168. *Syntheses from Phthalimido-acids. Part IX.* Model Compounds for a Synthesis of Glutathione, and Phthalyl-L-glutamic Anhydride as a Source of α-Glutamylpeptides.*

By F. E. KING, J. W. CLARK-LEWIS, and ROY WADE.

Some exploratory experiments which led to the synthesis of glutathione recorded in the preceding paper are described. The phosphorus trichloride coupling procedure introduced by Süs caused racemisation when applied to the 3-formyl-2 : 2-dimethylthiazolidine derived from L-cysteine. *N*-Phthalyl- and other *N*-acyl-L-cysteine derivatives are found to be too labile for preferential hydrolysis of ester groups under the acid conditions which have been used successfully in other cases for preparation of phthalylpeptides from their esters. γ-Benzyl and γ-methyl hydrogen phthalyl-L-glutamate are prepared, and are shown to be useful intermediates in the synthesis of α-L-glutamyl-amide and -peptide derivatives.

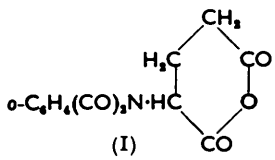
PHTHALYL-L-GLUTAMIC ANHYDRIDE has been used extensively for preparation of γ-L-glutamyl peptides since its introduction by King and Kidd,[1] and their demonstration that the anhydride reacts with bases to give phthalyl-γ-L-glutamyl derivatives directly by preferential fission of the γ-carbonyl-to-oxygen bond. Alcohols react similarly, and phthalyl-α-L-glutamyl peptides may be obtained from the γ-esters by coupling the free α-carboxyl group by either the mixed anhydride or the acid chloride coupling procedure. Phthalyl-L-glutamic acid is easily converted into the anhydride, and has usually been prepared by acid hydrolysis of diethyl phthalyl-L-glutamate as originally described,[1] except that better results are obtained when the hydrolysis time is reduced to 75 minutes.[2,3] Phthalyl-L-glutamic anhydride may also be obtained in similar over-all yield by a modification of the fusion technique described by Sheehan and Bolhofer [4] (see Experimental section). Measurements of the optical rotation of phthalyl-L-glutamic acid dissolved in aqueous sodium carbonate [1] are unreliable because the initial rotation depends upon the concentrations of sodium carbonate and of acid. Moreover the observed rotation gradually falls owing to hydrolysis of phthalylglutamic acid and, if sufficient sodium carbonate is present, finally attains a positive value corresponding to that of an equivalent solution of L-glutamic acid (complete hydrolysis). Dioxan is therefore preferred as a solvent for polarimetry of phthalyl-L-glutamic acid.

Soon after the discovery that phthalyl-L-glutamic anhydride (I) provides a convenient route to γ-glutamyl derivatives experiments were commenced on the incorporation of this feature into a synthesis of glutathione (II), the most important γ-glutamyl peptide. Early experiments (1949) centred on the preparation of *S*-benzyl-L-cysteinylglycine (III) for conversion into glutathione by reaction with phthalylglutamic anhydride and removal of the protecting phthalyl and benzyl groups. *S*-Benzyl-*N*-phthalyl-L-cysteine (IV) was obtained in 33% yield by fusion of *S*-benzyl-L-cysteine and phthalic anhydride at 110—115°, and was shown to be optically pure by removal of the phthalyl group, to afford *S*-benzyl-L-cysteine. Reaction at 135—140° gave a higher yield (70%) of an optically impure product containing 45—55% of the racemic compound, and reaction in acetic acid [5] gave a product of even lower optical purity (85% racemic compound). Balenović and Fleš [6] have since described the preparation of this compound with specific rotation −82·3°, later [7] amended to −151° without comment, although the authors have informed

* Part VIII, preceding paper.

[1] King and Kidd, *J.*, 1949, 3315; *Nature*, 1948, **162**, 776.
[2] D. A. A. Kidd, Thesis, Oxford, 1949.
[3] Clark-Lewis and Fruton, *J. Biol. Chem.*, 1954, **207**, 477.
[4] Sheehan and Bolhofer, *J. Amer. Chem. Soc.*, 1950, **72**, 2470.
[5] Wanag and Veinbergs, *Ber.*, 1942, **75**, 1558.
[6] Balenović and Fleš, *J. Org. Chem.*, 1952, **17**, 347.
[7] *Idem*, *J.*, 1952, 2447.

us that the optically pure material was obtained by fusion of the reactants at the lower temperature. *S*-Benzyl-*N*-phthalylcysteine was converted by acid chloride and mixed anhydride couplings into *S*-benzyl-*N*-phthalylcysteinylglycine which was converted into *S*-benzylcysteinylglycine (III) (accompanied by the corresponding dioxopiperazine) during model experiments conducted with optically impure *S*-benzyl-*N*-phthalylcysteine. These experiments showed that this route to *S*-benzyl-L-cysteinylglycine (III) is inferior to that described by Loring and du Vigneaud [8] and were not therefore repeated with optically pure *S*-benzyl-*N*-phthalyl-L-cysteine (IV). Difficulties encountered in the preparation of the latter, and the poor yields obtained by previous workers in debenzylation of *N*-benzyloxycarbonyl-*S*-benzylglutathione (see preceding paper), led us to abandon the use of *S*-benzyl intermediates in a conventional type of glutathione synthesis.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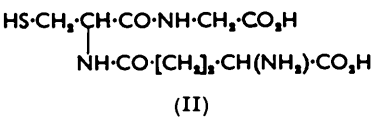
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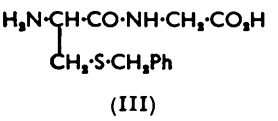
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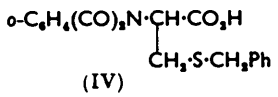
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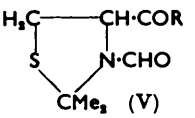
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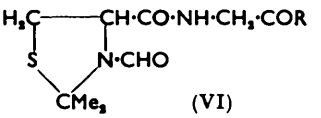
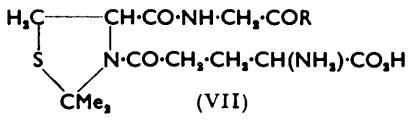

