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The Synthesis of a Sweet Peptide, α-L-Aspartyl-L-phenylalanine Methyl Ester, without the Use of Protecting Groups

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The large-scale preparation of α-aspartyl peptides which are entirely free from the corresponding β-isomers is not easily accomplished. In a previous paper the present authors reported a convenient one-step preparation of the hydrochloride and hydrobromide of L-aspartic anhydride, which might be useful intermediates for preparing aspartyl peptides.[1] It then appeared of interest to prepare α-L-aspartyl-L-phenylalanine methyl ester (α-APM), which has a sweet taste,[2] by the direct condensation of L-aspartic anhydride hydrochloride with methyl L-phenylalaninate. Although Kovacs *et al.* described the condensation of L-aspartic anhydride hydrobromide with ethyl glycinate to give a mixture of α- and β-aspartyl glycine ethyl esters,[3] its practical use for peptide synthesis has not yet been reported in the literature.

Simply by mixing L-aspartic anhydride hydrochloride and methyl L-phenylalaninate in an organic solvent, we can obtain a mixture of α- and β-APM in a fairly good yield. This coupling reaction, however, is always accompanied with side-reactions, such as the self-condensation of L-aspartic anhydride and the further condensation between the unchanged anhydride and the resulting dipeptide ester, since the amino group of the aspartyl residue is unprotected. In order to overcome these disadvantages and, in particular, to increase the formation of the α-isomer, the reaction conditions, such as the temperature, the solvent effects, and the molar ratios of the two reactants, were investigated. It was found that the reaction could be effected by adding such weak acids as acetic acid, by employing an excess of methyl L-phenylalaninate, and by conducting it at a low temperature. This method would appear to have considerable promise for conveniently preparing α-APM on a large scale, but it is necessary to separate the α-APM from the reaction mixture.

In the course of investigations of this peptide, we had occasion to attempt to hydrolyze the methyl ester group of α-APM in dilute hydrochloric acid. Contrary to expectations, α-APM hydrochloride (α-APM·HCl) readily crystallized out when α-APM was dissolved in dilute hydrochloric acid. This finding is interesting, since most peptides usually increase their

1) Y. Ariyoshi, T. Yamatani, N. Uchiyama, and N. Sato, This Bulletin, **45**, 2208 (1972).

2) R. H. Mazur, J. M. Schlatter, and A. H. Goldkamp, *J. Amer. Chem. Soc.*, **91**, 2684 (1969).

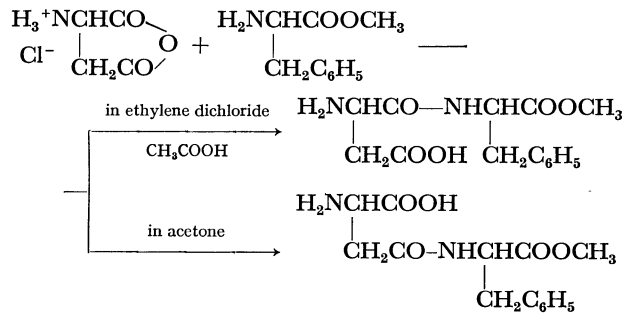
3) J. Kovacs, H. N. Kovacs, and R. Ballina, *ibid.*, **85**, 1839 (1963).

solubilities in such an acidic solution; *e.g.*, some closely related compounds, such as α-L-aspartyl-D-phenylalanine methyl ester hydrochloride and α-L-aspartyl-L-phenylalanine ethyl ester hydrochloride, are too soluble to be isolated. α-APM was also sparingly soluble in aqueous hydrobromic acid, while β-APM was almost freely soluble in both dilute hydrochloric and hydrobromic acids.

This characteristic was used successfully in the separation of α- and β-APM. After the separation, the recovery of the α-APM from the salt, and that of the β-APM from the mother liquor, could be accomplished easily and in an excellent yield by conventional neutralization. The ester group and the peptide bond were stable enough for the separation procedure.

