Optical Resolution of Amino Acid Esters by *N*-Protected Aspartylphenylalanine Esters

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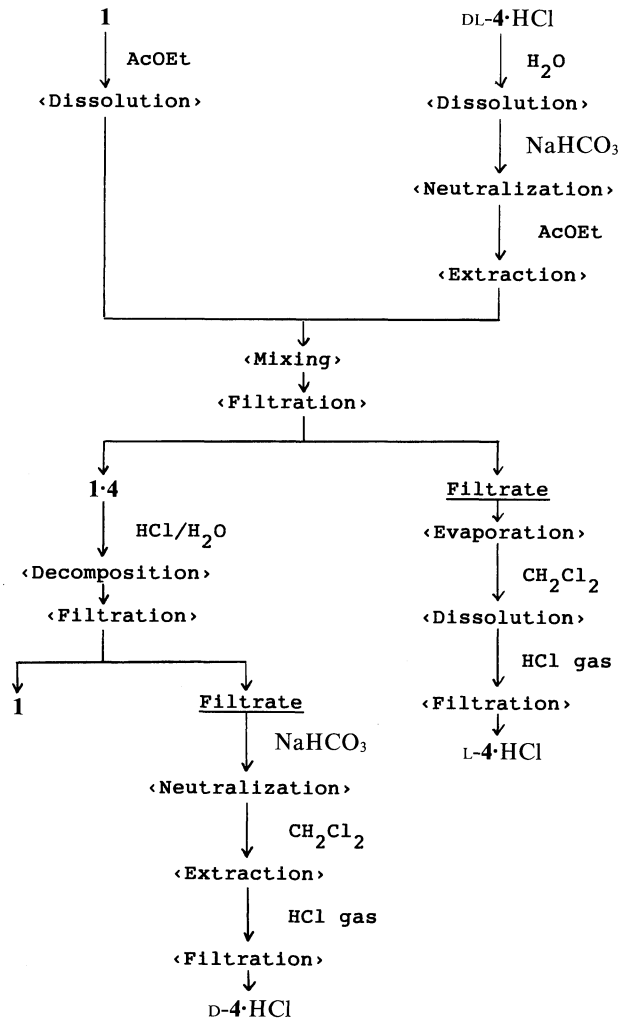
A new method was studied for optical resolution of racemic amino acid esters by using, as resolving agents, *N*-protected L-α-aspartyl-L-phenylalanine alkyl esters, typically, *N*-benzyloxycarbonyl-L-α-aspartyl-L-phenylalanine methyl ester, the precursor to the synthetic sweetener aspartame. Thus, when the dipeptide and racemic amino acid ester were mixed in solvents, precipitation took place by formation of salt preferentially with D-amino acid ester to effect the optical resolution.

The optical resolution of recemates is of great importance in preparation of optically active compounds. In the resolution of amino acids, preferential crystallization, diastereomeric procedure, enzymatic degradation, etc., are commonly used.[1)]

We reported in 1979 novel enzymatic reactions in which *N*-protected aspartic and glutamic acids whose side-chain carboxylates were unprotected underwent reactions with amino acid esters.[2)] It was also found that, when racemic substrates were used, the reaction gave an L–L dipeptide, which precipitated as a salt exclusively with a D-amino acid ester through the unprotected side-chain carboxylate of the dipeptide.[3)] Such reactions are very interesting not only from the point of view of the synthesis of L–L dipeptides from racemic substrates but also from the point of view of optical resolution of racemic amino acid esters. If the selective salt formation with D-amino acid esters should not involve the enzyme, then the dipeptides may find themselves very useful as the resolving agents for racemic amino acid esters. Therefore, optical resolution of amino acid esters by using side-chain unprotected aspartyl dipeptides were investigated.