(VI)

The new route to glutathione [9] necessitated coupling L-3-formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid (V; R = OH) with glycine methyl ester, to give L-3-formyl-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine (VI; R = OMe), and this was accomplished by the mixed carbonic anhydride method (with *iso*butyl chloroformate) after attempts to prepare the acid chloride (V; R = Cl) had failed. The alternative phosphorus trichloride coupling procedure introduced by Süs and Hoffmann [10] caused racemisation of L-3-formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid (V; R = OH) and gave the above methyl ester (VI; R = OMe) and the corresponding ethyl ester (VI; R = OEt) in racemic form. These two esters, and also benzoylglycylglycine ethyl ester, were obtained in 40—50% yield, but when aniline was used instead of amino-esters anilides were formed in yields of 90%, *e.g.*, benzoylglycine anilide and phthalylglycine anilide, which have not previously been prepared in this way (see, however, Goldschmidt and Lautenschlager [11]). 3-Formyl-2 : 2-dimethyl-4-phenylcarbamoylthiazolidine (V; R = NHPh) (90%), prepared from the L-acid (V; R = OH) and aniline by the phosphorus trichloride method, had an optical purity of 65% compared with L-anilide obtained by the mixed anhydride procedure. Süs and Hoffmann [10] illustrated their method with optically inactive acylamino-acids and our results demonstrate the need for establishing the optical purity of peptides derived from active amino-acids by this method. Goldschmidt and Jutz [11] claim that racemisation does not occur under the milder conditions which they have employed.

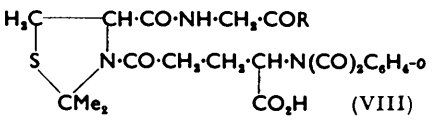
An undesirable feature of the synthesis of glutathione from 2 : 2-dimethylthiazolidines described in the preceding paper is the preferential alkaline hydrolysis of the ester (VII; R = OMe) to the acid (VII; R = OH), and it was thought that this might be avoided by deamidation of the phthalyl-γ-L-glutamylamide (VIII; R = NH_2) with nitrous acid. DL- and L-4-Carbamoylmethylcarbamoyl-3-formyl-2 : 2-dimethylthiazolidine (VI; R = NH_2) were obtained quantitatively by ammonolysis of the DL- and the L-ester

[8] Loring and du Vigneaud, *J. Biol. Chem.*, 1935, **111**, 385.
[9] Preceding paper.
[10] Süs and Hoffmann, *Annalen*, 1951, **572**, 96.
[11] Goldschmidt and Jutz, *Chem. Ber.*, 1953, **86**, 1116; cf. Goldschmidt and Lautenschlager, *Annalen*, 1953, **580**, 68.

(VI; R = OMe), and the L-amide with identical specific rotation was also obtained by mixed anhydride coupling of glycinamide and L-3-formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid (V; R = OH). Deformylation of the amide (VI; $R = NH_2$) with

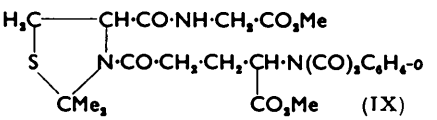


(VII)

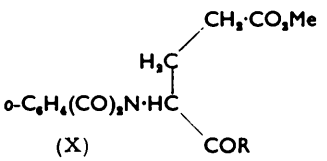


(VIII)

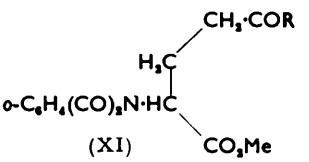
methanolic hydrogen chloride, however, gave ammonium chloride and 4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine hydrochloride instead of the 4-carbamoylmethylcarbamoyl-2 : 2-dimethylthiazolidine required for conversion into (VIII; $R = NH_2$). Hydrolysis of phthalyl-γ-DL-glutamylglycine methyl ester with a mixture of acetone and dilute hydrochloric acid [12] gave the corresponding acid, phthalyl-γ-DL-glutamylglycine, but a similar hydrolysis of L-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethyl-3-phthalyl-γ-L-glutamylthiazolidine (VIII; R = OMe) gave phthalyl-L-glutamic acid.[9] This indication of the lability of 3-acyl derivatives of the thiazolidine nucleus received confirmation by the isolation of α-methyl hydrogen phthalyl-L-glutamate after similar hydrolysis of the analogous dimethyl ester (IX). Acid hydrolysis of glutathione itself gives initially glutamic acid and cysteinylglycine.



(IX)



(X)



(XI)

α-Glutamyl-peptides can be prepared from phthalyl-L-glutamic anhydride (I) by reaction with alcohols and then coupling the γ-esters with amino-acid derivatives by the acid chloride or the mixed anhydride method. Phthalyl-L-glutamic anhydride with methanol and with benzyl alcohol gave γ-methyl hydrogen phthalyl-L-glutamate (X; R = OH) and the corresponding γ-benzyl ester. The latter was converted with diazomethane into γ-benzyl α-methyl phthalyl-L-glutamate (XI; $R = O{\cdot}CH_2Ph$) which gave α-methyl hydrogen phthalyl-L-glutamate (XI; R = OH) by hydrogenolysis. The isomeric monomethyl esters (X and XI; R = OH) were coupled with aniline and with amino-esters by the mixed anhydride method and by the acid chloride method. The acid chlorides (X and XI; R = Cl) were obtained from the acids (X and XI; R = OH) by the action of thionyl chloride in the cold as it was found that heating during the preparation of the acid chlorides led to the formation of racemic products in subsequent condensations. Methyl hydrogen phthalyl-L-glutamates (X and XI; R = OH) with unchanged specific rotations were regenerated by hydrolysis of the acid chlorides which had been prepared in the cold. The acid chloride from γ-methyl hydrogen phthalyl-L-glutamate was treated with glycine methyl ester, ethyl *p*-aminobenzoate, and aniline to yield respectively phthalyl-α-L-glutamylglycine dimethyl ester (X; $R = NH{\cdot}CH_2{\cdot}CO_2Me$), the α-*p*-ethoxycarbonylanilide (X; $R = NH{\cdot}C_6H_4{\cdot}CO_2Et$-*p*), and the α-anilide (X; R = NHPh), and the last was also prepared by the mixed anhydride method. The isomeric γ-anilides (XI; R = NHPh, and XI; $R = NH{\cdot}C_6H_4{\cdot}CO_2Et$-*p*) were prepared similarly from α-methyl hydrogen phthalyl-L-glutamate (XI; R = OH), which was also converted by the mixed anhydride