The treatment of L-aspartic anhydride hydrochloride with 4 molecular equivalents of methyl L-phenylalaninate in the presence of acetic acid in ethylene dichloride at −20 °C gave a mixture of α- and β-APM, from which α-APM was isolated in a 37% yield (from the anhydride) by separation through its hydrochloride, followed by the neutralization of the salt with sodium carbonate and subsequent column chromatography.

On the other hand, when the reaction was conducted in acetone in the absence of acids, β-APM was formed predominantly and was isolated in a 36% yield.



Experimental

All the melting points are uncorrected. The peptides used here were left over from a previous investigation.[4] The optical rotations were measured by means of a visual polarimeter. The determination of the α- and β-APM by paper electrophoresis was carried out according to the method described in a previous paper.[5]

α-L-Aspartyl-L-phenylalanine Methyl Ester Hydrochloride Dihydrate (α-APM·HCl). α-APM (5.0 g) was dissolved in 1 M hydrochloric acid (50 ml) with slight warming, and then the solution was kept in a refrigerator overnight. The crystals thus formed were collected by filtration; yield, 5.2 g (86%); mp 127—128 °C (decomp., partly melted at 103 °C); $[\alpha]_D^{25}$ +1.3° (*c* 2, water). Lit,[6] mp 105—110 °C. Found: C, 45.86; H, 6.18; N, 7.55; Cl, 9.73%. Calcd for $C_{14}H_{18}O_5N_2 \cdot HCl \cdot 2H_2O$: C, 45.84; H, 6.32; N, 7.64; Cl, 9.67%.

α-L-Aspartyl-L-phenylalanine Methyl Ester Hydrobromide Dihydrate (α-APM·HBr). This salt was obtained from a solution of α-APM (5.0 g) in 1M hydrobromic acid (100 ml) by the procedure used in the preparation of α-APM·HCl; yield, 4.3 g (63%); mp 155 °C (decomp.); $[\alpha]_D^{2.5}$ +1.0° (*c* 2, water). Found: C, 41.13; H, 5.76; N, 6.85; Br, 19.39%. Calcd for $C_{14}H_{18}O_5N_2 \cdot HBr \cdot 2H_2O$: C, 40.89; H, 5.64; N, 6.81; Br, 19.43%.

Separation of α- and β-APM. α-APM (10 g) and β-APM (10 g) were dissolved in 1 M hydrochloric acid (100 ml) with slight warming. The solution was then kept in a refrigerator overnight. The crystals thus formed were collected by filtration; yield, 9.4 g (78%). The IR spectrum (Nujol) of the crystals was identical with that of the authentic α-APM·HCl. The crystals (9.3 g) were dissolved in water (90 ml), and the solution was adjusted to pH 4.8 with a 5% aqueous sodium bicarbonate solution while being stirred at 50 °C. The solution was then kept in a refrigerator overnight. The crystals thus formed were collected by filtration and washed with a small amount of chilled water; yield, 6.7 g (67%). The crystals were found by paper electrophoresis to be free from the β-isomer. For elemental analysis, the crystals were recrystallized from water; mp 235—236 °C (decomp.); $[\alpha]_D^{25}$ +32.0° (*c* 1, acetic acid). Lit,[2] mp 95 °C and 245—247 °C. Lit,[7] mp 246—247 °C. Lit,[4] mp 235—236 °C; $[\alpha]_D$ +32° (*c* 1, acetic acid). Found: C, 55.52; H, 6.13; N,9.32%. Calcd for $C_{14}H_{18}O_5N_2 \cdot 1/2H_2O$: C, 55.44; H, 6.31; N, 9.24%.

