*Amino-acids and Peptides. Part XII.** α- *and* β-L-*Aspartyl*-L-*valine.*

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Benzyloxycarbonyl-L-aspartic anhydride has been shown to be dimorphous. With L-valine methyl ester it gave α- and β-coupling products, from which α- and β-L-aspartyl-L-valine have been obtained in the usual way. Unexpectedly, the α-dipeptide yields 1 mol. of carbon dioxide on prolonged heating with ninhydrin. There is also an indication, from paper chromatography, that when heated for some hours in aqueous solution it is partly converted into the β-isomer.

In Part VI (Le Quesne and Young, *J.*, 1952, 24) it was shown that benzyloxycarbonyl-L-aspartic anhydride reacts with amino-esters to give both α- and β-aspartyl derivatives, which were separated by fractional extraction from an organic solvent with aqueous sodium carbonate. This method has now been used to prepare α- and β-L-aspartyl-L-valine.

In Part VI (*loc. cit.*) we recorded a melting point of 111° for benzyloxycarbonyl-L-

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aspartic anhydride, for which Bergmann, Zervas, and Salzmann (*Ber.*, 1933, **66**, 1288) gave a melting point of 124°. We have now shown that this compound is dimorphous, and in contact with organic solvents the lower-melting slowly changes into the higher-melting form.

Coupling of the anhydride with L-valine methyl ester was effected both in anhydrous ethyl acetate and in the presence of aqueous sodium hydrogen carbonate. In both cases nearly quantitative yields of the mixed products were obtained, and in view of the economy with the ester the latter method was used subsequently. The isomers so formed were separated by fractional extraction from ethyl acetate with small portions of aqueous sodium carbonate (Le Quesne and Young, *J.*, 1950, 1954). Repeated fractionation gave crystalline benzyloxycarbonyl-α- and -β-L-aspartyl-L-valine methyl esters, which were hydrolysed to the acids; hydrogenation gave α- and β-L-aspartyl-L-valine. Paper chromatography revealed only one component in each case, the isomers being well separated when a mixture of *n*-butanol, acetic acid, and water was used as the mobile phase.

We remarked in Part VI (*loc. cit.*) on the characteristic blue colour given with ninhydrin by the β-aspartyl-peptides described there. This was observed on paper chromatograms, and is given by β-L-aspartyl-L-valine also. However, when aqueous solutions of the β-peptides are heated with ninhydrin, in the presence of sodium acetate, a red-brown colour is obtained, and a similar colour is given under Moore and Stein's conditions (*J. Biol. Chem.*, 1948, **176**, 367); asparagine behaves similarly. α-Aspartyl-peptides give the usual purple colour with ninhydrin on paper, and blue with Moore and Stein's solution.

We have determined the carbon dioxide evolved when the dipeptides react with ninhydrin under the conditions of Van Slyke, Dillon, MacFadyen, and Hamilton (*ibid.*, 1941, **141**, 627). The β-aspartyl-peptide, having a free α-amino-carboxyl group, gave 1·0—1·1 mols. of carbon dioxide (at pH 2·5). α-Aspartyl-peptides have only a β-amino-carboxyl group, but α-L-aspartyl-L-valine evolved appreciable amounts of carbon dioxide in 7 min.; when heating was continued for 20 min., *ca.* 1 mol. of carbon dioxide was obtained. No aspartic acid or valine (or β-dipeptide, see below) could be detected by chromatography on paper of a solution of the dipeptide which had been heated similarly, without the addition of ninhydrin. Although β-alanine evolves 0·16 mol. of carbon dioxide after 6 minutes' heating at pH 4·7, none is evolved at pH 2·5 (*idem, loc. cit.*).