[12] Sheehan, Chapman, and Roth, *J. Amer. Chem. Soc.*, 1952, **74**, 3822.

method into the dimethyl ester (IX) of L-2 : 2-dimethyl-3-phthalyl-γ-L-glutamylthiazolidine-4-carboxyglycine by reaction with L-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine. Hydrolysis of the diester (IX) gave α-methyl hydrogen phthalyl-L-glutamate (XI; R = OH) as already mentioned. α-Benzyl hydrogen phthalyl-L-glutamate should prove particularly useful for the preparation of α-glutamyl-peptides as the ester group is removable by hydrogenolysis at room temperature.

Experimental

Phthalyl-L-glutamic Acid and Anhydride (I).—The anhydride was prepared by the method reported previously [1] except that the time of hydrolysis was reduced to 75 minutes,[2,3] and also by modification of Sheehan and Bolhofer's method [4] of fusing equimolecular quantites of phthalic anhydride and L-glutamic acid. By carefully controlling the oil-bath temperature between 135° and 145° and by restricting to 3 min. the time of treatment of the product with acetic anhydride at 100°, phthalyl-L-glutamic anhydride (50—55%) was obtained with $[\alpha]_D^{20}$ −42·1° (2% in dioxan). Specimens of low optical activity were purified by hydrolysis and recrystallisations of the acid from ethyl acetate–light petroleum. The more soluble fractions were discarded and the crystalline acid was converted with acetic anhydride at 100° in 3 min. into phthalyl-L-glutamic anhydride, $[\alpha]_D^{20}$ −43·5° (3% in dioxan).

Hydrolysis [1] of the anhydride gave phthalyl-L-glutamic acid, $[\alpha]_D^{25}$ −48·3° (3% in dioxan).[3] This acid had $[\alpha]_D^{20}$ −23·7° (0·491 g. in 15 c.c. of 0·333N-Na_2CO_3) (previously reported : [1] $[\alpha]_D^{18}$ −27·4°, from measurements at the same concentration) which fell to an equilibrium value of $[\alpha]_D^{19}$ −17·7°. Similarly, another sample showed initially $[\alpha]_D^{24}$ −18·8° (0·545 g. in 25 c.c. of 0·602N-Na_2CO_3), $[\alpha]_D^{24}$ +7·5° after 20 hr., and reached a steady value of +7·75° ± 0·1° after 36 hr. owing to complete hydrolysis. The value $[\alpha]_D^{24}$ +7·75° ± 0·1° (calc. as phthalylglutamic acid) corresponds to $[\alpha]_D^{24}$ +14·6° ± 0·2° (calc. as glutamic acid), and an equivalent solution prepared by dissolving phthalic acid (0·1939 g.) and L-glutamic acid (0·1775 g.) in 15 c.c. of 0·602N-sodium carbonate had $[\alpha]_D^{23\cdot5}$ +14·7°.

S-*Benzyl*-N-*phthalyl*-L-*cysteine* (IV).—An intimate mixture of finely powdered *S*-benzyl-L-cysteine [13] (2·35 g.), $[\alpha]_D^{20}$ +24·1° (1·8% in N-NaOH),[14] and phthalic anhydride (1·7 g.) was heated for 30 min. at 110—115° (oil-bath temp.). The solid was digested with warm benzene (40 c.c.), and the filtrate from insoluble material (0·5 g.) was concentrated to 10 c.c. The solution was stored at 0° for three days and then filtered from *S*-benzyl-*N*-phthalyl-L-cysteine (0·9 g.), and the filtrate when stored at 0° deposited a further crop (0·4 g., total 1·3 g., 32%), prisms, m. p. 128—129°, $[\alpha]_D^{20}$ −150·1° (1·1% in MeOH) (lit., [6,7] m. p. 128—129·5°, $[\alpha]_D$ −151°). The *S*-benzylthiuronium salt melted at 153—154°. A solution of *S*-benzyl-*N*-phthalyl-L-cysteine (1·7 g.) and 10M-hydrazine hydrate (1·5 c.c.) in ethanol (50 c.c.) was boiled for 2 hr. before evaporation to dryness under reduced pressure. A suspension of the residue in 2N-hydrochloric acid (25 c.c.) was warmed to 50° and then kept at 0° for 4 hr. before filtration from phthalhydrazide, and the filtrate was concentrated to 10 c.c., and adjusted to pH 5—6 by addition of aqueous sodium acetate. *S*-Benzyl-L-cysteine separated from the solution in plates, m. p. 215° (decomp.), $[\alpha]_D^{20}$ +23·9° (1·7% in N-NaOH). Wood and du Vigneaud [14] record $[\alpha]_D^{20}$ +23·5° (1% in N-NaOH).

