α-L- and D-Aspartylglycine and D-α-Asparagine

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α-L- and D-aspartylglycine and D-α-asparagine have been prepared *via* the mixed anhydride of N-benzyl-L- (D-) aspartic acid and chloroformic acid.

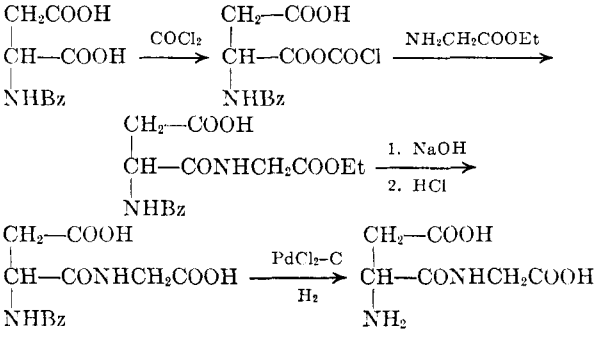
The preparation of optically active α-aspartylpeptides which are entirely free from the corresponding β-isomers is not easily accomplished. The two most important methods so far devised for their synthesis have been worked out by Young.[1-3] In the first method benzyloxycarbonyl-L-aspartic anhydride was coupled with an amino acid ester[1,2] whereby a mixture of α- and β-aspartyldipeptides was produced which was then separated by repeated extraction with sodium bicarbonate. The difficulties inherent in this procedure are illustrated by the fact that the separation of the α- and β-aspartylglycines was only achieved after thirty successive extractions[4] and the aspartylleucines could not be separated satisfactorily.[3] Alternatively, β- and α-benzyl benzyloxycarbonyl-L-aspartate were coupled with the amino acid benzyl esters, yielding the α- and β-aspartyldipeptide esters, respectively.

Recently, Weygand demonstrated[5] that α-aspartylpeptides are produced when N-trifluoroacetyl-L-aspartic anhydride reacted with amino acid esters at room temperature. However, yields were low, probably due to preferential formation of β-aspartyldipeptides which were not isolated.

We had previously found, while preparing racemic α- and β-aspartyldipeptides *via* the mixed anhydride of N-benzyl-DL-aspartic acid and chloroformic acid,[6,7] that N-benzyl-α-aspartylglycine is insoluble in water and is, therefore, precipitated after hydrolysis of its ethyl ester with sodium hydroxide and acidification with hydrochloric acid, in contradistinction to N-benzyl-β-aspartylglycine which is water-soluble. This method suffered from the disadvantage that only racemic substances could be prepared. However, since N-benzyl-DL-aspartic acid had been resolved recently,[8] we intended to apply this method to the synthesis of α-L- and D-aspartylglycine and D-α-asparagine (these D-isomers have not been reported in the literature, so far), hoping that the above-mentioned solubility differences between the α- and β-derivatives would also be maintained in the optically active series.

In fact, according to the properties of the final substances, it appeared that only the α-isomers were produced. Reaction of the optically active N-benzylaspartic acids with phosgene in dioxane at room temperature proceeds much more quickly than with the racemic compound at 50–60°. It must, however, be emphasized that good results in this reaction depend on the use of high grade reagents; this especially holds true for the phosgene. In contradistinction to the racemic N-benzylaspartylglycine ester which precipitated mainly together with the ethyl glycinate hydrochloride, the two respective enantiomorphs were exclusively isolated from the dioxane solution. They were thus essentially pure and not contaminated with the glycine ester hydrochloride which is almost insoluble in dioxane. Therefore, it was practical to hydrolyze this substance with sodium hydroxide without further purification, since only N-benzyl-α-D- (or L-) aspartylglycine was precipitated after acidification with hydrochloric acid. Hydrogenolysis of the N-benzyl group was then carried out in absolute ethanol using a palladium chloride on Norit catalyst (30%), since it was observed that glacial acetic acid, used in the racemic series,[6] caused partial hydrolysis of the optically active dipeptides. No racemization seemed to take place in the coupling reaction with the mixed anhydride and the α-D-aspartylglycine had a specific rotation of $[\alpha]^{25}D$ −35.2° which agrees with the values given for its enantiomorph in the literature, although of course, of opposite sign. On paper chromatograms it appeared as a purple spot[1] and it gave a positive biuret reaction.[6]

D-α-Asparagine, whose enantiomorph has recently been prepared by improved methods, *via*



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p-toluenesulfonyl-L-aspartic anhydride[9] and through N-trifluoroacetyl-L-aspartic anhydride,[10] was synthesized by the above route in optically and chromatographically pure condition.

