616. *The Synthesis of* N-p-*Aminobenzoyl*-DL- *and* -L-*glutamine and* -DL-iso*glutamine.*

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The dehydration of *N*-*p*-nitrobenzoyl-L-glutamic acid gives 4-2′-carboxyethyl-2-*p*-nitrophenyloxazol-5-one, which by treatment with ammonia and reduction is converted into *N*-*p*-aminobenzoyl-DL-*iso*glutamine. With benzyl alcohol the oxazolone forms α-benzyl *N*-*p*-nitrobenzoyl-DL-glutamate which has been used for the preparation of *N*-*p*-aminobenzoyl-DL-glutamine. An alternative synthesis of the latter and of the corresponding L-isomer from the respective γ-monoethyl glutamates is also described.

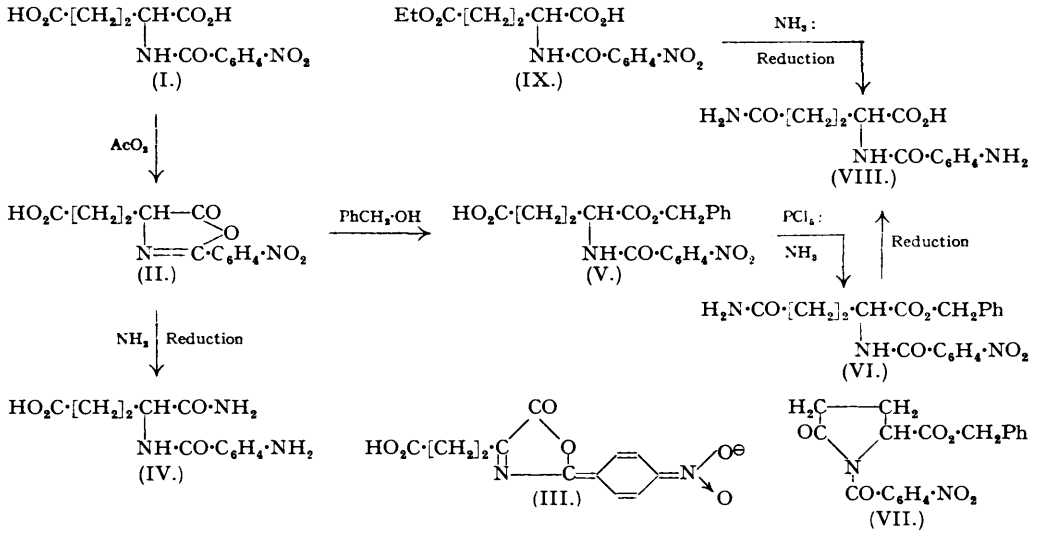
IT has been reported by Auhagen (*Z. physiol. Chem.*, 1943, **277**, 197) that in its capacity for reversing the inhibitory action of sulphanilamide on the growth of *Streptobacterium plantarum* 10S, *N*-*p*-aminobenzoyl-L-glutamic acid is 8—10 fold as effective as *p*-aminobenzoic acid. With the subsequent elucidation of the structure of folic acid, this observation appeared to link *p*-aminobenzoic acid with folic acid synthesis, but although Auhagen's results have recently been confirmed for the related organism, *S. plantarum* 5S (Nimmo-Smith, Lascelles, and Woods, *Brit. J. Exp. Path.*, 1948, **29**, 264), they do not appear to apply to other similar bacterial species (see, for example, Williams, *J. Biol. Chem.*, 1944, **156**, 85). Alternatively, in view of the considerable biological significance of glutamine, it may be that *p*-aminobenzoic acid is utilised in a synthesis requiring *N*-*p*-aminobenzoylglutamine; Woods (private communication) has, in fact, postulated the existence of pteroylglutamine in order to explain certain data concerning the growth requirements of bacteria. The following synthesis of *N*-*p*-aminobenzoyl-glutamine and -*iso*glutamine was therefore undertaken to provide material for microbiological investigation.