When the preparation of *S*-benzyl-*N*-phthalylcysteine was repeated with oil-bath temperature 135—140° there resulted a higher yield (2·8 g., 70%) of partly racemised material, m. p. 128—129°, $[\alpha]_D^{20}$ between −67° and −85° (different samples). A mixture of *S*-benzyl-L-cysteine (15·7 g.), phthalic anhydride (11·1 g.), and glacial acetic acid [5] (150 c.c.) was boiled until a test portion failed to react with ninhydrin (9 hr.). Water (500 c.c.) was added to the solution; the precipitated oil solidified when triturated and was then collected, washed with water, and dried in a desiccator ($CaCl_2$). Recrystallisation from ethyl acetate–light petroleum gave *S*-benzyl-*N*-phthalylcysteine (17·1 g., 68%), m. p. 128—129°, $[\alpha]_D^{20}$ −29·5° (2% in dioxan), $[\alpha]_D^{20}$ −22·1° (2% in MeOH). A small yield (1·1 g., 30%) of partly racemised *S*-benzyl-*N*-phthalylcysteine, m. p. 128—129°, $[\alpha]_D^{20}$ −28° (0·8% in MeOH), was also obtained after *S*-benzyl-L-cysteine (2·1 g.) and phthalic anhydride (1·5 g.) had been heated in toluene (40 c.c.) and dimethylformamide (10 c.c.) at the b. p. for 15 min.

[13] du Vigneaud, Audrieth, and Loring, *J. Amer. Chem. Soc.*, 1930, **52**, 4500.
[14] Wood and du Vigneaud, *J. Biol. Chem.*, 1939, **130**, 109.

S-*Benzyl*-N-*phthalylcysteinylglycine*.—Partly racemised *S*-benzyl-*N*-phthalylcysteine (10 g.), $[\alpha]_D$ −35° (optical purity *ca.* 25%), was converted by the acid chloride method into S-*benzyl*-N-*phthalylcysteinylglycine* (6·9 g., 60%), needles, m. p. 160—164° raised by recrystallisation from aqueous ethanol to m. p. 164—165°, which was also prepared (1·4 g., 70%) by the mixed anhydride method from starting material (1·7 g.) of the same specific rotation (Found: C, 60·0; H, 4·5; N, 6·6. $C_{20}H_{18}O_5N_2S$ requires C, 60·3; H, 4·6; N, 7·0%). A solution of *S*-benzyl-*N*-phthalylcysteinylglycine (10 g.), potassium carbonate (1·75 g.), 32·7% aqueous hydrazine (2·46 c.c.), and water (60 c.c.) was kept at room temperature for 3 days and then acidified with 12N-hydrochloric acid before filtration from phthalhydrazide (3·2 g., 80%). The filtrate was passed through a column of Amberlite IR-4B resin, and evaporation of eluate fractions gave *S*-benzylcysteinylglycine (2 g., 30%), m. p. 200° (decomp.) (Found: C, 53·5; H, 6·0; N, 10·2. Calc. for $C_{12}H_{16}O_3N_2S$: C, 53·7; H, 6·0; N, 10·4%). *S*-Benzyl-L-cysteinylglycine [8, 15] has been reported with m. p. 166—167°, and with m. p. 163·5—164·5°, $[\alpha]_D^{18}$ +48·6° (in 0·5N-HCl). Some eluate deposited sparingly soluble 3-benzylthiomethyl-2 : 5-dioxopiperazine, m. p. 190°, which was also deposited in the resin (Found: C, 57·1; H, 5·2. Calc. for $C_{13}H_{14}O_2N_2S$: C, 57·5; H, 5·6%) (recorded for the L-isomer,[10, 16] m. p. 190° and 198°).

Benzoylglycine Anilide, Phthalylglycine Anilide, and Ethyl Benzoylglycylglycinate by the Süs Coupling Procedure.—(a) *Benzoylglycine anilide*. A suspension of benzoylglycine (3·6 g.) in a mixture of dry benzene (100 c.c.), aniline (2·8 c.c.), and phosphorus trichloride (1·5 c.c.) was boiled for 2 hr. The clear yellow solution was evaporated to dryness under reduced pressure and the residue was digested with aqueous sodium carbonate (15%) before collection of the crystalline solid, which on being washed with water and crystallised from ethanol gave benzoylglycine anilide (5 g., 95%) in prisms, m. p. 208—209° (lit.,[17] m. p. 208·5°).

(b) *Phthalylglycine anilide*. Phthalylglycine (2·05 g.) was boiled for 4 hr. with a mixture of aniline (1·5 c.c.), phosphorus trichloride (0·8 c.c.), and dry benzene (50 c.c.), and the solvent was evaporated under reduced pressure. The residue was digested with saturated aqueous sodium hydrogen carbonate, and the insoluble solid was collected by filtration and washed with water. The phthalylglycine anilide [18] crystallised from acetic acid in needles (2·5 g., 90%), m. p. 213—232°. The anilide (75%) was also prepared by the mixed anhydride method (with *iso*butyl chloroformate).

(c) *Benzoylglycylglycine ethyl ester*. Freshly distilled glycine ethyl ester (3 g.), benzoylglycine (4·5 g.), phosphorus trichloride (2·5 c.c.), and dry benzene (100 c.c.) were shaken in a closed flask for 3 min. (the temperature of the mixture rose to 40°) and then boiled for 2 hr., whereupon the solution became brown and a tarry deposit was formed. The solvent was evaporated under reduced pressure and the dark brown residue was digested with 15% aqueous sodium carbonate before filtration, and the residue was washed with water. Crystallisation of the solid from water (charcoal) gave yellow needles, m. p. 108—112°, and recrystallisation (charcoal) gave benzoylglycylglycine ethyl ester (3 g., 50%) in needles, m. p. 119—120° (lit.,[10] m. p. 119—120°).