Experimental

Microcombustion analyses are by Drs. Weiler and Strauss (Oxford). Melting points were determined in a Fisher-Johns apparatus. The ascending method of paper partition chromatography was used (80% phenol). Hydrogenolyses were carried out in a Parr low pressure hydrogenation apparatus at 70–80°.

Mixed Anhydride of N-Benzyl-D-aspartic Acid and Chloroformic Acid.—N-Benzyl-D-aspartic acid[8] (7.5 g.) was suspended in dry dioxane (150 ml.) in a three-necked, round-bottomed flask, equipped with a gas leading tube, a reflux condenser protected by a calcium chloride tube, and a mechanical stirrer. Phosgene (The Matheson Co., Inc.) was introduced with stirring at room temperature and a clear solution resulted after about 5 min. After altogether 35 min. the introduction of phosgene was discontinued. Excess phosgene was removed at room temperature by bubbling dry nitrogen through the solution until the odor of phosgene was no longer perceptible. The mixed anhydride was always freshly prepared before coupling with amino acid esters and used directly in dioxane solution.

Ethyl N-Benzyl-α-D-aspartylglycinate.—To the above solution of the mixed anhydride, cooled in an ice bath, was added dropwise with stirring freshly prepared ethyl glycinate (8.5 g.) and the reaction mixture was left overnight. The precipitate which had formed meanwhile, consisting almost exclusively of ethyl glycinate hydrochloride, was filtered off and the solution evaporated *in vacuo*. Acetone was added to the residual oil and the substance which crystallized was filtered off. Recrystallization from ethanol gave long needles, m.p. 137° (5.5 g., 53%); $[\alpha]^{23}D$ +24° (*c* 6.25, in water).

Anal. Calcd for $C_{15}H_{20}O_5N_2$: C, 58.2; H, 6.5; N, 9.1. Found: C, 58.3; H, 6.5; N, 8.9.

N-Benzyl-α-D-aspartylglycine.—Ethyl N-benzyl-α-D-aspartylglycinate (3.5 g.) was dissolved in 1 *N* sodium hydroxide solution (24 ml.) and left at room temperature for 90 min. On acidification with hydrochloric acid to pH 4, N-benzyl-α-D-aspartylglycine was precipitated. After cooling for about 0.5 hr. it was filtered off. Recrystallization from aqueous ethanol gave m.p. 198° (3 g., 94%); $[\alpha]^{21}D$ +17.6 (*c* 12.5, in 5% sodium bicarbonate).

Anal. Calcd. for $C_{13}H_{16}O_5N_2$: N, 10. Found: N, 10.0.

α-D-Aspartylglycine.—N-Benzyl-α-D-aspartylglycine (2 g.) was suspended in absolute ethanol (100 ml.) and the catalyst (0.3 g. of 30% palladium chloride on Norit) added. After 10 hr. of hydrogenolysis the dipeptide which adhered to the catalyst was filtered off and separated from the latter by recrystallization from water; m.p. 178° (0.81 g., 60%); $[\alpha]^{25}D$ −35.2° (*c* 10.06, in 1 *N* hydrochloric acid). The substance gave a positive biuret reaction and a purple spot on paper chromatograms (R_f 0.17).

Anal. Calcd. for $C_8H_{10}O_5N_2$: C, 37.8; H, 5.3; N, 14.7. Found: C, 37.4; H, 5.6; N, 14.4.