Le Quesne (Thesis, Oxford, 1950) observed that when aqueous solutions of α-L-aspartyl-L-tyrosine and -L-glutamic acid had been heated for some hours at 100°, the colour given with ninhydrin by the remaining dipeptide on the paper chromatogram was blue, not purple. Similar treatment of solutions in 0·5N-hydrochloric acid resulted only in hydrolysis, and it was suggested that in the former case conversion into the β-isomer had occurred. We have now further indications of such isomerisation in the case of α-L-aspartyl-L-valine. Partition chromatography of an aqueous solution which had been heated for 6 hr. at 100° showed the presence of aspartic acid and valine, and of a substance corresponding in position to β-L-aspartyl-L-valine, and giving a blue colour with ninhydrin. In a similar experiment in the presence of sodium hydrogen carbonate, neither amino-acid nor β-dipeptide was detected, the α-dipeptide apparently remaining unchanged. Chromatography of an aqueous solution of the β-dipeptide which had been heated at 100° for 6 hr. revealed little change. There is some analogy with the rearrangement observed when the ethyl ester of benzoyl-DL-*iso*asparagine is heated with sodium carbonate (Battersby and Robinson, *Chem. and Ind.*, 1954, 45), and we hope to examine the reaction further.

Experimental

Benzyloxycarbonyl-L-aspartic Anhydride.—Finely powdered benzyloxycarbonyl-L-aspartic acid (10·2 g.) was dissolved in acetic anhydride (7 ml.) by warming to 45° with shaking. After 2 hr. the mixture had solidified; the crystals were washed with cold, dry ether and dissolved in hot ether. After removal of some solvent *in vacuo*, light petroleum (b. p. 60—80°) was added, giving colourless needles, m. p. 109—110° (7·3 g., 78%). Recrystallisation from ether–light petroleum gave long needles, m. p. 111°, $[\alpha]_D^{15}$ −38·6° (*c*, 2·25 in glacial acetic acid). In later

preparations, the crude product was dissolved in ether and allowed to crystallise below 0°; prisms were deposited, m. p. 123°, raised to 124° by recrystallisation from ether–light petroleum, $[\alpha]_D^{15}$ −37·3° (*c*, 2·88 in glacial acetic acid) (Found: C, 58·0; H, 4·6; N, 5·2. Calc. for $C_{12}H_{11}O_5N$: C, 57·8; H, 4·5; N, 5·6%). A saturated solution of the higher-melting form in glacial acetic acid at 16° had n_D^{16} 1·3961; a similar solution prepared from a mixture of both forms had n_D^{16} 1·3979 (see Williams and Young, *J.*, 1951, 1745). Infra-red absorption spectra of the two solids were nearly identical.

Benzyloxycarbonyl-α- and -β-aspartyl-L-valine Methyl Esters.—(*a*) L-Valine methyl ester (3·9 g.) in ethyl acetate was added to benzyloxycarbonyl-L-aspartic anhydride (2·0 g.) in ethyl acetate. After 48 hr. at room temperature the acidic products were extracted into aqueous sodium carbonate, from which they were removed after acidification by extraction with ethyl acetate. Removal of the solvent *in vacuo* left a solid mixture of the α- and β-isomers (2·9 g., 95%).

(*b*) L-Valine methyl ester hydrochloride (3·4 g.) and potassium hydrogen carbonate (6 g.) were dissolved in water (45 ml.) under ethyl acetate (30 ml.). Benzyloxycarbonyl-L-aspartic anhydride (5·0 g.) in ethyl acetate (30 ml.) was added, and stirring was continued for 2 hr. at room temperature. Next morning the aqueous layer was separated and acidified and the mixed isomers were extracted with ethyl acetate as before (7·3 g., 95%).

The crude products (27·6 g.) obtained by both methods were fractionated by dissolving them in ethyl acetate and extraction with 10-ml. portions of N-sodium carbonate. Acidification of the extracts gave oils which slowly solidified. Continued refractionation of the early fractions gave *benzyloxycarbonyl-β-L-aspartyl-L-valine methyl ester*, m. p. 160—162° (7·9 g., 27%), raised to 163—164·5° by recrystallisation from ethyl acetate, $[\alpha]_D^{19}$ −13·3° (*c*, 2·20 in H_2O containing 1 equiv. of sodium hydroxide) (Found: C, 56·7; H, 6·4; N, 7·1. $C_{18}H_{24}O_7N_2$ requires C, 56·8; H, 6·4; N, 7·4%).