The method envisaged consisted in the use of *N*-*p*-nitrobenzoylglutamic anhydride, as in Bergmann's synthesis of glutamine from carbobenzyloxy-L-glutamic anhydride, or of that starting from phthalylglutamic anhydride which as recently shown (King and Kidd, *J.*, 1949, 3315), by giving exclusively γ-derivatives, offers a direct route to the naturally-occurring amide. However, the dehydration of *N*-*p*-nitrobenzoyl-L-glutamic acid (I) with acetic anhydride gave a racemic product, and since loss of optical activity under these conditions is believed to indicate oxazolone formation, the resultant anhydro-compound was regarded as 4-2′-carboxyethyl-2-*p*-nitrophenyloxazol-5-one (II). Proof of its structure was to be found in the intense violet-blue colour produced on contact with alkalis, a reaction which is the basis of the Waser test for α-amino-acids and is ascribed by Karrer and Keller (*Helv. Chim. Acta*, 1943, **26**, 50) to mesomerism associated with the anion (III).

The effect of aqueous or liquid ammonia on the oxazolone (II) was to form an ammonium salt, *N*-*p*-nitrobenzoyl-DL-*iso*glutamine, from which was derived by catalytic reduction *N*-*p*-aminobenzoyl-DL-*iso*glutamine (IV) characterised by its mono-hydrochloride and -picrate.

To obtain the comparable acylglutamine the oxazolone (II) was first heated in dioxan solution with one molecular proportion of benzyl alcohol to form α-benzyl *N*-*p*-nitrobenzoyl-DL-glutamate (V); use of excess of the alcohol in the absence of solvent gave the crystalline αγ-dibenzyl ester. By use of the technique of Bergmann, Zervas, and Fruton (*J. Biol. Chem.*, 1936, **115**, 608) for carbobenzyloxyleucine chloride (see also Synge, *Biochem. J.*, 1948, **42**, 99),

the monobenzyl ester was converted into its acid chloride which without isolation was treated with ammonia giving α-benzyl *N*-*p*-nitrobenzoyl-DL-gluta mine (VI). A halogen-free product obtained from *N*-*p*-nitrobenzoylglutamate α-benzyl ester by warming it with excess of thionyl chloride is believed to be 2-carbobenzyloxy-1-*p*-nitrobenzoylpyrrolid-5-one (VII): a similar cyclisation has recently been observed with α-benzyl *N*-carbobenzyloxyglutamic acid (Berenbom and White, *J. Amer. Chem. Soc.*, 1949, **71**, 2246). Catalytic reduction of the nitro-group in (VI) was accompanied by hydrogenolysis of the benzyl ester thus giving the required *N*-*p*-amino-benzoyl-DL-glutamine (VIII).



The aminobenzoylglutamine was synthesised by an alternative route, starting from γ-ethyl DL-glutamate which was prepared by the method employed by Abderhalden and Nienberg (*Z. physiol. Chem.*, 1933, **219**, 155) for the γ-L-ester. The incorrect constitution assigned to the latter by these authors was revised by Bergmann and Zervas (*ibid.*, 1933, **221**, 51), and the corrected structure later confirmed by Nienberg (*Ber.*, 1935, **68**, 2232), so that there appeared to be little doubt that the monoethyl DL-glutamate belonged to the γ-series. Acylation in aqueous bicarbonate with *p*-nitrobenzoyl chloride gave γ-ethyl *N*-*p*-nitrobenzoyl-glutamate (IX) which reacted with liquid ammonia to form *N*-*p*-nitrobenzoyl-DL-glutamine. By catalytic hydrogenation the corresponding amino-compound was obtained which was identical with the *N*-*p*-aminobenzoyl-DL-glutamine (VIII) previously synthesised, thus confirming earlier suppositions as to the constitution of the anhydro-*p*-nitrobenzoylglutamic acid and its reaction products. From γ-ethyl *N*-*p*-nitrobenzoyl-L-glutamate, which was reduced incidentally to γ-ethyl *N*-*p*-aminobenzoyl-L-glutamate, *p*-nitro- and *p*-amino-benzoyl-L-glutamine were also synthesised.

