56. *Syntheses from Phthalimido-acids. Part II. Further Reactions of Phthalylglutamic Anhydride.*

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The action of methanol and of benzyl alcohol on phthalyl-DL-glutamic anhydride gives monoesters, shown to be γ-derivatives by hydrolysis of the phthalyl group with hydrazine to give compounds of recognisable constitution. This parallels the formation of γ-amides from phthalyl-DL- and -L-glutamic anhydrides with ammonia, etc., which has resulted in an improved synthesis of glutamine (King and Kidd, *Nature*, 1948, **162**, 776; *J.*, 1949, 3315).

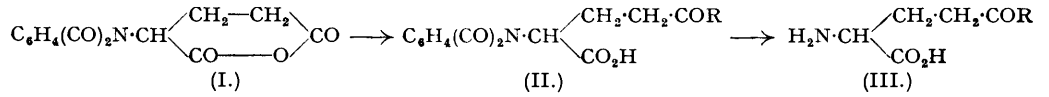
Hydrazine reacts preferentially with the anhydride ring of phthalylglutamic anhydride, as is evident in the production of a hydrazide which is converted *via* the azide into the known phthalyl-γ-DL-glutamylanilide.

IN a recent communication (King and Kidd, *J.*, 1949, 3315) which is to be regarded as Part I of this series, an account was given of the preparation and some reactions of phthalyl-DL- and

-L-glutamic anhydrides already cursorily reported (*Nature*, 1948, **162**, 776). Remarkable among the properties of these anhydrides is their conversion, by ammonia and by certain amines and amino-acids, into γ-amides, thus providing—in contrast to the usual method employing carbobenzyloxyglutamic anhydride (Bergmann and Zervas, *Ber.*, 1933, **66**, 1288)—a direct synthesis of glutamine.

The reactions of phthalyl-DL-glutamic anhydride have since been further investigated, and the results indicate that cleavage of the anhydride ring to form γ-derivatives is a general property. For example, the crystalline product obtained by heating a solution of the DL-anhydride in methanol is γ-methyl hydrogen phthalyl-DL-glutamate (II; R = OMe), and it can be hydrolysed with aqueous hydrazine under the mild conditions described in Part I to γ-methyl hydrogen DL-glutamate (III; R = OMe). The constitution of this acid ester, and hence of its precursor, rests on its identity with that prepared by partial esterification of DL-glutamic acid following the conditions first determined by Abderhalden and Nienberg (*Z. physiol. Chem.*, 1933, **219**, 155) for the ethyl ester of the L-isomer. Though wrongly oriented at the time, the product thus prepared is the γ-ester (see Bergmann and Zervas, *ibid.*, 1933, **221**, 51; Nienberg, *Ber.*, 1935, **68**, 2232), a fact which is further demonstrated by the recent application of the method to the synthesis of what is undoubtedly γ-ethyl hydrogen DL-glutamate (King and Spensley, *J.*, 1950, 3159).

Similarly, the anhydride gave a high yield of γ-benzyl hydrogen phthalyl-DL-glutamate (II; R = O·CH_2Ph) when heated at 100° with excess of benzyl alcohol and, by adopting the well-known procedure of Bergmann and Zervas (*loc. cit.*) for the synthesis of L-glutamine, this derivative was made the starting point for a series of reactions leading to the hitherto



unknown DL-*iso*glutamine. The ester-acid chloride prepared with phosphorus pentachloride was treated with ethereal ammonia, so forming phthalyl-DL-*iso*glutamine benzyl ester. Hydrogenolysis afforded phthalyl-DL-*iso*glutamine, and removal of the phthalyl group with hydrazine left DL-*iso*glutamine. With the exception of the acid chloride, which was not purified, all stages were isolated as crystalline compounds in very satisfactory yields. The properties of the intermediate phthalylglutamide show it to be distinct from the authentic phthalyl-DL-glutamine (King and Kidd, *loc. cit.*), thus confirming the supposition, based on analogy with the methanol reaction, that treatment of phthalyl-DL-glutamic anhydride with benzyl alcohol yields the γ-glutamate.

The effect of hydrazine on the phthalylglutamic anhydride was also examined to ascertain whether a hydrazide might be obtained without severing the protecting group. The product obtained by treating a dioxan solution of the anhydride with one molecular portion of hydrazine hydrate was conveniently recovered by shaking the product with benzaldehyde, whereby phthalyl-DL-glutamyl-γ-benzylidenehydrazide (II; R = NH·N:CHPh) (85%) was obtained. This derivative was also formed when phthalyl-DL-glutamic anhydride in ethyl acetate–chloroform was shaken with aqueous sodium hydrogen carbonate and benzylidenehydrazine. The constitution of the hydrazide was determined by dissolving it in hydrochloric acid and adding sodium nitrite. The ether-soluble portion (31%) of the product displayed the normal reactions of an azide, and when mixed with aniline gave phthalyl-DL-glutamyl-γ-anilide (II; R = NHPh) identical with that prepared and oriented as described in Part I.