Ethyl α-D-Aspartylglycinate.—Ethyl N-benzyl-α-D-aspartylglycinate (2 g.) was dissolved in ethanol (50 ml.) and the catalyst (0.3 g.) added. After 6 hr. of hydrogenolysis, the catalyst was filtered off and the solvent evaporated *in vacuo*. The residue was recrystallized from ethanol; m.p. 167°; (0.7 g., 50%). The substance gave a positive biuret reaction.

Anal. Calcd. for $C_8H_{14}O_5N_2$: C, 44.0; H, 6.4; N, 12.8. Found: C, 43.6; H, 6.2; N, 12.8.

N-Benzyl-D-α-asparagine.—To an ice-cooled solution of the mixed anhydride in dioxane, prepared from 5.6 g. of N-benzyl-D-aspartic acid, was added gaseous ammonia until no more precipitate formed. After filtration the crude substance containing ammonium chloride was dried in a vacuum desiccator and recrystallized from ethanol; 167° (2.9 g., 52%); $[\alpha]^{21}D$ +24° (*c* 12.5, in water). The substance gave a positive biuret reaction.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.5; H, 6.3; N, 12.6. Found: C, 59.1; H, 6.3; N, 12.9.

D-α-Asparagine.—N-Benzyl-D-α-asparagine (0.4 g.) was dissolved in absolute ethanol (25 ml.) and the catalyst (0.1 g.) added. After 8 hr. of hydrogenolysis, the substance was freed from the catalyst to which it adhered by dissolving it in a small quantity of water and adding a large volume of absolute ethanol, after filtration. It crystallized in long needles which gave a red biuret reaction. On paper chromatograms it gave a purple spot (R_f 0.41); (0.18 g., 67%); $[\alpha]^{23}D$ −14.2° (*c* 3, in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_4H_8O_3N_2 + H_2O$: C, 32.0; H, 6.2; N (total), 18.7; N (Van Slyke), 9.3. Found: C, 31.8; H, 6.2; N (total), 18.3; N (Van Slyke), 9.1.

Ethyl N-Benzyl-α-L-aspartylglycinate.—An ice-cooled solution of the mixed anhydride in dioxane, prepared from N-benzyl-L-aspartic acid[8] (9 g.), was coupled with ethyl glycinate (12 g.). The substance was isolated in the same way as its enantiomorph; m.p. 136°; (6.4 g., 51%); $[\alpha]^{24}D$ −23.8° (*c* 4.5, in water).

Anal. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.2; H, 6.5; N, 9.1. Found: C, 58.4; H, 6.5; N, 9.0.

N-Benzyl-α-L-aspartylglycine.—Ethyl N-benzyl-α-L-aspartylglycinate (3.4 g.) was hydrolyzed to yield N-benzyl-α-L-aspartylglycine. After recrystallization from aqueous ethanol m.p. 197°; (2.8 g., 93%); $[\alpha]^{23}D$ −17.0° (*c* 12.5, in 5% sodium bicarbonate).

Anal. Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.6; H, 5.7; N, 10.0. Found: C, 55.2; H, 5.7; N, 9.9.

α-L-Aspartylglycine.—After hydrogenolysis of N-benzyl-α-L-aspartylglycine (1.9 g.), the dipeptide was isolated and purified in the same manner as its enantiomorph; m.p. 187°; (0.8 g., 57%). The substance gave a positive biuret reaction and a purple spot on paper chromatograms (R_f 0.17); $[\alpha]^{24}D$ +31.5° (*c* 2.5, in 1 *N* hydrochloric acid).

Anal. Calcd. for $C_8H_{10}O_5N_2 + H_2O$: C, 34.6; H, 5.8; N, 13.6. Found: C, 35.0; H, 5.6; N, 13.3.

Ethyl α-L-Aspartylglycinate.—Ethyl N-benzyl-α-L-aspartylglycinate (2.5 g.) was hydrogenolyzed as above. The dipeptide ester gave a positive biuret reaction; m.p. 166–167° (0.8 g., 45%).

Anal. Calcd. for $C_8H_{14}O_5N_2$: C, 44.0; H, 6.4; N, 12.8. Found: C, 43.5; H, 6.4; N, 12.8.

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