L-3-*Formyl*-2 : 2-*dimethyl*-4-*phenylcarbamoylthiazolidine* (V; R = NHPh).—(a) *Mixed anhydride method*. A solution of L-3-formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid [9] (0·95 g.) and triethylamine (0·7 c.c.) in pure dry dioxan (20 c.c.) was stirred and cooled to 10° (dioxan commenced to solidify) while *iso*butyl chloroformate (0·63 c.c.) was added. The suspension of triethylamine hydrochloride in the mixed anhydride solution was stirred at 10° for 10 min. and then during the addition of aniline (0·6 c.c.) (slow evolution of carbon dioxide). Next day the filtrate from triethylamine hydrochloride was evaporated to dryness. The gummy residue of anilide solidified when triturated, and crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave L-3-*formyl*-2 : 2-*dimethyl*-4-*phenylcarbamoylthiazolidine* (1 g., 75%) in leaflets, m. p. 189—190°, $[\alpha]_D^{20}$ −179° (0·07% in EtOH), $[\alpha]_D^{20}$ −280° (2% in $CHCl_3$) (Found: C, 59·2; H, 6·1; N, 10·3. $C_{13}H_{16}O_2N_2S$ requires C, 59·1; H, 6·1; N, 10·6%).

(b) *Sus coupling method*.[10] Heat was evolved when L-3-formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid (1·5 g.) was added to a solution of aniline (1·1 c.c.) and phosphorus trichloride (0·9 c.c.) in dry benzene (50 c.c.). The mixture was boiled until evolution of hydrogen chloride ceased (3 hr.) and the residue remaining after removal of solvent by evaporation under reduced

[15] Kögl and Akkermann, *Rec. Trav. chim.*, 1946, **65**, 216.
[16] King and Suydam, *J. Amer. Chem. Soc.*, 1952, **74**, 5499.
[17] Curtius, *J. prakt. Chem.*, 1895, **52**, 257.
[18] Scheiber, *Ber.*, 1913, **46**, 1100.

pressure was treated with 15% aqueous sodium carbonate. The brown solid collected by filtration was dissolved in the minimum volume of hot ethanol and, after cautious dilution of the solution with water, the partly racemised anilide (1·9 g., 90%) crystallised in prisms, m. p. 187—188°, $[\alpha]_D^{20}$ −115·5° (1·1% in EtOH) (Found: C, 59·0; H, 6·2; N, 10·4%). A solution of the anilide (0·5 g.), $[\alpha]_D^{20}$ −115·5°, in dry methanol (25 c.c.) containing hydrogen chloride (2%) was warmed on a steam-bath for 5 min. and then evaporated to dryness under reduced pressure. The residue was dissolved in a little methanol, and the filtrate was diluted with dry ether; 2 : 2-*dimethyl-4-phenylcarbamoylthiazolidine hydrochloride* (*ca.* 100%) crystallised in needles, m. p. 120° (decomp.) (Found: N, 10·1. $C_{12}H_{16}ON_2S$,HCl requires N, 10·3%). The optical purity of the product, based on that of the formyl derivative, was *ca.* 65%.

DL-*4-Ethoxycarbonylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* (VI; R = OEt).—Phosphorus trichloride (2·5 c.c.) was added to a solution of freshly distilled glycine ethyl ester (3·9 g.) in dry benzene (30 c.c.) and dioxan (30 c.c.), and the mixture was shaken for 5 min. in a stoppered flask (exothermic reaction and separation of gelatinous solid). L-3-Formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid (5·5 g.) was added and the mixture was boiled gently until evolution of hydrogen chloride ceased (2 hr.; the solution became dark brown). The residue obtained by evaporation of the solvent under reduced pressure was triturated with 15% aqueous sodium carbonate, and the oil which separated was extracted into ethyl acetate. Evaporation of the ethyl acetate solution left a brown oil which crystallised when triturated, and crystallisation of the solid from ethyl acetate (charcoal) gave DL-*4-ethoxycarbonylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* (3·3 g., 40%) in prisms, m. p. 94—95° (Found: C, 48·5; H, 6·5; N, 9·8. $C_{11}H_{18}O_4N_2S$ requires C, 48·2; H, 6·6; N, 10·2%). This material was optically inactive in ethanol and in chloroform.

3-Formyl-DL-*4-methoxycarbonylmethylcarbamoyl-2* : 2-*dimethylthiazolidine* (VI; R = OMe).—The methyl ester was prepared by the Süs method as described above for the ethyl ester, except that glycine methyl ester (5·5 g.) was used. Recrystallisation of the product from ethyl acetate (charcoal) afforded 3-*formyl*-DL-*4-methoxycarbonylmethylcarbamoyl-2* : 2-*dimethylthiazolidine* (4·3 g., 55%), m. p. 107—108° (Found: N, 10·5. $C_{10}H_{16}O_4N_2S$ requires N, 10·8%). Solutions of the compound in methanol and in chloroform showed no observable rotation. The L-isomer [9] has $[\alpha]_D^{20}$ −161·2° (1·5% in $CHCl_3$).

DL-*4-Methoxycarbonylmethylcarbamoyl-2* : 2-*dimethylthiazolidine Hydrochloride*.—The foregoing methyl ester (0·5 g.) was deformylated with methanolic hydrogen chloride as described above for the anilide. Crystallisation of the product from a methanol solution diluted with several volumes of dry ether gave DL-*4-methoxycarbonylmethylcarbamoyl-2* : 2-*dimethylthiazolidine hydrochloride* (0·45 g., 90%) in deliquescent needles, m. p. 128° (decomp.) (Found: C, 40·4; H, 6·4. $C_9H_{16}O_3N_2S$,HCl requires C, 40·2; H, 6·3%).

DL-*4-Carbamoylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* (VI; R = NH_2).—A solution of DL-3-formyl-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine (0·5 g.) in methanol (3 c.c.) was diluted with methanol (25 c.c.) previously saturated at 0° with dry ammonia, and stored in a closed container for three days. DL-*4-Carbamoylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* (*ca.* 100%) crystallised from the concentrated solution in prisms, m. p. 164—165° (Found: C, 44·5; H, 6·2; N, 16·8. $C_9H_{15}O_3N_3S$ requires C, 44·1; H, 6·1; N, 17·1%).

L-*4-Carbamoylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* (VI; R = NH_2).—(a) L-3-Formyl-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine was converted into the amide (*ca.* 100%) by ammonolysis as described above for the DL-compound. L-*4-Carbamoylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* crystallised from methanol in prisms, m. p. 188—189°, $[\alpha]_D^{20}$ −95·6° (1·4% in MeOH) (Found: C, 44·5; H, 6·2; N, 16·8%).