When the phthalylglutamic anhydride was treated with excess of hydrazine, the formation of phthalhydrazide showed, as was to be expected, that hydrolysis of this phthalyl group had also occurred.

Experimental.

γ-Methyl Hydrogen Phthalyl-DL-glutamate (II; R = OMe).—Phthalyl-DL-glutamic anhydride (I) (5 g.) was refluxed with methyl alcohol (65 c.c.), heating being continued for ½ hour after the solid had dissolved. Removal of excess of methanol under reduced pressure left a syrup which was extracted with aqueous sodium hydrogen carbonate. The filtered solution was acidified with hydrochloric acid to Congo-red and kept overnight at 0°. Colourless needles of the *ester hydrate* separated, m. p. 60—62° (Found: loss in a vacuum at 110°, 8·1. $C_{14}H_{13}O_6N,1\frac{1}{2}H_2O$ requires loss, 8·4%), which when dried at 100° under reduced pressure gave γ-methyl hydrogen phthalyl-DL-glutamate as prisms (from benzene–light petroleum), m. p. 114° (Found: C, 57·4; H, 5·0; N, 4·8. Calc. for $C_{14}H_{13}O_6N$: C, 57·7; H, 4·5; N, 4·8%). Sheehan and Bolhofer (*J. Amer. Chem. Soc.*, 1950, **72**, 2471) give m. p. 119·5—120·5° (corr.).

γ-Methyl Hydrogen DL-*Glutamate* (III; R = OMe).—(i) DL-Glutamic acid hydrochloride (8·45 g.) was shaken with methanol (70 c.c.) containing hydrogen chloride (6 g.), and after 20 minutes the solution was filtered and concentrated at 20° under diminished pressure. Addition of dry ether gave a syrup which was dissolved in methanol (50 c.c.) containing aqueous ammonia (2·5 c.c.; *d* 0·88). After this had been kept at 0°, a white precipitate (2 g., 27%) appeared, which when crystallised from aqueous methanol gave *γ-methyl hydrogen* DL-*glutamate*, m. p. 183° (decomp.) (Found: C, 44·9; H, 7·0; N, 8·5. $C_6H_{11}O_4N$ requires C, 44·8; H, 6·9; N, 8·7%). The pure ester gave a violet colour with ninhydrin reagent.

(ii) γ-Methyl hydrogen phthalyl-DL-glutamate (2 g.), dissolved in water (25 c.c.) containing sodium carbonate (0·2 g.), was treated with hydrazine hydrate (0·7 g.; 50%), and the mixture left at room temperature for 48 hours. The precipitate of phthalhydrazide which appeared on acidification with hydrochloric acid was removed and the filtrate evaporated to dryness at low pressure. Trituration of the residue with cold methyl alcohol and concentration of the alcoholic extract gave the hydrochloride of γ-methyl hydrogen DL-glutamate which on treatment with anhydrous ether formed a flocculent highly deliquescent solid (0·8 g.). Dissolved in methanol and treated with aqueous ammonia (0·24 g.; *d* 0·88), it gave, after 12 hours at 0°, the monoester (0·6 g., 54%), m. p. and mixed m. p., after crystallisation from aqueous ethanol, 183°.

*γ-Benzyl Hydrogen Phthalyl-*DL-*glutamate* (II; R = O·CH_2Ph).—Phthalyl-DL-glutamic anhydride (8 g.) was heated with benzyl alcohol (12 g.) at 100° until a clear solution was obtained. Ether was then added and the ethereal solution extracted with aqueous sodium hydrogen carbonate. The combined extracts were washed with ether and acidified with hydrochloric acid, the precipitated oil being taken up in ether. Drying and evaporation of the ethereal solution left a gum (10·2 g., 90%) which solidified in contact with benzene, and crystallisation from benzene–light petroleum gave *γ-benzyl hydrogen phthalyl-*DL-*glutamate* as colourless soft needles, m. p. 85—86° (Found: C, 65·7; H, 4·75; N, 4·1. $C_{20}H_{17}O_6N$ requires C, 65·5; H, 4·6; N, 3·8%).

*Phthalyl-*DL-iso*glutamine Benzyl Ester.*—A solution of the γ-benzyl hydrogen phthalylglutamate (6·7 g.) in dry ether (50 c.c.) was shaken with phosphorus pentachloride (3·92 g.), with occasional cooling in ice-water, until reaction was complete. The pale yellow syrup left on removal of the ether was well washed with light petroleum and then redissolved in ether. Ethereal ammonia was added until precipitation ceased and, after collection, the solid was triturated with dilute aqueous sodium hydrogen carbonate. The residue of *phthalyl-*DL-iso*glutamine benzyl ester* (6·1 g., 91%) crystallised from water in minute felted needles, m. p. 126—128° (Found: C, 66·0; H, 4·9; N, 7·5. $C_{20}H_{18}O_5N_2$ requires C, 65·5; H, 4·9; N, 7·6%).

