Syntheses from Phthalimido-acids. Part VI. Further Products from Phthalyl-*DL*-aspartic Anhydride, and the Preparation of Phthalyl-glycyl-*DL*-asparagine and -*DL*-serine.*

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Both α- and β-methyl esters are obtained from phthalyl-DL-aspartic anhydride and methanol. That which is formed in preponderating amount is the α-methyl compound since it is converted into an ester-amide identical with phthalylasparagine methyl ester.

Phthalylglycyl chloride, the azide, and the ethyl carbonic anhydride have each been used in the preparation of phthalylglycyl-asparagine and -DL-serine.

Examples described in earlier papers of this series (*J.*, 1949, 3315; 1951, 243; 1954, 1044) show that the action of amino-compounds and also of methanol and benzyl alcohol on phthalyl-DL- and -L-glutamic anhydride leads, at least qualitatively, to the formation of γ-derivatives. Only a limited investigation has so far been made of the reactions of

* Part V, preceding paper.

phthalyl-DL- and -L-aspartic anhydride, but as already recorded in Part III (*J.*, 1951, 2976) the ammonolysis of these two compounds yields β-amides, a result which is comparable with the behaviour of the corresponding phthalylglutamic anhydrides.

Included in the present communication on the preparation of new derivatives of DL-aspartic acid is an account of the action of methanol on phthalyl-DL-aspartic anhydride. A methanol solution of the anhydride afforded two products, (A) m. p. 182° (yield 57%), crystallising from the cold solution, and (B) m. p. 147—148° (yield 36%), which was obtained when the remaining liquid was concentrated. Both were acid esters and were converted through their acid chlorides into amido-esters, which were also formed from the corresponding azides and from the mixed carbonic anhydrides. The structures of the isomeric amido-esters were determined by esterifying, with diazomethane, the phthalyl-DL-asparagine prepared as in Part III (*loc. cit.*), whereupon a product was obtained identical with that originating from (A); this identified the compounds (A) and (B) as α- and β-methyl phthalyl-DL-aspartate respectively. The formation of two products, the α-isomer predominating, is thus in marked contrast to the conversion of the phthalylaspartic anhydride into phthalylasparagine and of the phthalylglutamic anhydride into γ-derivatives. Amides of the methyl ester (A) were prepared from *p*-aminobenzoic acid and its ethyl ester by the mixed carbonic anhydride synthesis.

Phthalylglycyl-DL-asparagine was prepared by coupling DL-asparagine with phthalylglycyl chloride in alkaline solution; treating the product with hydrazine gave glycyl-DL-asparagine, the racemic form of a component of the structure attributed by Woolley (*J. Biol. Chem.*, 1948, **176**, 1291) to lycomarasmin, *viz.*,

$$HO_2C\cdot CMe(OH)\cdot NH\cdot CH_2\cdot CO\cdot NH\cdot CH(CO_2H)\cdot CH_2\cdot CO\cdot NH_2.$$

Phthalylglycyl derivatives of serine and of its methyl ester which were synthesised in this way were also obtained from the azide and the ethyl carbonic anhydride of phthalylglycine.

Experimental

α-Methyl and β-Methyl Hydrogen Phthalyl-DL-aspartate.—A mixture of phthalyl-DL-aspartic anhydride (15 g.; Part III, *J.*, 1951, 2976) and dry methanol (150 c.c.) was boiled for 30 min. after dissolution was complete, and then kept at 0°. Crystallisation afforded *α-methyl hydrogen phthalyl-DL-aspartate* (9·9 g., 58%) as colourless prisms, m. p. 182° (Found: C, 56·1; H, 4·0; N, 4·91. $C_{13}H_{11}O_6N$ requires C, 56·3; H, 4·0; N, 5·1%).

The residue obtained by evaporating the filtrate from the α-ester was extracted with aqueous sodium hydrogen carbonate. Acidification of the extract (Congo-red) afforded *β-methyl hydrogen phthalyl-DL-aspartate* (6·1 g., 36%), m. p. 148—152°, as an oil which rapidly crystallised. The ester was very soluble in methanol; it crystallised from water in plates, m. p. 147—148° (Found: C, 56·3; H, 3·8; N, 5·0. $C_{13}H_{11}O_6N$ requires C, 56·3; H, 4·0; N, 5·1%).

Phthalyl-DL-asparagine Methyl Ester.—(*a*) Evaporation of the solution obtained by treating a suspension of phthalyl-DL-asparagine (1 g.; Part III, *loc. cit.*) in methanol (25 c.c.) with excess of ethereal diazomethane afforded a crystalline residue, which was washed with aqueous sodium hydrogen carbonate and with water. *Phthalyl-DL-asparagine methyl ester* crystallised from water in long rods, m. p. 170° (Found: C, 56·5; H, 4·4; N, 9·9. $C_{13}H_{12}O_5N_2$ requires C, 56·5; H, 4·4; N, 10·1%).