(b) L-3-Formyl-2 : 2-dimethylthiazolidine-4-carboxylic acid (1·9 g.) in dry dioxan (30 c.c.) at 10° was converted into the mixed anhydride with triethylamine and *iso*butyl chloroformate. The mixed anhydride solution was stirred at 10° for 10 min. and then during the addition of glycine amide (0·74 g.) in dimethylformamide (10 c.c.). Next day the solution was filtered from triethylamine hydrochloride, and evaporation of the filtrate under reduced pressure left a solid residue of the amide which crystallised from methanol in prisms (2·2 g., 90%), m. p. and mixed m. p. 188—189°, $[\alpha]_D^{20}$ −95·7° (2% in $CHCl_3$).

Deformylation of L-*4-Carbamoylmethylcarbamoyl-3-formyl-2* : 2-*dimethylthiazolidine* (VI; R = NH_2).—The above amide (1 g.) was heated for 5 min. on a steam-bath with methanol (80 c.c.) containing hydrogen chloride (2%), and the solution was then evaporated to dryness

under reduced pressure. The residue was dissolved in a little dry methanol, and dilution of the solution with an equal volume of dry ether precipitated ammonium chloride (0·14 g., 66%). The filtrate was further diluted with dry ether, which caused the separation of white, hygroscopic needles (0·73 g.), m. p. 120° (decomp.), which were identified as L-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine hydrochloride by conversion with sodium formate (0·75 g.) and 90% formic acid (10 c.c.) in acetic anhydride (4·5 c.c.) into the 3-formyl derivative, prisms, m. p. 104—107° raised by recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) to m. p. 108—109° alone and when mixed with authentic L-3-formyl-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine.[9]

γ-Methyl Hydrogen Phthalyl-L-glutamate (X; R = OH) *and its Acid Chloride* (X; R = Cl).—Phthalyl-L-glutamic anhydride (15 g.) was boiled with methanol (200 c.c.) for 15 min. after the solid had dissolved, and the solution was then evaporated to dryness under reduced pressure. Crystallisation of the solid residue from benzene–light petroleum (b. p. 60—80°) gave *γ-methyl hydrogen phthalyl-L-glutamate* (13·5 g., 80%) in prisms, m. p. 135—136° raised by several crystallisations from ethyl acetate–light petroleum to m. p. 136—137°, $[\alpha]_D^{20}$ −40·5° (27% in $CHCl_3$) (Found: C, 57·9; H, 4·4; N, 4·7. $C_{14}H_{13}O_6N$ requires C, 57·7; H, 4·5; N, 4·8%).

γ-Methyl hydrogen phthalyl-L-glutamate was converted into the acid chloride by allowing the acid to stand with thionyl chloride (3 c.c. per g.) at room temperature for 14 hr. Thionyl chloride was removed by distillation under reduced pressure, and the last traces were removed by two similar distillations after the addition of dry benzene (bath-temp. 30° throughout). The acid chloride, which did not crystallise, was hydrolysed when stirred with water for 2 hr., and the crystalline acid was collected and dried at 100° for 15 min. Crystallisation of the solid from benzene–light petroleum gave γ-methyl hydrogen phthalyl-L-glutamate, $[\alpha]_D^{20}$ −40·5° (2·7% in $CHCl_3$), m. p. and mixed m. p. 136—137°. γ-Methyl hydrogen phthalyl-L-glutamate was racemised when heated for 1 hr. with thionyl chloride as the acid chloride so formed gave racemic products.

γ-Methyl Phthalyl-L-glutamate α-Anilide (X; R = NHPh).—(a) *Acid chloride method.* The acid chloride prepared as described above from γ-methyl hydrogen phthalyl-L-glutamate (2 g.) was dissolved in dry benzene (15 c.c.), the solution was added dropwise to a stirred solution of aniline (2 c.c.) in benzene (10 c.c.), and stirring was continued for 6 hr. The solid was collected and washed with water, aqueous sodium hydrogen carbonate, and water, and then drained at the pump. Crystallisation of the residue (2·2 g.) from aqueous methanol gave *γ-methyl phthalyl-L-glutamate α-anilide* in rods, m. p. 138—139°, $[\alpha]_D^{20}$ −1·0° (2% in $CHCl_3$) (Found: C, 65·4; H, 5·1; N, 8·0. $C_{20}H_{18}O_5N_2$ requires C, 65·6; H, 4·9; N, 7·7%). γ-Methyl hydrogen phthalyl-L-glutamate (2 g.) was boiled with thionyl chloride (10 c.c.) for 1 hr. before evaporation to dryness under reduced pressure and coupling with aniline as described above. An optically inactive anilide was obtained which crystallised from benzene–light petroleum (b. p. 60—80°) in rods, m. p. 136—140° unchanged by repeated crystallisation from this and other solvent mixtures.

(b) *Mixed anhydride method.* A solution of γ-methyl hydrogen phthalyl-L-glutamate (1 g.) and triethylamine (0·48 c.c.) in dry chloroform (15 c.c.) was cooled to 5° before addition of *iso*butyl chloroformate (0·43 c.c.). The solution was stirred at 5° for 10 min. before the addition of aniline (0·6 c.c.), and the solution was allowed to warm to room temperature (evolution of carbon dioxide). Next day the solution was washed with water, aqueous sodium hydrogen carbonate, and water before evaporation to dryness. The residual gum was dissolved in the minimum volume of hot methanol, and the solution was diluted with water until faintly turbid. The α-anilide, $[\alpha]_D^{20}$ −1·2° (2% in $CHCl_3$), crystallised in rods, m. p. 138—139° alone and when mixed with that prepared by method (a).

