline material which separated during the evaporation was collected and dissolved in water, and the solution made alkaline. The brown flocculent precipitate was removed and extracted with boiling water. When the solution was cooled, ethyl *p*-glycylaminobenzoate monohydrate (0·50 g., 18%) separated as rods, m. p. 96—97° (Found: N, 11·6%).

p-*Ethoxycarbonyl(hydroxyimino)acetanilide*.—To a solution of chloral hydrate (9 g.) in water (120 c.c.) were added crystalline sodium sulphate (130 g.), ethyl *p*-aminobenzoate (8·26 g.) dissolved in water (60 c.c.) containing the necessary volume of concentrated hydrochloric acid for solution, and hydroxylamine hydrochloride (11 g. in 50 c.c. of water). The mixture was rapidly heated and boiled for 2 min., an orange oil separating. When cooled, the oil solidified, and by crystallising it from boiling water p-*ethoxycarbonyl(hydroximino)acetanilide* (3·9 g., 33%) was obtained as pale yellow plates, m. p. 148—149°. Recrystallisation from water (charcoal) yielded colourless needles, m. p. 173—175°, possibly a stereoisomer of the product of m. p. 148—149° (Found: C, 56·0; H, 5·3; N, 12·1. $C_{11}H_{12}O_4N_2$ requires C, 55·9; H, 5·1; N, 11·9%).

Ethyl p-(*Benzoylglycylamino*)*benzoate*.—(i) A suspension of benzoylglycine (3·0 g.) in dry benzene (100 c.c.) containing ethyl *p*-aminobenzoate (3·2 g.) was treated with freshly distilled phosphorus trichloride (1·5 c.c.), heat being evolved and a gelatinous solid precipitated. The mixture was heated under reflux on a steam-bath for 2 hr. while hydrogen chloride was continuously evolved. The solvent was then evaporated under reduced pressure and the residue washed with aqueous sodium carbonate. The undissolved material was dried and crystallised from ethyl acetate, *ethyl* p-(*benzoylglycylamino*)*benzoate* (4·0 g., 73%) separating as felted needles, m. p. 169—170° raised by recrystallisation to 170—171°. A further quantity (0·45 g.), m. p. 169—170°, was obtained by concentration of the mother-liquors (total yield 81%) (Found: C, 66·4; H, 5·3; N, 8·7. $C_{18}H_{18}O_4N_2$ requires C, 66·3; H, 5·6; N, 8·6%).

(ii) A solution of ethyl *p*-glycylaminobenzoate (0·3 g.) in pure dry chloroform (10 c.c.) was treated with excess of benzoyl chloride and triethylamine and shaken for 15 min. The solvent was removed *in vacuo* and the residue washed with dilute hydrochloric acid, aqueous sodium carbonate, and water. The residue of ethyl *p*-(benzoylglycylamino)benzoate crystallised from ethyl acetate in felted needles, m. p. and mixed m. p. 170°.

Ethyl p-(*Acetylglycylamino*)*benzoate*.—(i) A suspension of acetylglycine (1·2 g.) in benzene (100 c.c.) containing ethyl *p*-aminobenzoate (1·92 g.) was treated with freshly distilled phosphorus

trichloride (0·9 c.c.), and the mixture heated under reflux on a steam-bath for 2 hr. The solvent was then evaporated under reduced pressure and the residue extracted with aqueous sodium carbonate. The residue of *ethyl* p-(*acetylglycylamino*)*benzoate* crystallised from ethyl acetate or moist ethanol (charcoal) in plates (1·7 g., 64%), m. p. 224° (Found: C, 59·1; H, 6·1; N, 10·7. $C_{13}H_{16}O_4N_2$ requires C, 59·1; H, 6·1; N, 10·6%).

(ii) A solution of acetylglycine (1·17 g.) in dry chloroform (20 c.c.) containing triethylamine (1·4 c.c.) was cooled to 0° and stirred during the addition of ethyl chloroformate (0·96 c.c.). After the solution had been kept at 0° for a further 10 min., a solution of ethyl *p*-aminobenzoate (1·65 g.) in chloroform (20 c.c.) was added. Effervescence was slight and the solution was left overnight at room temperature. The precipitate was collected and crystallised from ethyl acetate (1·17 g., 44%), separating as plates, m. p. and mixed m. p. 224°. A further quantity (0·2 g.) was obtained by concentration of the chloroform solution (total yield 52%).

p-(*Acetylglycylamino*)*benzoic Acid.*—A solution of acetylglycine (1·17 g.) in pure dry dioxan (10 c.c.) containing triethylamine (1·4 c.c.) was cooled to 10° and stirred during the addition of ethyl chloroformate (0·96 c.c.). Triethylamine hydrochloride was precipitated immediately and after a further 10 min. at 10° a solution of *p*-aminobenzoic acid (1·37 g.) in N-sodium hydroxide (10 c.c.) was added. When effervescence had ceased aqueous sodium hydroxide was added and the solution was thrice extracted with ether. The aqueous phase was acidified with 5N-hydrochloric acid and stored at 0° for several hours. The precipitate of p-(*acetylglycylamino*)*benzoic acid* (1·24 g., 52%) crystallised from aqueous ethanol or aqueous dioxan as plates, m. p. varying from 260° to 270° (decomp.) with rate of heating (Found: C, 56·2; H, 5·3; N, 11·5. $C_{11}H_{12}O_4N_2$ requires C, 56·0; H, 5·1; N, 11·8%).