*Phthalyl-*DL-iso*glutamine.*—The foregoing benzyl ester (4 g.) in aqueous ethanol (100 c.c.) containing acetic acid (0·5 c.c.) was hydrogenated over palladium–charcoal at 1—2 atmospheres pressure and 40—60°. The residue obtained on evaporating the filtered solution to dryness was shaken with aqueous sodium hydrogen carbonate, and the soluble portion precipitated with hydrochloric acid. The deposited *phthalyl-*DL-iso*glutamine* (2·6 g., 87%) crystallised from aqueous alcohol in fine needles, m. p. 170—172° with shrinkage at 100—120° (Found: loss on drying in a vacuum at 110°, 5·9. $C_{13}H_{12}O_5N_2,H_2O$ requires loss, 6·1%. Found, in dried material: C, 57·1; H, 4·3; N, 10·0. $C_{13}H_{12}O_5N_2$ requires C, 56·5; H, 4·4; N, 10·1%).

DL-iso*Glutamine.*—Phthalyl-DL-*iso*glutamine (1·3 g.) was dissolved in aqueous sodium carbonate (1 equiv. of 0·5N.) to which hydrazine hydrate (0·45 g.; 50%) was then added. After 48 hours at 0°, the solution was acidified with excess of 3N-hydriodic acid, and the precipitate of phthalhydrazide collected. The filtrate was exactly neutralised with 0·5N-sodium carbonate, and the solution evaporated to small bulk at low pressure. Addition of acetone precipitated DL-iso*glutamine* (0·35 g., 55%) which could be obtained in clusters of needles (Found: N, 19·3. $C_5H_{10}O_3N_2$ requires N, 19·2%) when rapidly crystallised from aqueous acetone but was contaminated by ammonium glutamate when crystallisation was slow. Alternatively, phthalyl-DL-*iso*glutamine (1 g.) was dissolved in aqueous hydrazine (1·11 g. of 19·6%, 2 mols.) and after 48 hours at 0° the phthalylhydrazide was precipitated with acetic acid, the *iso*glutamine being obtained from the filtrate by the addition of acetone. DL-*iso*Glutamine does not melt but slowly chars above 180°. It gives a magenta colour with ninhydrin reagent.

*Phthalyl-*DL-*glutamyl-γ-benzylidenehydrazide* (II; R = NH·N:CHPh).—(i) A solution of phthalyl-DL-glutamic anhydride (2 g.) in dioxan (25 c.c.) was treated with hydrazine hydrate (1·8 g. of 50%, 2 mols.) in dioxan (25 c.c.), added in portions with water-cooling. Anhydrous ether was then introduced and, after being kept at 0°, the deliquescent precipitate was separated by decantation and dissolved in water (25 c.c.). After the addition of sodium hydrogen carbonate (0·65 g.) and benzaldehyde (2·6 g.), the mixture was shaken for an hour and the precipitate of benzaldazine removed. Acidification with hydrochloric acid gave *phthalyl-*DL-*glutamyl-γ-benzylidenehydrazide* as a pale straw-coloured mass (2·5 g., 85%) which after being washed with ether and crystallised from aqueous alcohol or ethyl acetate–light petroleum formed minute prisms, m. p. 136—138° (Found: C, 63·4; H, 4·8; N, 11·0. $C_{20}H_{17}O_5N_3$ requires C, 63·4; H, 4·5; N, 11·1%).

(ii) A mixture of the glutamic anhydride (2 g.) in chloroform–ethyl acetate (50 c.c. of each) and of sodium hydrogen carbonate (1 g.) in water (25 c.c.) was vigorously shaken with benzylidenehydrazine (1 g.), added in portions. On removal of the aqueous layer and acidification with hydrochloric acid, the phthalylglutamylbenzylidenehydrazide separated and after crystallisation had m. p. and mixed m. p., 136—137°.

When a suspension of the anhydride in water was treated with excess of hydrazine, dissolution quickly occurred, and acidification with hydrochloric acid followed by the addition of sodium acetate caused the precipitation of phthalhydrazide. No attempt was made to isolate the amino-acid moiety.

*Phthalyl-*DL-*glutamyl-γ-anilide* (II; R = NHPh).—The phthalylglutamic anhydride (2 g.) was treated with hydrazine hydrate as described above, and the deliquescent solid precipitated by ether

was dissolved without removal of the organic layer in 2N-hydrochloric acid (100 c.c.). Aqueous sodium nitrite (10%) was added, using starch–iodide indicator, and the ethereal layer separated, washed, and dried (Na_2SO_4). Evaporation at low temperature left minute white prisms (0·7 g., 31%), decomposing at *ca.* 60° with effervescence. When aniline (0·8 g.) was added to the ethereal solution, which was then set aside for 1—2 days, a pale buff powder was slowly deposited. This could be crystallised from water containing a little hydrochloric acid (charcoal) to yield colourless prisms, m. p. 106—108°, alone or mixed with phthalyl-DL-glutamyl-γ-anilide prepared from phthalyl-DL-glutamic anhydride and aniline by King and Kidd (*loc. cit.*, p. 3318).

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