Dimethyl Phthalyl-α-L-glutamylglycinate (X; R = $NH{\cdot}CH_2{\cdot}CO_2Me$).—A solution of the acid chloride from γ-methyl hydrogen phthalyl-L-glutamate (2 g.) in dry benzene (15 c.c.) was added dropwise to a stirred solution of glycine methyl ester hydrochloride (0·9 g.) and triethylamine (2·1 c.c., 2·2 equiv.) in dry chloroform (10 c.c.). Next day the solvent was distilled under reduced pressure and the residue was washed with water, aqueous sodium hydrogen carbonate, and water. *Phthalyl-α-L-glutamylglycine dimethyl ester* (2·2 g., 85%) crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in needles, m. p. 80—82° raised by recrystallisation to m. p. 82—83°, $[\alpha]_D^{21}$ −3·1° (1·4% in $CHCl_3$) (Found: C, 55·7; H, 5·3; H, 7·6. $C_{17}H_{18}O_7N_2$ requires C, 56·3; H, 5·0; N, 7·7%). A similar reaction with L-4-methoxycarbonylmethylcarbamoyl-2 : 2-dimethylthiazolidine hydrochloride gave a non-crystalline neutral product which was not further investigated.

Ethyl p-(*γ-Methyl Phthalyl-α-L-glutamyl*)*aminobenzoate* (X; R = NH·C_6H_4·CO_2Et-*p*).—A solution of the acid chloride from γ-methyl hydrogen phthalyl-L-glutamate (2 g.) in dry benzene (20 c.c.) was added to a stirred solution of ethyl *p*-aminobenzoate (1·8 g.) in chloroform (20 c.c.). Next day the filtrate from ethyl *p*-aminobenzoate hydrochloride was evaporated to dryness, and crystallisation of the residue from ethyl acetate–light petroleum (b. p. 60—80°) gave *ethyl* p-(*γ-methyl phthalyl-α-L-glutamyl*)*aminobenzoate* (1·8 g., 65%) in prisms, m. p. 124—126° raised by recrystallisation to m. p. 126—127°, $[\alpha]_D^{20}$ −1·5° (2% in $CHCl_3$) (Found: C, 62·8; H, 4·6; N, 6·8. $C_{23}H_{22}O_7N_2$ requires C, 63·0; H, 5·0; N, 6·4%).

γ-Benzyl Hydrogen Phthalyl-L-glutamate.—Phthalyl-L-glutamic anhydride (15 g.) dissolved when heated with freshly distilled benzyl alcohol (22 c.c.) on a steam-bath for 2 hr., and the solution was then extracted with aqueous sodium hydrogen carbonate. The aqueous extract was washed with ether before acidification with 2N-hydrochloric acid and extraction with ether, and evaporation of the solvent from the ether extract left a residue of *γ-benzyl hydrogen phthalyl-L-glutamate* which crystallised slowly from benzene–light petroleum (b. p. 60—80°) in waxy needles (16·4 g.) containing benzene of crystallisation, m. p. 66—67°, $[\alpha]_D^{20}$ −30·4° (3% in $CHCl_3$) (Found: C, 69·8; H, 5·0; N, 3·11. $C_{20}H_{17}O_6N,C_6H_6$ requires C, 70·1; H, 5·2; N, 3·15. Found, after drying in a vacuum-desiccator over hard paraffin and sulphuric acid for 48 hr.: C, 65·4; H, 4·7; N, 3·4. $C_{20}H_{17}O_6N$ requires C, 65·4; H, 4·7; N, 3·8%).

α-Methyl Hydrogen Phthalyl-L-glutamate (XI; R = OH) *and its Acid Chloride* (XI; R = Cl).—Ethereal diazomethane was added to γ-benzyl hydrogen phthalyl-L-glutamate (10 g.) until the solution remained faintly yellow and the excess of diazomethane was decomposed by addition of a few drops of acetic acid before evaporation of the solution to dryness. The oily residue of α-methyl γ-benzyl phthalyl-L-glutamate was hydrogenolysed in ethyl acetate at room temperature and pressure over palladised charcoal (5% of Pd; prepared from the oxide). When absorption of hydrogen ceased (*ca.* 50% of the theoretical volume) the solution was filtered from catalyst and the filtrate, after extraction with aqueous sodium hydrogen carbonate, was again hydrogenolysed and extracted with aqueous sodium hydrogen carbonate. The combined aqueous extracts were acidified with 2N-hydrochloric acid, and the precipitated methyl ester was collected and drained at the pump. *α-Methyl hydrogen phthalyl-L-glutamate* crystallised from ethyl acetate–light petroleum in rods (5·2 g., 65%), m. p. 136—137°, $[\alpha]_D^{20}$ −39·8° (2·8% in dioxan) (Found: C, 58·0; H, 4·4; N, 5·0. $C_{14}H_{13}O_6N$ requires C, 57·7; H, 4·5; N, 4·8%), and from ethyl acetate in needles, m. p. 138°, $[\alpha]_D^{26}$ −55·9° (3·2% in EtOAc) (Found: C, 57·7; H, 4·5; N, 4·8%). Mixed anhydride couplings of α-methyl hydrogen phthalyl-L-glutamate with glycine ethyl ester and with L-cysteine (sodium salt) gave products which did not crystallise.

The α-methyl ester γ-acid chloride (XI; R = Cl) was prepared with cold thionyl chloride as described above for the isomeric γ-methyl ester α-acid chloride, and was used in benzene solution for coupling reactions. α-Methyl hydrogen phthalyl-L-glutamate of the same m. p. and specific rotation was obtained after its conversion into the acid chloride and hydrolysis of the latter by stirring with water for 2 hr.