The results of bacteriological tests demonstrate that *N*-*p*-aminobenzoyl-DL-*iso*glutamine is unable to replace *p*-aminobenzoic acid or *N*-*p*-aminobenzoyl-L-glutamic acid as a growth factor for *S. plantarum* 5S or 10S, and that *N*-*p*-aminobenzoyl-L-glutamine has only very feeble growth activity for these organisms. We are indebted to Dr. H. H. Nimmo-Smith and Dr. D. D. Woods, Department of Biochemistry, Oxford University, for this summary of their results.

Experimental.

4-2′-*Carboxyethyl*-2-p-*nitrophenyloxazol*-5-*one* (II).—Anhydrous *N*-*p*-nitrobenzoyl-L-glutamic acid (25 g.) and acetic anhydride (80 c.c.) were heated on a steam-bath for ½ hour with occasional shaking. The resulting solution was filtered, and when cold was treated with dry ether (150 c.c.) followed by light petroleum (b. p. 40—60°; 250 c.c.). The *oxazolone* separated as a brownish oil which solidified in the course of a day or so at 0°. It was collected, washed with ether–petroleum mixture (50 : 50) and stored over phosphoric oxide in a vacuum. The product (16·2 g., 70%), m. p. 149°, was sufficiently pure for most purposes, but when recrystallised from a small quantity of acetic anhydride and washed with benzene it gave minute elongated prisms, m. p. 151° (Found: C, 51·8; H, 3·8; N, 9·8. $C_{12}H_{10}O_6N_2$ requires C, 51·8; H, 3·6; N, 10·1%). The oxazolone was also somewhat soluble in ethyl acetate. It dissolved in aqueous alkali to an intense violet solution; with aqueous pyridine the compound gave a rather bluer shade.

N-p-*Nitrobenzoyl*-DL-iso*glutamine*.—4-2′-Carboxyethyl-2-*p*-nitrophenyloxazol-5-one (7·2 g., 1 mol.) was dissolved in warm anhydrous dioxan (70 c.c.), and, to the solution cooled in ice-water, aqueous ammonia (*d* 0·88; 3 c.c., 2 mols.) was slowly added. The intense violet coloration which accompanied the formation of a gummy precipitate disappeared in approximately 1 hour leaving a crystalline product (7·2 g.). This was collected and washed with ether, and when it was dissolved in water (20 c.c.) and treated with concentrated hydrochloric acid (2·5 c.c., 1 mol.) N-p-*nitrobenzoyl*-DL-iso*glutamine* was precipitated, which on 2 crystallisations from water gave large, irregular-shaped prisms (5·1 g., 67%), m. p. 185°, or fine elongated prisms from methanol (Found : C, 48·8; H, 4·3; N, 14·1. $C_{12}H_{13}O_6N_3$ requires C, 48·8; H, 4·4; N, 14·2%). In a variation of the above method of preparation, in which the anhydride was dissolved in liquid ammonia, a good yield of the ammonium salt was again formed which was worked up as before.

N-p-*Aminobenzoyl*-DL-iso*glutamine* (IV).—*N*-*p*-Nitrobenzoyl-DL-*iso*glutamine (3·1 g.) was dissolved in methanol (75 c.c.) and hydrogenated at 2—3 atmospheres and room temperature over palladised charcoal. Removal of the catalyst and the solvent left N-p-*aminobenzoyl*-DL-iso*glutamine* as a solid, which was crystallised from water giving small rectangular prisms (2·2 g., 80%), m. p. 182—183° (Found : C, 54·3; H, 5·4; N, 16·3. $C_{12}H_{15}O_4N_3$ requires C, 54·3; H, 5·7; N, 15·9%). A solution of *p*-aminobenzoyl-DL-*iso*glutamine in ethanolic hydrogen chloride was treated with dry ether; the *hydrochloride* was precipitated as a gum which solidified on being rubbed with a little alcohol; it crystallised from 90% ethanol in microscopic crystals, softening at 161—173°, m. p. *ca.* 235° (decomp.) (Found : C, 47·5; H, 5·6. $C_{12}H_{15}O_4N_3$,HCl requires C, 47·8; H, 5·3%). A mixture of saturated ethanolic picric acid (12 c.c.) and of *p*-aminobenzoyl-DL-*iso*glutamine (0·5 g.) in water (5 c.c.) after several days deposited yellow needles of the *picrate* which crystallised from ethanol as an alcoholate, m. p. 132—133° (Found : C, 44·5; H, 4·2; N, 15·7. $C_{12}H_{15}O_4N_3$,$C_6H_3O_7N_3$,C_2H_6O requires C, 44·4; H, 4·4; N, 15·6%).