Formylglycine Hydrazide.—A solution of formylglycine ethyl ester (20·2 g.) in ethanol (50 c.c.) was treated with hydrazine hydrate (15 c.c. of 60%), an exothermic reaction taking place. After some hours the *hydrazide* slowly separated in colourless rectangular prisms (13·6 g., 74%), which after crystallisation from ethanol had m. p. 130° (Found: C, 31·2; H, 5·7; N, 35·5. $C_3H_7O_2N_3$ requires C, 30·8; H, 6·0; N, 35·9%).

Formylglycine Anilide.—A solution of formylglycine hydrazide (4 g.) in N-hydrochloric acid (40 c.c.) at 0° was stirred during the addition of 1 mol. of aqueous sodium nitrite (10%). The solution was neutralised with sodium carbonate and then treated with aniline (10 c.c.). Stirring was continued for 2 hr., after which the precipitate was collected, washed with dilute hydrochloric acid, and crystallised from water. *Formylglycine anilide* was thus obtained in plates, m. p. 152° (Found: C, 60·5; H, 5·7; N, 15·9. $C_9H_{10}O_2N_2$ requires C, 60·5; H, 5·7; N, 15·7%).

p-(*Formylglycylamino*)*benzoic Acid.*—(i) *N*-Formylglycine hydrazide (5 g.) in N-hydrochloric acid (50 c.c.) was converted into the azide, and the solution made just alkaline and treated at 0° with *p*-aminobenzoic acid (5·4 g.) dissolved in aqueous sodium carbonate (50 c.c. of 5%) added during 1 hr. The addition of 5N-hydrochloric acid then precipitated the p-(*formylglycylamino*)*benzoic acid* as a cream-coloured solid (4·4 g.) which when collected crystallised from a very large volume of water in small glistening prisms, decomp. 253—254° (Found: C, 53·9; H, 4·6; N, 12·2. $C_{10}H_{10}O_4N_2$ requires C, 54·0; H, 4·5; N, 12·6%).

(ii) A solution of formylglycine (2·06 g.) in acetone (30 c.c.) containing triethylamine (2·8 c.c.) was cooled to 0° and stirred during the addition of ethyl chloroformate (1·92 c.c.). After a further 10 min. at 0°, *p*-aminobenzoic acid (2·74 g.) in aqueous N-sodium hydroxide (20 c.c.) was added, and when the reaction ceased the solution was acidified with 5N-hydrochloric acid and the acetone was evaporated under reduced pressure. The precipitate of *p*-(formylglycylamino)benzoic acid (2·1 g., 47%) was collected after 15 hr. at 0°; it crystallised from water or from aqueous dioxan in prisms, decomp. *ca.* 250° depending on the rate of heating.

The mixed anhydride from *N*-formylglycine and ethyl chloroformate prepared as in (ii) and kept at 0° for 10 min. was treated with a solution of ethyl *p*-aminobenzoate (3·3 g.) in acetone. No effervescence was observed, and later, when the solution was evaporated to dryness and the residue washed with dilute hydrochloric acid and aqueous sodium carbonate, the product was found to be ethyl *p*-(ethoxycarbonylamino)benzoate (2·73 g., 55%), m. p. 128—130°. Crystallised from aqueous ethanol it formed plates, m. p. 130° alone or with an authentic specimen. The same product was obtained when chloroform was used as solvent.

p-*Glycylaminobenzoic Acid.*—A suspension of the formyl-dipeptide (1·5 g.) in ethanol (15 c.c.) saturated at 0° with dry hydrogen chloride was momentarily warmed to boiling and set aside for 4—5 hr. The microcrystalline ester hydrochloride was collected, dissolved in a little cold water, and treated with 2·5N-sodium hydroxide (10 c.c.). The voluminous precipitate was then redissolved by the addition of a little acetone, and the solution set aside for

30 min. to complete hydrolysis of the ester. Addition of acetic acid slowly precipitated *p*-glycylaminobenzoic acid (0·5 g.) which crystallised from hot water as needles, m. p. 300° (decomp.), consisting either of a *dihydrate* (Found : C, 47·2; H, 6·1; N, 12·3. $C_9H_{10}O_3N_2,2H_2O$ requires C, 47·0; H, 6·1; N, 12·2%) or of the monohydrate (cf. Tropp, *loc. cit.*) (Found : C, 50·5; H, 5·7; N, 13·2. Calc. for $C_9H_{10}O_3N_2,H_2O$: C, 50·9; H, 5·7; N, 13·2. Found, in specimen dried at 150° *in vacuo* : C, 55·6; H, 5·5; N, 14·1. Calc. for $C_9H_{10}O_3N_2$: C, 55·7; H, 5·2; N, 14·4%).

