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The Separation of α- and β-L-Aspartyl-L-phenylalanine Methyl Ester with Aromatic Acids

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Although aspartyl peptides can be conveniently prepared by the coupling of *N*-protected aspartic anhydrides with amino acids or amino acid esters, followed by deprotection, this method usually gives a mixture of α- and β-aspartyl peptides.[1–3] Furthermore, the separation of the isomers is generally so difficult and so complicated that only a few methods have been reported; in them techniques of fractional extraction and column chromatography were used.[2,3] Some aromatic acids have been used as specific precipitating reagents for amino acids.[4,5] However, no specific precipitating reagent for the α- and β-aspartyl peptides has been reported in the literature.

Table 1. Separation of α- and β-APM with various precipitating reagents

Reagent	APM	Grams of α- and β-APM in 100 m*l* of aqueous solution: Initial solution	Mother liquor
[a]3,5-Dinitrobenzoic acid	α-APM	1.00	0.10
	β-APM	1.00	0.97
[b]β-Phenylpropionic acid	α-APM	1.00	0.26
	β-APM	1.00	0.91
[a]Cinnamic acid	α-APM	1.00	0.20
	β-APM	1.00	0.92
[b]*p*-Hydroxybenzoic acid	α-APM	1.00	0.30
	β-APM	1.00	1.00
[b]Gentisic acid	α-APM	1.00	0.16
	β-APM	1.00	1.00
[b]β-Resorcylic acid	α-APM	1.00	0.01
	β-APM	1.00	0.96
[b]5-Chlorosalicylic acid	α-APM	1.00	0.10
	β-APM	1.00	1.00
[a]2,5-Dichlorophenol	α-APM	1.00	0.06
	β-APM	1.00	1.00
[b]β-Naphthoic acid	α-APM	1.00	0.28
	β-APM	1.00	1.00
[b]β-Naphthoxyacetic acid	α-APM	1.00	0.27
	β-APM	1.00	0.99

To α-APM in the initial solution, 1.2[a] or 2.4[b] molecular equivalents of the reagents were used.

α-L-Aspartyl-L-phenylalanine methyl ester (α-APM), which has a sweet taste very similar to that of sucrose, has been found as a new sweetening agent.[6] When α-APM was prepared by the condensation of such *N*-protected L-aspartic anhydrides as benzyloxycarbonyl-L-aspartic anhydride with methyl L-phenylalaninate and by subsequent deprotection, the corresponding β-derivative, β-APM, was also produced as a by-product. α-APM must be sufficiently purified from β-APM, since β-APM has a weakly bitter taste. Purification by recrystallization from water is ineffective because α- and β-APM have almost equal solubilities, and because α-APM is easily changed to the diketopiperazine derivative by heating.

In the course of studies of the sweet peptide, it was

Table 3. Separation of α- and β-APM with various reagents on preparative scale

Mixture α-APM g	Mixture β-APM g	Reagent	g	Water m*l*	Recovery α-APM g (%)	Recovery β-APM g (%)
5.0	5.0	3,5-Dinitrobenzoic acid	4.2	500	3.1 (62)	2.5 (50)
5.0	5.0	Cinnamic acid	3.0	500	3.2 (64)	3.2 (64)
5.0	5.0	Gentisic acid	6.0	500	3.1 (62)	4.4 (88)
5.0	5.0	β-Resorcylic acid	12.0	500	3.5 (70)	3.9 (78)

Table 2. Characters of precipitated compounds

Compound	Reagent	Component ratio α-APM:Reagent	Mp °C	Elementary analysis (%)	
I	3,5-Dinitrobenzoic acid	1:1	141—142	$C_{21}H_{22}O_{11}N_4 \cdot H_2O$	Found: C, 48.09; H, 4.81; N, 10.68 Calcd: C, 48.09; H, 4.61; N, 10.68
II	Cinnamic acid	1:1	140—141	$C_{23}H_{26}O_7N_2 \cdot H_2O$	Found: C, 59.80; H, 6.29; N, 6.35 Calcd: C, 59.99; H, 6.13; N, 6.08
III	Gentisic acid	1:1	133—134	$C_{21}H_{24}O_9N_2 \cdot H_2O$	Found: C, 54.27; H, 5.53; N, 5.75 Calcd: C, 54.07; H, 5.62; N, 6.01
IV	β-Resorcylic acid	1:2	182—186	$C_{28}H_{30}O_{13}N_2$	Found: C, 55.79; H, 5.04; N, 4.83 Calcd: C, 55.81; H, 5.02; N, 4.65

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found that α-APM forms sparingly soluble compounds in water with 3,5-dinitrobenzoic acid, β-phenylpropionic acid, cinnamic acid, *p*-hydroxybenzoic acid, gentisic acid, β-resorcylic acid, 5-chlorosalicylic acid, 2,5-dichlorophenol, β-naphthoic acid, and β-naphthoxyacetic acid. β-APM, on the contrary, did not form such compounds with these reagents. This characteristic was successfully used for the separation of the two isomers of APM. When a mixture of α- and β-APM was placed in contact with these reagents in water, α-APM was selectively precipitated with the reagents, while β-APM remained almost quantitatively in the mother liquor (Table 1).