α-*Benzyl* N-p-*Nitrobenzoyl*-DL-*glutamate* (V).—4-2′-Carboxyethyl-2-*p*-nitrophenyloxazol-5-one (16·2 g., 1 mol.) was dissolved in hot anhydrous dioxan (20 c.c.) and after addition of benzyl alcohol (6·3 c.c., 1 mol.) the mixture was heated under reflux in an oil-bath at 120° for 8 hours. Removal of the solvent under reduced pressure and trituration of the residual gum with dry ether gave a solid which was ground in a mortar with a solution of sodium hydrogen carbonate (5 g.) in water (100 c.c.). Insoluble material (2·8 g.) was separated by filtration, and the filtrate acidified (Congo red) with dilute hydrochloric acid. The precipitate of α-*benzyl* N-p-*nitrobenzoyl*-DL-*glutamate* (8·3 g., 37%) when crystallized from a very large volume of 10% aqueous ethanol gave feathery clusters of tiny needles, m. p. 146° (Found : C, 59·1; H, 4·6; N, 7·6. $C_{19}H_{18}O_7N_2$ requires C, 59·1; H, 4·7; N, 7·3%).

Dibenzyl N-p-*Nitrobenzoyl*-DL-*glutamate*.—When 4-2′-carboxyethyl-2-*p*-nitrophenyloxazol-5-one was heated with excess of benzyl alcohol without additional solvent at 100—110° for 3 hours, a very low yield of the α-monobenzyl ester remained on trituration with ether. Evaporation of the combined ether washings left a viscous yellow oil which largely solidified in a few days. When this was carefully washed with a little ethanol a crystalline powder remained, insoluble in acid and alkali. Recrystallisation from ethanol gave minute elongated prisms, m. p. 84° and mixed m. p. with benzyl *p*-nitrobenzoate (m. p. 84°) 66°. Analysis showed the compound to be *dibenzyl* N-p-*nitrobenzyl*-DL-*glutamate* (Found : C, 64·9; H, 4·9; N, 6·2. $C_{26}H_{24}O_7N_2$ requires C, 65·5; H, 5·0; N, 5·9%).

N-p-*Nitrobenzoyl*-DL-*glutamine* α-*Benzyl Ester* (VI).—α-Benzyl *N*-*p*-nitrobenzoyl-DL-glutamate (1 g., 1 mol.), suspended in dry chloroform (25 c.c.) cooled to 0°, was treated with powdered phosphorus pentachloride (0·6 g., 1·1 mol.). The mixture was shaken until no further solid dissolved and then filtered, and the filtrate, after being twice very rapidly washed with ice-cold water, was dried (Na_2SO_4) for a few minutes. When the drying agent had been removed, ether (10 c.c.) saturated at 0° with dry ammonia was added. Shortly after, the white precipitate was collected and thoroughly ground in a mortar under aqueous sodium hydrogen carbonate. The insoluble N-p-*nitrobenzoyl*-DL-*glutamine* α-*benzyl ester* (0·45 g., 43%) was collected, washed with water, and dried. It had m. p. 173—175° after softening at 128° and was apparently a hydrate. Recrystallisation from ethanol gave matted microscopic needles, m. p. 174—176° (Found : C, 59·7; H, 4·9; N, 10·8. $C_{19}H_{19}O_6N_3$ requires C, 59·2; H, 4·9; N, 10·9%).

