523. *Amino-acids and Peptides. Part XIII.* γ-L-Glutamyl-L-aspartic Acid.*

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The synthesis of γ-L-glutamyl-L-aspartic acid through benzyloxycarbonyl-γ-L-glutamyl azide proved unsatisfactory,[1] so we attempted the synthesis by other routes. Even under vigorous conditions, diethyl and dibenzyl L-aspartate failed to react satisfactorily with 1-toluene-*p*-sulphonyl-5-oxopyrrolidine-2-carboxylic acid,[2] and finally we used the reaction of phthaloyl-L-glutamic anhydride with dibenzyl L-aspartate. The coupling product was crystallised as the dicyclohexylammonium salt, which was hydrogenated to phthaloyl-γ-L-glutamyl-L-aspartic acid. This was purified by the bisdiphenylmethylammonium hydrogen salt; removal of the phthaloyl group by hydrazine gave the crystalline dipeptide monohydrate. No impurity was detected in this material by the ninhydrin reaction after electrophoresis on paper under conditions which gave a clear separation from authentic α-L-glutamyl-L-aspartic acid,[3] and from the constituent amino-acids, but the dipeptide obtained from the crude coupling product was shown to contain a little α-isomer, indicating that the anhydride ring does not open exclusively in one direction in this case.

Shiba, Yamakita, and Kaneko [4] reported the preparation of this dipeptide monohydrate by fractionation of the products of reaction of benzyloxycarbonyl-L-glutamic anhydride with diethyl L-aspartate; they do not record the specific rotation of their product, but give a melting point of 217—218°, in comparison with 177—182°.[1] In the present synthesis the product melted at 194·5—196°. In our experience, the melting points of free peptides are of little significance for characterisation. The clear separation between the isomeric α- and γ-dipeptides effected by paper electrophoresis provides a reliable method for the detection of the α-isomer, which was absent from our product.

Experimental.—Melting points were determined on a Kofler block.

Phthaloyl-γ-L-glutamyl(dibenzyl L-aspartate) (dicyclohexylammonium salt). Phthaloyl-L-glutamic anhydride [3·57 g.; m. p. 204—205°, $[\alpha]_D^{17}$ −44·2° (*c*, 3·2 in dioxan)] and dibenzyl L-aspartate toluene-*p*-sulphonate (13·4 g.) were dissolved in purified tetrahydrofuran with the addition of dried triethylamine (2·80 g.), and the solution was set aside at room temperature for

* Part XII, *J.*, 1954, 2870.

[1] Le Quesne and Young, *J.*, 1950, 1959; Rowlands and Young, *Biochem. J.*, 1957, **65**, 516.
[2] Winn and Young, unpublished work.
[3] Le Quesne and Young, *J.*, 1950, 1954.
[4] Shiba, Yamakita, and Kaneko, *J. Inst. Polytechnics, Osaka City Univ.*, Ser. C., 1956, **5**, 144.

40 hr. The solvent was removed *in vacuo* and the residue was taken up in ethyl acetate. Some dibenzyl L-aspartate toluene-*p*-sulphonate separated at this stage and was recovered. The filtrate was washed with dilute hydrochloric acid and with water, and dried ($MgSO_4$). A slight excess of dicyclohexylamine was added, and addition of light petroleum (b. p. 60—80°) to the warm solution caused crystallisation of the *dicyclohexylammonium salt* of phthaloyl-γ-L-glutamyl-(dibenzyl L-aspartate); a further quantity was obtained by evaporation of the mother-liquors. The crude product (8·7 g., 84%) recrystallised from ethyl acetate as needles, m. p. 145—146°, $[\alpha]_D^{18}$ −19·6° (*c* 1·99 in ethanol), (Found: C, 68·4; H, 6·7; N, 5·7. $C_{43}H_{51}O_4N_3$ requires C, 68·5; H, 6·8; N, 5·6%).

Phthaloyl-γ-L-glutamyl-L-aspartic acid (bisdiphenylmethylammonium hydrogen salt). Phthaloyl-γ-L-glutamyl(dibenzyl L-aspartate) was liberated from a solution of the dicyclohexylammonium salt (4·56 g.) in 70% ethanol by the cation-exchange resin Amberlite IR-120 (H^+ form). The solution so obtained was hydrogenated at atmospheric pressure in the presence of palladium black (0·5 g.). The catalyst was filtered off and the filtrate was evaporated to dryness in a desiccator, yielding phthaloyl-γ-L-glutamyl-L-aspartic acid as a glass (2·4 g.). This was dissolved in dried ethanol, and diphenylmethylamine (purified by recrystallisation of the toluene-*p*-sulphonate, m. p. 246—246·5°) was added to pH 8 (moistened indicator paper). The solution was poured into dried ether, and the precipitated salt reprecipitated after treatment with charcoal. Analysis showed the product (3·65 g., 76%) to be *bisdiphenylmethylammonium hydrogen phthaloyl-γ-L-glutamyl-L-aspartate dihydrate*, m. p. 116—119° [Found: C, 65·2; H, 5·7; N, 6·7%; equiv. (titration), 798. $C_{43}H_{42}O_9N_4,2H_2O$ requires C, 65·0; H, 5·8; N, 7·05%; equiv., 794·5].

γ-L-Glutamyl-L-aspartic acid. The last-mentioned salt (1·15 g.) was dissolved in aqueous ethanol, and purified diphenylmethylamine was added to raise the pH to 8 (moistened indicator paper). Hydrazine hydrate (72·4 mg.) was added and the solution was left at room temperature for 4 days. Dilute hydrochloric acid was then added to pH 2, followed by diphenylmethylammonium acetate to pH 3·5; after filtration, ethanol was added until cloudiness appeared, whereafter at 0° γ-L-glutamyl-L-aspartic acid monohydrate separated. It was reprecipitated from water by ethanol, and for analysis was dried for 18 hr. at 45°/0·3 mm. (Found: C, 38·8; H, 5·4; N, 9·7. Calc. for $C_9H_{14}O_7N_2,H_2O$: C, 38·6; H, 5·8; N, 10·0%); it had m. p. 194·5—196°, $[\alpha]_D^{18}$ +24·6° (*c* 0·44 in water), $[\alpha]_D^{18}$ +18·6° (*c* 2·26 in 0·5N-hydrochloric acid). Paper chromatography showed a single spot (detected by ninhydrin): in butan-1-ol–water–acetic acid (62 : 26 : 12) R_F 0·13 (flow relative to aspartic acid, R_{asp}, 0·83; α-glutamylaspartic acid [3] had R_{asp} 1·3); in phenol–water (80 : 20), R_F 0·04 (R_{asp}, 0·66; α-glutamylaspartic acid had R_{asp} 0·68). After electrophoresis of the product on paper (Durrum-type apparatus), in 10% acetic acid, ninhydrin revealed only one component, travelling towards the cathode 0·76 of the distance of aspartic acid, while glutamic acid and α-glutamylaspartic acid travelled 1·22 and 1·72 times the distance of aspartic acid, respectively.

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