The separation of the isomers on a preparative scale was achieved with 3,5-dinitrobenzoic acid, cinnamic acid, gentisic acid, and β-resorcylic acid. The elementary analyses showed that the precipitates consist of α-APM and the reagent in a 1: 1 molar ratio, except for the precipitate with β-resorcylic acid, which consists of α-APM and β-resorcylic acid in a 1: 2 molar ratio (Table 2).

The precipitates and the mother liquors were separately acidified with hydrochloric acid. After removing the liberated reagents by extraction with ether, the acidic solutions were adjusted to the isoelectric point of APM with sodium bicarbonate. The α- and β-APM thus crystallized out were recovered by filtration in fairly high yields (Table 3).

Experimental

The melting points are uncorrected.

α- and β-APM. α- and β-APM were prepared by the method described by Mazur *et al.*[6] α-APM: mp 235—236°C (dec.); $[\alpha]_D^{25}+32.0°$ (*c* 1, acetic acid); $[\alpha]_D^{24}\pm 0°$ (*c* 1, water). lit, mp 246—247°C (sintered at 190°C); $[\alpha]_D$ 0° (water).[6] lit, mp 246—247°C; $[\alpha]_D-2.3°$ (*c* 1, N HCl).[7] β-APM: mp 198—199°C (dec.); $[\alpha]_D^{28}+42.5°$ (*c* 1, acetic acid); $[\alpha]_D^{24}$ $+4.0°$ (*c* 1, water). lit, mp 196—197°C; $[\alpha]_D+4°$ (water).[6]

Determination of α- and β-APM. Paper electrophoresis was carried out in aqueous acetic acid of pH 2.77 at 40 V/cm for 1.5 hr; the paper was stained with a cadmium-ninhydrin reagent.[8] The two spots corresponding to α- and β-APM were individually cut off and eluted with methanol, and the absorbances of the eluates were measured at 510 mμ.

Crystalline Compounds of α-APM with the Various Reagents (I, II, III, and IV in Table 2). *α-APM Compound with 3,5-Dinitrobenzoic Acid (I):* To a stirred solution of 2.0 g of α-APM in 200 m*l* of water, 1.7 g of 3,5-dinitrobenzoic acid was added at room temperature. The solution was kept in a refrigerator overnight. The crystals thus formed as needles were collected by filtration; yield, 3.2 g (89%).

α-APM compound with cinnamic acid (II) was obtained from a mixture of 1.0 g of α-APM and 1.0 g of cinnamic acid in 100 m*l* of 50% aqueous ethanol; yield, 0.9 g (60%).

α-APM compounds with gentisic acid (III) and with β-resorcylic acid (IV) were obtained form a mixture of 1.0 g of α-APM and 0.5 g of gentisic acid or β-resorcylic acid in 100 m*l* of water; yield of III, 0.8 g (50%); yield of IV, 0.5 g (28%). For anal. and mp, see Table 2.

Separation of α- and β-APM with the Various Precipitating Reagents. A typical run was as follows (Table 3): To 500 m*l* of an aqueous solution containing 5.0 g of α-APM and 5.0 g of β-APM, 3.0 g of cinnamic acid was added with stirring at room temperature. The mixture was stirred for 5 hr at room temperature and then kept in a refrigerator overnight. The precipitate was collected by filtration and suspended in 500 m*l* of water. The mixture was adjusted to pH 2.0 with hydrochloric acid. The cinnamic acid thus liberated was removed by extraction with ether. The aqueous solution was adjusted to pH 5.0 with sodium bicarbonate and evaporated *in vacuo* to dryness. The residual crystals were recrystallized from 100 m*l* of water; yield, 3.2 g (64%); mp 235—236°C (dec.); $[\alpha]_D^{26}+32.0°$ (*c* 1, acetic acid). Found: C, 55.50; H, 6.21; N, 9.35%. Cacld for $C_{14}H_{18}O_5N_2\cdot 1/2\,H_2O$: C, 55.44; H, 6.31; N, 9.24%. The compound was confirmed by paper electrophoresis to be free from the β-isomer.

The mother liquor, excluding the α-isomer, was treated as above to give β-APM; yield, 3.2 g (64%); mp 198—199°C (dec.); $[\alpha]_D^{26}+43.0°$ (*c* 1, acetic acid). (Found: N, 9.30%). The compound was confirmed by paper electrophoresis to be pure β-APM.

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