2-*Carbobenzyloxy*-1-p-*nitrobenzoylpyrrolid*-5-*one* (VII).—During attempts to prepare α-benzyl *N*-*p*-nitrobenzoylglutamic acid chloride using thionyl chloride, the acid was warmed with excess of the reagent to 50°. The product isolated after removal of excess of thionyl chloride had m. p. 103° and was halogen-free; it was insoluble in sodium carbonate solution, and from analyses appeared to be 2-*carbobenzyloxy*-1-p-*nitrobenzoylpyrrolid*-5-*one* (Found : C, 62·0; H, 4·3; N, 8·0. $C_{19}H_{16}O_6N_2$ requires C, 62·0; H, 4·4; N, 7·6%).

N-p-*Aminobenzoyl*-DL-*glutamine* (VIII).—(i) *N*-*p*-Nitrobenzoyl-DL-glutamine α-benzyl ester (0·3 g.) in warm ethanol (15 c.c.) was hydrogenated with palladised charcoal catalyst at room temperature and 2—3 atmospheres pressure. The product separated on the catalyst which was collected and extracted with hot water; N-p-*aminobenzoyl*-DL-*glutamine* (0·15 g., 75%) crystallised on cooling in minute elongated prisms, m. p. 222° after recrystallisation (Found : C, 54·0; H, 5·7; N, 15·7. $C_{12}H_{15}O_4N_3$ requires C, 54·3; H, 5·7; N, 15·9%).

(ii) The hydrogenation of *N*-*p*-nitrobenzoyl-DL-glutamine (1·5 g.) (see below) in ethanol (100 c.c.) in presence of palladised charcoal at room temperature and 2—3 atmospheres led to the deposition of a product (yield, after crystallisation, 0·6 g., 45%) identical with that from method (i) (Found : C, 54·5; H, 5·8; N, 15·8%). *N*-*p*-Aminobenzoyl-DL-glutamine failed to give a picrate under conditions which were successful for the corresponding derivative of *iso*glutamine.

γ-*Ethyl* DL-*Glutamate*.—Powdered DL-glutamic acid (10 g.) added in portions was shaken with a solution of hydrogen chloride (6 g.) in absolute ethanol (100 c.c.). After standing for 15—30 minutes, the liquid was filtered and concentrated in a vacuum below 30° to about 20 c.c. It was then triturated

with dry ether (150 c.c.) and the crystalline *γ-ethyl* DL-*glutamate hydrochloride* (13·3 g., 93%) collected and washed with ether. The hygroscopic product, m. p. 105—107°, was purified for analysis by precipitation with dry ether from a cold ethanolic hydrogen chloride solution (Found: C, 39·7; H, 6·9; Cl, 16·7. $C_7H_{13}O_4N$,HCl requires C, 39·7; H, 6·6; Cl, 16·8%). The hydrochloride (13·3 g.) dissolved in methanol (80 c.c.) was exactly neutralised with ammonia (3·5 c.c.; *d* 0·88). The precipitated *γ-ethyl* DL-*glutamate* (8·3 g., 70% from glutamic acid) crystallised from aqueous ethanol in fine pearly plates, m. p. 187° (decomp.) (Found: C, 48·1; H, 7·2; N, 8·3. $C_7H_{13}O_4N$ requires C, 48·0; H, 7·4; N, 8·0%).

γ-Ethyl N-p-*Nitrobenzoyl*-DL-*glutamate* (IX).—γ-Ethyl DL-glutamate (7·0 g., 1 mol.) dissolved in water (120 c.c.) containing sodium hydrogen carbonate (3·36 g., 1 mol.) was treated at room temperature with *p*-nitrobenzoyl chloride (7·5 g., 1 mol.) dissolved in dry dioxan (to total volume of 40 c.c.), and N-aqueous sodium hydroxide (40 c.c., 1 mol.) added simultaneously with stirring during 1 hour. The deep reddish-purple colour which developed as the reaction proceeded gradually faded during a further 1 hour's stirring, after which the solution was filtered and acidified (Congo red) with concentrated hydrochloric acid. The precipitated oil was collected by three extractions with chloroform, the combined extracts being washed with 2N-hydrochloric acid and water, and then dried (Na_2SO_4). Evaporation under reduced pressure left *γ-ethyl* N-p-*nitrobenzoyl*-DL-*glutamate* as a gum which soon solidified, and on crystallisation from a large quantity of 10% aqueous ethanol (*ca.* 400 c.c.) formed rectangular prisms (6·5 g., 50%), m. p. 112—115° (Found: C, 52·0; H, 4·5; N, 8·7. $C_{14}H_{16}O_7N_2$ requires C, 51·9; H, 4·9; N, 8·6%).