(*b*) α-Methyl hydrogen phthalyl-DL-aspartate was converted into the acid chloride (59%) with phosphorus pentachloride in ether, or more conveniently with thionyl chloride at the b. p. The acid chloride crystallised from light petroleum (b. p. 40—60°) in rosettes of needles, m. p. 77—78°. Addition of an excess of ethereal ammonia to the acid chloride (1 g.), in anhydrous ether, gave an immediate flocculent precipitate which was collected and washed with aqueous sodium hydrogen carbonate, and with water. Crystallisation from water afforded phthalyl-DL-asparagine methyl ester (0·82 g., 88%) in rods, m. p. 170°, not depressed by admixture with the amide described above.

(*c*) Addition of an excess of aqueous sodium azide (*ca.* 25%) to a concentrated acetone solution of the acid chloride [1 g.; prepared as in (*b*)] precipitated the α-methyl aspartate β-azide as an oil which rapidly crystallised. Extraction of the azide with ether and treatment with ethereal ammonia at 0° for 12 hr. afforded the amide (0·79 g., 85%), which crystallised from water in rods, m. p. and mixed m. p. 170°.

Phthalyl-DL-iso*asparagine Methyl Ester.*—(*a*) The acid chloride was obtained as an oil from β-methyl hydrogen phthalyl-DL-aspartate by the methods described above for the α-ester. When treated with ethereal ammonia the acid chloride afforded *phthalyl*-DL-iso*asparagine methyl ester* (50% from the acid) which crystallised from water in needles, m. p. 141° (Found : C, 56·4; H, 4·4; N, 10·1. $C_{13}H_{12}O_5N_2$ requires C, 56·5; H, 4·4; N, 10·1%).

(*b*) Addition of aqueous sodium azide (*ca.* 25%) to the foregoing acid chloride, in acetone, afforded the β-ester α-azide as an oil which, with ethereal ammonia at 0°, gave phthalyl-DL-*iso*asparagine methyl ester, m. p. 141°, identical with that obtained by method (*a*).

(*c*) A solution of β-methyl hydrogen phthalyl-DL-aspartate (1·4 g.) and triethylamine (0·7 c.c.) in dry dioxan (10 c.c.) was cooled to 10° and kept at this temperature for 10 min. after the addition of ethyl chloroformate (0·48 c.c.). The solution of the mixed anhydride was then treated with ethereal ammonia, and the flocculent amide was collected and washed with aqueous sodium hydrogen carbonate, and with water. Crystallisation of the residue (0·86 g., 61%) from water afforded phthalyl-DL-*iso*asparagine methyl ester in needles, m. p. 141° alone or when mixed with specimens prepared by methods (*a*) and (*b*).

Methyl β-(p-*Carboxyphenylcarbamyl*)-α-*phthalimidopropionate and its Ethyl Ester.*—A solution of the mixed anhydride, prepared from α-methyl hydrogen phthalyl-DL-aspartate (1·39 g.) and ethyl chloroformate as described under (*c*) above, was treated with a solution of *p*-aminobenzoic acid (0·69 g.) in N-sodium hydroxide (5 c.c.). Acidification (Congo-red) of the alkaline solution, after ether-extraction, with 5N-hydrochloric acid and storage at 0° afforded *methyl* β-(p-*carboxyphenylcarbamoyl*)-α-*phthalimidopropionate* (1·3 g., 66%), m. p. 260—262° (decomp.), raised to 264—265° (decomp.) by recrystallisation (plates) from aqueous ethanol or aqueous dioxan (Found : C, 61·1; H, 4·2; N, 7·4. $C_{20}H_{16}O_7N_2$ requires C, 60·6; H, 4·1; N, 7·1%).

In a similar manner the addition of a chloroform solution of ethyl *p*-aminobenzoate (0·83 g.) afforded the corresponding ethyl ester (1·84 g., 87%), which was isolated by evaporation of the solvents. *Methyl* β-(p-*ethoxycarbonylphenylcarbamoyl*)-α-*phthalimidopropionate* crystallised from aqueous 2-ethoxyethanol in plates, m. p. 205—207° (Found : C, 62·6; H, 4·5; N, 6·4. $C_{22}H_{20}O_7N_2$ requires C, 62·3; H, 4·7; N, 6·6%).

Phthalylglycyl-DL-*asparagine.*—Solutions of phthalylglycyl chloride (4·47 g.) in dioxan (15 c.c.) and of N-sodium hydroxide (20 c.c.) were added simultaneously during 1 hr. to a stirred solution of DL-asparagine (2·64 g.) in N-sodium hydroxide (20 c.c.) at 0°. The solution was acidified (litmus) and concentrated to small bulk under reduced pressure and stored at 0° after the addition of a little 2N-hydrochloric acid. Crystallisation of the precipitate afforded *phthalylglycyl*-DL-*asparagine* (2·6 g., 41%) in needles, m. p. 205—206° (decomp.), raised to 208—209° (decomp.) by recrystallisation from water (Found : C, 53·0; H, 4·2; N, 12·8. $C_{14}H_{13}O_6N_3$ requires C, 52·7; H, 4·1; N, 13·2%).