α-Methyl Phthalyl-L-glutamate γ-Anilide (XI; R = NHPh).—(a) *Acid chloride method.* α-Methyl hydrogen phthalyl-L-glutamate (2 g.) was converted into the anilide as already described for the γ-methyl ester α-anilide [method (a)]. *α-Methyl phthalyl-L-glutamate γ-anilide*, $[\alpha]_D^{20}$ −29·5° (0·6% in MeOH), crystallised from benzene in prisms (2·3 g., 90%), m. p. 151—152° raised to m. p. 152—153° by recrystallisation from ethyl acetate–light petroleum (Found: C, 65·8; H, 4·9; N, 7·7. $C_{20}H_{18}O_5N_2$ requires C, 65·6; H, 4·9; N, 7·65%). A solution of α-methyl hydrogen phthalyl-L-glutamate (1 g.) in thionyl chloride (5 c.c.) was boiled until evolution of hydrogen chloride ceased (40 min.) and the acid chloride was isolated by removal of the thionyl chloride under reduced pressure. The residue was dissolved in dry benzene (10 c.c.), and the solution was added dropwise to a stirred solution of aniline (1 c.c.) in benzene (10 c.c.). Stirring was continued for 4 hr. before collection of the solid (0·85 g.), which was washed with water, aqueous sodium hydrogen carbonate, and water. Crystallisation of the residue from benzene (thrice) gave leaflets, m. p. 70—74°, which after two crystallisations from aqueous ethanol gave *α-methyl phthalyl-DL-glutamate γ-anilide* in prisms, m. p. 110° (Found: C, 65·6; H, 4·7; N, 7·9%). Evaporation of the filtrate of the original reaction mixture gave a neutral residue (0·15 g.) which, after several crystallisations from ethyl acetate–light petroleum (b. p. 60—80°), gave α-methyl phthalyl-L-glutamate γ-anilide in prisms, m. p. and mixed m. p. 152—153°.

(b) *Mixed anhydride method.* The α-methyl ester γ-anilide was also obtained by the mixed anhydride method as described for the γ-methyl ester α-anilide [method (b)]. The product had the same specific rotation and m. p. as that prepared by method (a) and there was no depression in the mixed m. p.

*Preparation and Acid Hydrolysis of Phthalyl-γ-*DL*-glutamylglycine Methyl Ester.*—Phthalyl-DL-glutamic anhydride [1] (5·2 g.) was added to a stirred solution of glycine methyl ester hydrochloride (2·5 g.) and triethylamine (2·8 c.c.) in dry chloroform (50 c.c.). The exothermic reaction raised the temperature of the mixture to 45°. Next day the solvent was removed, the residue was extracted with aqueous sodium hydrogen carbonate, and the solution was filtered before acidification with 2N-hydrochloric acid, which precipitated a gum that crystallised when kept. Recrystallisation of the product from water gave *α-hydrogen phthalyl-γ-*DL*-glutamylglycine methyl ester* (5·3 g., 75%) in aggregates of leaf-like plates, m. p. 155—156° raised by recrystallisation to m. p. 157—158° (Found: C, 54·9; H, 4·4; N, 8·1. $C_{16}H_{16}O_7N_2$ requires C, 55·2; H, 4·6; N, 8·1%).

A solution of the ester (1 g.) in acetone (12 c.c.) and 4N-hydrochloric acid (12 c.c.) was boiled for 30 min. and then concentrated to 10 c.c. Next day the crystalline solid (0·9 g.) was collected and washed with a little water, and was identified as phthalyl-γ-DL-glutamylglycine [1] which crystallised from water in prisms, m. p. and mixed m. p. 206—207°.

L-2 : 2-*Dimethyl*-3-*phthalylglycylthiazolidine*-4-*carboxylic Acid.*—A solution of phthalylglycyl chloride (2·25 g.) in pure dry chloroform (15 c.c.) was added dropwise to a stirred solution of L-2 : 2-dimethylthiazolidine-4-carboxylic acid hydrochloride [9] (2·0 g.) and triethylamine (1·4 c.c.) in dry chloroform (50 c.c.). Next day the solution was evaporated and the clear, glassy residue was digested with saturated aqueous sodium hydrogen carbonate. Acidification of the filtered aqueous extract with 4N-hydrochloric acid precipitated L-2 : 2-*dimethyl*-3-*phthalylglycylthiazolidine*-4-*carboxylic acid* which crystallised from aqueous ethanol in leaflets (3·3 g., 95%), m. p. 202—203°, $[\alpha]_D^{20}$ −38·5° (2% in EtOH) (Found: C, 54·9; H, 4·2; N, 7·6. $C_{16}H_{16}O_5N_2S$ requires C, 55·2; H, 4·6; N, 8·0%).

Preparation and Acid Hydrolysis of L-2 : 2-*Dimethyl*-3-(*phthalyl-γ-*L*-glutamyl*)*thiazolidine*-4-*carboxyglycine Dimethyl Ester* (IX).—α-Methyl hydrogen phthalyl-L-glutamate (1 g.) in chloroform (15 c.c.) at 5° was converted into the mixed anhydride with triethylamine (0·48 c.c.) and *iso*butyl chloroformate (0·43 c.c.). A solution of L-2 : 2-dimethyl-4-methoxycarbonylmethylcarbamoylthiazolidine hydrochloride (0·9 g.) and triethylamine (0·48 c.c.) in chloroform (10 c.c.) was added to the mixed anhydride solution (carbon dioxide was evolved) and next day the solution was washed with water, aqueous sodium hydrogen carbonate, and water. Evaporation of the organic solution left a neutral residue of L-2 : 2-dimethyl-3-(phthalyl-γ-L-glutamyl)-thiazolidine-4-carboxyglycine dimethyl ester (IX), which was not obtained crystalline. The neutral ester (0·6 g.) was boiled in acetone (12 c.c.) and 4N-hydrochloric acid (12 c.c.) for 30 min. before evaporation to small bulk (5 c.c.) under reduced pressure, which caused separation of an oily acid that solidified (0·3 g.) when triturated. Recrystallisation of the solid from water and then from ethyl acetate–light petroleum (b. p. 60—80°) gave α-methyl hydrogen phthalyl-L-glutamate in rods, m. p. 135—136° not depressed with the authentic material of m. p. 136—137° (Found: C, 57·7; H, 4·1; N, 5·0%).

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