N-p-*Nitrobenzoyl*-DL-*glutamine*.—γ-Ethyl N-*p*-nitrobenzoyl-DL-glutamate (4·8 g.) was dissolved in excess of liquid ammonia, and the solution put in an autoclave at room temperature. Next day, the pressure was released and the ammonia allowed to evaporate. The residual gum was dissolved in a small amount of water, and the solution acidified (Congo red) with 5N-hydrochloric acid, the crude product (4·4 g.), which rapidly hardened, then being taken up in hot water. The warm solution was decanted from resinous material (later identified as impure ethyl *p*-nitrobenzoylglutamate) which first separated, and was left at 0° overnight. The microcrystalline N-p-*nitrobenzoyl*-DL-*glutamine* (2·5 g., 57%) had m. p. 178—181°, raised by repeated crystallisation from water to 191—193°. The m. p. of a mixture with *N-p*-nitrobenzoyl-DL-*iso*glutamine (m. p. 185°) was *ca.* 174° (Found: C, 48·7; H, 4·5; N, 14·3. $C_{12}H_{13}O_6N_3$ requires C, 48·8; H, 4·4; N, 14·2%).

γ-Ethyl N-p-*Nitrobenzoyl*-L-*glutamate*.—The same procedure was adopted as for the DL-compound above, the yield of *γ-ethyl* N-p-*nitrobenzoyl*-L-*glutamate* after one crystallisation from 10%-aqueous ethanol being 67%. Two crystallisations gave hair-like needles, m. p. 114—115°, approximately 1½ times as soluble in 10% ethanol as the DL-compound (Found: C, 51·9; H, 4·6; N, 8·8%).

γ-Ethyl N-p-*Aminobenzoyl*-L-*glutamate*.—γ-Ethyl *N-p*-nitrobenzoyl-L-glutamate was dissolved in ethanol and hydrogenated over palladised charcoal. Removal of catalyst and solvent left a brownish gum which when dissolved in acetone and carefully treated with benzene gave *γ-ethyl* N-p-*aminobenzoyl*-L-*glutamate*, m. p., after recrystallisation from water, 128—129° (Found: C, 57·5; H, 6·1; N, 9·4. $C_{14}H_{18}O_5N_2$ requires C, 57·1; H, 6·1; N, 9·5%).

N-p-*Nitrobenzoyl*-L-*glutamine*.—The method of preparation corresponded with that used for the DL-compound, *N-p*-nitrobenzoyl-L-glutamine, m. p. 173—175° (81%), being obtained almost pure after 1 crystallisation from water. Recrystallisation from water gave irregular-shaped crystals, m. p. 175—176°, $[\alpha]_D^{19}$ in N-sodium hydroxide, 20·1° (Found: C, 49·1; H, 4·4%).

N-p-*Aminobenzoyl*-L-*glutamine*.—*N-p*-Nitrobenzoyl-L-glutamine (3·0 g.) was dissolved in warm methanol (60 c.c.) and hydrogenated over palladised charcoal at room temperature and 2—3 atmospheres pressure. After evaporation of most of the solvent, product and catalyst were collected and extracted with a little hot water. On cooling, the solution deposited N-p-*aminobenzoyl*-L-*glutamine*, m. p. 197°, $[\alpha]_D^{18}$ in N-sodium hydroxide +25°, and in N-hydrochloric acid, −8° (Found: C, 54·2; H, 5·6; N, 15·6%).

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