Glycyl-DL-*asparagine.*—A solution of phthalyl-DL-asparagine (3·19 g.) and sodium carbonate (0·58 g.) in water (60 c.c.) was treated with aqueous hydrazine (33% ; 1 c.c.) at room temperature for 3 days, and then acidified (Congo-red) with hydriodic acid. After storing the solution at 0° phthalhydrazide was removed by filtration, the filtrate evaporated to dryness below 45°, and the residue was extracted with a small volume of water. Dilution of the extract with a large volume of ethanol precipitated *glycyl*-DL-*asparagine* (1·55 g., 82%), m. p. 204—206° (decomp.), raised by crystallisation from aqueous ethanol to 215—216° (decomp.), after discoloration at 210°. When heated rapidly it had m. p. 222° (decomp.), after discoloration at 216° (Found : C, 37·9; H, 6·2; N, 21·5. $C_6H_{11}O_4N_3$ requires C, 38·1; H, 5·9; N, 22·2%).

Phthalylglycyl-DL-*serine.*—(*a*) A solution of DL-serine (1·05 g.) and sodium hydrogen carbonate (1·85 g.) in water (20 c.c.) was cooled to 0° and stirred vigorously during the dropwise addition (1 hr.) of a solution of phthalylglycyl chloride (2·24 g.) in benzene (40 c.c.). After a further hour's stirring the aqueous phase was acidified with 5N-hydrochloric acid and storage at 0° for several hours gave a precipitate (1·8 g.), m. p. 193—195° (decomp.), which crystallised from water in small plates, m. p. 194° (decomp.). Extraction of this material with hot ethyl acetate and crystallisation from water afforded *phthalylglycyl*-DL-*serine monohydrate* (1·3 g., 42%) as plates, m.p. 199° (decomp.) (Found: C, 50·2; H, 4·8; N, 9·0; loss on drying *in vacuo*, 6·0. $C_{13}H_{12}O_6N_2,H_2O$ requires C, 50·3; H, 4·5; N, 9·0; loss, 5·8%). Emerson (U.S.P. 2,498,665; *Chem. Abs.*, 1950, **44**, 4926b) records m. p. 191° for phthalylglycyl-DL-serine prepared in a similar manner.

(*b*) Crystalline phthalylglycine azide from phthalylglycyl chloride (1·12 g.) was dissolved in dioxan and added to a solution of DL-serine (0·53 g., 1 equiv.) in N-sodium hydroxide (5 c.c.) at 0°, and a further 5 c.c. of N-sodium hydroxide was added after 2 hr. The mixture was kept at room temperature for 5 hr., extracted with ether, and acidified (Congo-red) with 5N-hydrochloric acid. Collection of the precipitate after storage at 0° for 12 hr. afforded phthalylglycyl-

DL-serine monohydrate (0·65 g., 42%), m. p. 192—193° (decomp.), raised to 199° (decomp.) by recrystallisation from water.

(*c*) A solution of DL-serine (0·53 g.) in N-sodium hydroxide was added to a solution of the mixed anhydride prepared in the usual way from phthalylglycine (1·03 g.) and ethyl chloroformate, at 10°. When gas evolution ceased the solution was extracted with ether and acidified (Congo-red) with 5N-hydrochloric acid. Crystallisation afforded phthalylglycyl-DL-serine monohydrate (0·94 g., 61%), m. p. 199° (decomp.) alone or mixed with the specimens prepared by methods (*a*) and (*b*).

*Phthalylglycyl-*DL-*serine Methyl Ester.*—(*a*) DL-Serine methyl ester hydrochloride, m. p. 135—136°, was prepared by the method of Fischer and Suzuki (*Ber.*, 1905, **38**, 4193) who give m. p. 114°. A chloroform solution of phthalylglycyl chloride (1 equiv.) was added during 1 hr. to a stirred solution of DL-serine methyl ester hydrochloride (1 equiv.) and triethylamine (2 equivs.) in chloroform. The residue obtained by evaporation of the solvent was washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and with water; crystallisation from water afforded *phthalylglycyl-*DL-*serine methyl ester* (76%) as needles, m. p. 225° (decomp.) (Found : C, 54·8; H, 4·9; N, 9·1. $C_{14}H_{14}O_6N_2$ requires C, 54·9; H, 4·6; N, 9·2%).

(*b*) A chloroform solution of phthalylglycine azide (from 2·24 g. of phthalylglycyl chloride) was added to a solution of serine methyl ester hydrochloride (1·56 g., 1 equiv.) and triethylamine (1·38 c.c., 1 equiv.) in chloroform (20 c.c.) and kept at room temperature for 24 hr. Evaporation of the solvent left a residue which, after washing and crystallisation as described under (*a*), afforded phthalylglycyl-DL-serine methyl ester (1·6 g., 52%), m. p. 224° (decomp.) alone or mixed with the ester obtained by method (*a*).

(*c*) Addition of a chloroform solution of DL-serine methyl ester (1·19 g., 1 equiv.) to the mixed anhydride from phthalylglycine (2·05 g.), ethyl chloroformate (0·96 c.c.), and triethylamine (1·4 c.c.), and isolation as described above afforded phthalylglycyl-DL-serine methyl ester (1·9 g., 62%), m. p. 222° (decomp.), raised to 224—225° by recrystallisation from water.

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