Refractionation of the middle and later fractions gave *benzyloxycarbonyl-α-L-aspartyl-L-valine methyl ester* (11·7 g., 40%), m. p. 102—104°, $[\alpha]_D^{19}$ −8·4° (*c*, 2·34 in H_2O containing 1 equiv. of sodium hydroxide) (Found: C, 56·5; H, 6·3; N, 7·3%).

Benzyloxycarbonyl-α-L-aspartyl-L-valine.—The corresponding ester was hydrolysed with 2N-sodium hydroxide (2·2 equiv.) at room temperature for 20 min. Acidification gave the *acid* as an oil which crystallised slowly (yield, 90%), m. p. 137—139°, raised to 138—140° by recrystallisation from ethyl acetate–light petroleum. No optical rotation was observed in water containing 1 equiv. of sodium hydroxide (*c*, 2·4) (Found: C, 55·3; H, 6·1; N, 7·6. $C_{17}H_{22}O_7N_2$ requires C, 55·7; H, 6·1; N, 7·6%).

Benzyloxycarbonyl-β-L-aspartyl-L-valine.—The corresponding ester similarly gave the *acid*, m. p. 171—172°, raised to 172—173° by recrystallisation from acetone, $[\alpha]_D^{20}$ −24·8° (*c*, 2·41 in H_2O containing 1 equiv. of sodium hydroxide) (Found: C, 56·0; H, 6·2; N, 7·3%).

α-L-Aspartyl-L-valine.—Hydrogenation of the benzyloxycarbonyl derivative in the presence of palladium black gave the *dipeptide* (yield, quantitative) which, recrystallised from aqueous *n*-propanol, had m. p. 161—164°; $[\alpha]_D^{20}$ 0° ± 1° (*c*, 2·34 in H_2O), $[\alpha]_D^{20}$ +14° (*c*, 2·34 in glacial acetic acid), R_F in phenol 0·35, in *n*-butanol–acetic acid–water (62 : 12 : 26 vol.) 0·46 (Found: C, 44·6; H, 7·1; N, 11·4. $C_9H_{16}O_5N_2,\frac{1}{2}H_2O$ requires C, 44·8; H, 7·1; N, 11·6%). Hydrolysis in 20% hydrochloric acid gave a solution with the expected optical activity.

β-L-*Aspartyl-L-valine.*—This *dipeptide* was obtained similarly and had m. p. 197—200°, $[\alpha]_D^{20}$ −10° (*c*, 1·48 in H_2O), R_F in phenol 0·39, in *n*-butanol–acetic acid–water (62 : 12 : 26 vol.) 0·33 (Found: C, 44·5; H, 6·9; N, 11·9%).

Decarboxylation of the Dipeptides by Ninhydrin.—Under the conditions of the α-amino-carboxyl determination (Van Slyke *et al.*, *loc. cit.*), β-L-aspartyl-L-valine gave 1·0—1·1 mol. of carbon dioxide in 7 min. at 100° and pH 2·5. When α-L-aspartyl-L-valine was heated similarly for 20 min., *ca.* 1·0 mol. of carbon dioxide was evolved; shorter periods of heating gave smaller and variable amounts of carbon dioxide. In a control experiment, the α-dipeptide was heated similarly for 20 min. but without the addition of ninhydrin; paper chromatography (with phenol and with *n*-butanol–acetic acid–water) failed to reveal aspartic acid, valine, or β-dipeptide in the product.

Effect of Heating Aqueous Solutions of the Dipeptides.—Solutions (1%) of the dipeptides were sealed into test tubes and heated in a boiling-water bath for 6 hr. The resulting solutions were examined by paper chromatography, with phenol and with *n*-butanol–acetic acid–water as solvents. α-L-Aspartyl-L-valine gave spots corresponding in position to aspartic acid, valine, unchanged dipeptide, and β-L-aspartyl-L-valine (blue); known samples were run beside the unknown. β-L-Aspartyl-L-valine, similarly treated, gave chromatograms showing unchanged

dipeptide only. A solution of the α-dipeptide in aqueous sodium hydrogen carbonate was heated at 100° for 6 hr.; the solution was then made acid to Congo-red and a little sodium acetate was added. Chromatography showed the presence of α-dipeptide only, the spot corresponding in position to that obtained from a solution prepared similarly but not heated.

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