The filtrate from the α-APM·HCl was adjusted to pH 4.8 with a 5% aqueous sodium bicarbonate solution while being stirred at 50 °C; then the solution was treated in the manner described above to give 5.3 g (53%) of β-APM as needles. These needles were found by paper electrophoresis to be free from the α-isomer; mp 198—199 °C; $[\alpha]_D^{23}$ +40.5° (*c* 1, acetic acid). Lit,[2] mp 196—197 °C. Lit,[4] mp 198—199 °C; $[\alpha]_D$ +40.5° (*c* 1, acetic acid). Found: C, 55.29; H, 6.36; N, 9.27%. Calcd for $C_{14}H_{18}O_5N_2 \cdot 1/2H_2O$: C, 55.44; H, 6.31; N, 9.24%.

Synthesis of α-APM. A solution of methyl L-phenylalaninate hydrochloride (90 g) in water (450 ml) was neutralized with sodium carbonate (24 g), and the resulting free ester was extracted with two 350-ml portions of ethylene dichloride. To the organic solution there were then added acetic acid (9 g) and methanol (8 ml). Into this solution, L-aspartic anhydride hydrochloride (15.2 g)[1] was stirred at −20 °C. After stirring for 30 min at this temperature, hot water (350 ml, 70—80 °C) and an aqueous solution (300 ml) of sodium carbonate (5.7 g) were added successively to the reaction mixture. After the removal of the excess of methyl L-phenylalaninate by two extractions with 150-ml portions of ethylene dichloride, the aqueous layer was adjusted to pH 4.8 with dilute hydrochloric acid. The solution was found by paper electrophoresis to contain 18.2 g (60%) of α-APM and 6.1 g (20%) of β-APM. The solution was concentrated *in vacuo* to about 100 ml, and 36% hydrochloric acid (30 ml) was added to the concentrate. The solution was kept in a refrigerator overnight. The crystals (α-APM·HCl, 21.3 g, 58%) thus formed were collected by filtration and dissolved in water (200 ml). The solution was adjusted to pH 4.8 with a 5% aqueous sodium carbonate solution while being stirred at 50 °C, and then kept in a refrigerator overnight. The crystals thus formed were collected by filtration; yield, 13.0 g (43%). The crystals were dissolved in water (500 ml) and passed through a column (1×20 cm) of Dowex 1×4 (acetate form) maintained at 45 °C, and then the column was washed with water (20 ml). The eluate and washings were concentrated *in vacuo* to give α-APM, which was recrystallized from water; yield, 11.2 g (37%); mp 235—236 °C (decomp.); $[\alpha]_D^{22}$ +32.0° (*c* 1, acetic acid).

4) Y. Ariyoshi and N. Sato, This Bulletin, **45**, 2015 (1972).
5) Y. Ariyoshi and N. Sato, *ibid.*, **45**, 942 (1972).
6) P. M. Hardy, G. W. Kenner, R. C. Sheppard, and J. S. Morley, Brit. 1042484 (1966).
7) J. M. Davey, A. H. Laird, and J. S. Morley, *J. Chem. Soc., C*, **1966**, 555.

Found: C, 55.30; H, 6.19; N, 9.36%.

Synthesis of β-APM. To a stirred solution of methyl L-phenylalaninate (72 g) in acetone (750 ml), there was added L-aspartic anhydride hydrochloride (30.3 g) at −30 °C. After having been stirred for 1 hr at this temperature, the reaction mixture was allowed to attain room temperature and then concentrated *in vacuo*. The residue was dissolved in water (750 ml) containing sodium bicarbonate (20 g). The aqueous solution was extracted with two 500-ml portions of ethylene dichloride to remove the excess methyl L-phenylalaninate. The aqueous layer was adjusted to pH 4.8 with dilute hydrochloric acid and then concentrated to about 400 ml *in vacuo*. After storage in a refrigerator overnight, the crystals thus formed were collected by filtration; yield, 27.0 g (45%). Recrystallization from water gave 23.5 g (36%) of β-APM as needles, which were confirmed by paper electrophoresis to be free from the α-isomer; mp 198—199 °C; $[\alpha]_D^{23}$ +40.5° (*c* 1, acetic acid). Found: C, 55.79; H, 6.07; N, 9.39%.