Alternatively, the ester hydrochloride was dissolved in water and neutralised with aqueous sodium hydroxide. The liberated solid was washed with water and recrystallised from aqueous ethanol, and thus afforded colourless felted needles of ethyl *p*-glycylaminobenzoate monohydrate, m. p. 96—97°, described above (Found, in the dried material : C, 59·1; H, 6·4; N, 12·1%).

Ethyl p-(*Formylglycylamino*)*benzoate*.—Formylglycine hydrazide (9·4 g.), dissolved in a mixture of water (10 c.c.) and concentrated hydrochloric acid (7·1 c.c.), was treated with acetic acid (15 c.c.) and cooled to 0°. Aqueous sodium nitrite (7 g. in 11 c.c.) was run in and the solution immediately extracted four times with chilled ethyl acetate. The combined extracts were washed with cold dilute aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and added to an ethereal solution of ethyl *p*-aminobenzoate (12·2 g.). Within 2 hr., the *ethyl* p-(*formylglycylamino*)*benzoate* began to separate and after 16 hr. at 0° it was collected and crystallised from aqueous ethanol. The ester (5·4 g.), m. p. 175°, separated in small needles, m. p. after further crystallisation 178° (Found : C, 58·0; H, 5·6; N, 11·3. $C_{12}H_{14}O_4N_2$ requires C, 57·6; H, 5·6; N, 11·2%). An alternative procedure, in which the azide solution was not extracted, but was instead neutralised and added to an acetone solution of ethyl *p*-aminobenzoate under slightly alkaline conditions, gave a poorer yield (13%) of the formyldipeptide ester.

The ester was recovered unchanged after 2 hours' refluxing in ethanol solution with 100% hydrazine hydrate. When the ester (5·25 g.) was heated under reflux with 100% hydrazine hydrate (20 c.c.) in the absence of any other solvent, *p*-aminobenzhydrazide (2·8 g.), crystallising from ethanol in long needles, m. p. 220° (decomp.) [lit., m. p. 220° (decomp.)], was obtained (Found : C, 55·8; H, 6·0; N, 27·8. Calc. for $C_7H_9ON_3$: C, 55·6; H, 6·0; N, 27·8%).

p-(*Phthalylglycylamino*)*benzoyl-*L*-glutamic Acid*.—Aqueous sodium hydroxide (30 c.c. of 2%) and phthalylglycyl chloride (2·9 g.) (Harwood and Johnson, *J. Amer. Chem. Soc.*, 1933, **55**, 4178) in dioxan (30 c.c.) were added simultaneously with stirring to a solution of *p*-aminobenzoyl-L-glutamic acid (3·5 g.) (King, Acheson and Spensley, *J.*, 1949, 1401) in aqueous sodium hydroxide (1·1 g. in 30 c.c.) at room temperature during ½ hr. After 2 hr. the mixture was acidified with 5N-hydrochloric acid, this precipitating an oil which rapidly solidified and then separated from hot water as a gelatinous mass. The dried product formed a nearly white powder (2·7 g.), m. p. 178°. Recrystallisation gave the persistently gelatinous pure *phthalyltripeptide*, m. p. 189—190°, as a monohydrate (Found, in material dried at 15° : C, 56·4; H, 4·4; N, 8·6. $C_{22}H_{19}O_8N_3,H_2O$ requires C, 56·1; H, 4·5; N, 8·9%. Found, in material dried at 140° in a vacuum : C, 58·1; H, 4·3; N, 9·3. $C_{22}H_{19}O_8N_3$ requires C, 58·3; H, 4·2; N, 9·3%).

p-(Phthalylglycylamino)benzoic acid was dissolved in *NN*-dimethylformamide and treated with triethylamine and then with either ethyl or *iso*butyl chloroformate below 5°. A solution of L-glutamic acid in N-sodium hydroxide, added 10 min. later, caused effervescence but the product obtained by acidifying the ethyl acetate-washed aqueous liquid consisted of *p*-(phthalylglycylamino)benzoic acid, fine needles from aqueous 2-ethoxyethanol, m. p. 330° (decomp.). The phthalyl-dipeptide failed to react with glycine under similar conditions.

p-*Glycylaminobenzoyl-*L*-glutamic Acid*.—A solution of the phthalyl-tripeptide (2 g.) in aqueous sodium carbonate (0·5 g. in 15 c.c.) was left to react with hydrazine hydrate (0·25 g.) for 24 hr. at room temperature, and then exactly neutralised with hydriodic acid. After removal of the phthalhydrazide, the filtrate was evaporated to dryness and the gummy residue triturated with acetone. The sparingly soluble white powder crystallised slowly at 0° from an aqueous solution rendered turbid with ethanol at room temperature, the pure *tripeptide* affording fine colourless needles which decomposed with effervescence at 252—253° (Found : C, 52·3; H, 5·5; N, 13·3. $C_{14}H_{17}O_6N_3$ requires C, 52·0; H, 5·3; N, 13·0%).

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