Results and Discussion

Firstly we investigated whether the selective salt formation between dipeptides and D-amino acid esters during enzymatic peptide synthesis involves an enzyme or not. Thus, *N*-benzyloxycarbonyl-L-α-aspartyl-L-phenylalanine methyl ester (**1**), *N*-(*p*-methoxybenzyloxycarbonyl)-L-α-aspartyl-L-phenylalanine methyl ester (**2**), and *N*-benzyloxycarbonyl-L-α-aspartyl-L-phenylalanine ethyl ester (**3**) were treated with DL-phenylalanine methyl ester (DL-**4**) in the absence of enzyme. In accordance with the general procedure shown in Scheme 1 (represented by the example for **1** and DL-**4**), hot AcOEt solutions of DL-**4** and the protected dipeptides were mixed at mol ratio of 2 : 1 to obtain the salts, from which **4** was recovered as hydrochloride salts. The results are summarized in Table 1. It was found that D-**4**·HCl was recovered with quite high optical purities (87—98%) in all cases, indicating that an enzyme was not involved in the resolution. In 1,2-dichloroethane (EDC) and MeOH–Et_2O, it was also found that the salt was formed preferentially with D-**4**.



Scheme 1. General procedure for optical resolution.

The study was extended to see whether **1** could also effect the resolution of racemic amino acid esters other than **4**. Thus, resolutions of racemic H–Ala–OMe (**5**), H–Ala–OEt (**6**), H–Val–OMe (**7**), H–Val–OEt (**8**), H–

Table 1. Non-Enzymatic Resolution of Racemic Amino Acid Ester **4** by *N*-Protected Aspartyl Dipeptide Esters **1**, **2**, and **3**

Entry	Dipeptide	Solvent[a]	Salt						D-**4**·HCl[b]	
			Yield	Mp	$[\alpha]_D$[c]	Elemental analysis/%			$[\alpha]_D$[c]	Optical purity[d]
			%	°C	°	C	H	N	°	%
1	**1**	A	87.4	126—132	−6.5	63.05	6.15	7.01	−15.04	97.8
						(63.24	6.13	6.97)[e]		
2	**1**	B	73.2	124—128	−5.8	63.30	6.08	6.90	−13.72	89.2
3	**1**	C	79.8	125—130	−6.2	63.11	6.20	7.03	−14.40	93.6
4	**1**[f]	D	98.0	126—130	−6.4	63.20	6.23	7.10	−14.91	97.0[g]
5	**2**	A	96.5	111—122	−25.0	61.91	6.06	6.58	−13.40	87.1
						(62.15	6.16	6.59)[e]		
6	**3**	A	97.4	105—115	−8.2	63.38	6.32	6.78	−14.81	96.3
						(63.76	6.32	6.76)[e]		

a) A: ethyl acetate; B: 1,2-dichloroethane; C: methanol–H_2O; D: H_2O. b) Recovered from salts. c) c=1, MeOH, 25°C. d) $[\alpha]_D$ of authentic L-**4**·HCl is +15.38°.[c] e) Calculated. f) Na salt of **1** and hydrochloride of DL-**4** were used. g) From the filtrate, L-**4**·HCl with optical purity of 95% was recovered.

Table 2. Optical Resolution of Racemic Amino Acid Esters **5**—**12** by Aspartame Precursor **1**

Ester	Yield	Salt					D-Amino acid ester[a]		L-Amino acid ester[b]	
		Elemental analysis/%			Mp	$[\alpha]_D$[c]	$[\alpha]_D$[c]	Optical purity[d]	$[\alpha]_D$[c]	Optical purity[d]
	%	C	H	N	°C	°	°	%	°	%
5	84.1	58.89	6.02	7.96	107—119	−2.36	—[e]	—[e]	—[e]	—[e]
		(58.76	6.26	7.91)[f]						
6	95.4	59.26	6.67	7.91	114—120	−4.86	−2.03	13.9	+1.76	12.0
		(59.45	6.47	7.70)[f]						
7	99.1	60.07	6.37	7.49	131—135	−1.40	−22.02	90.1	+21.49	87.9
		(60.10	6.66	7.51)[f]						
8	97.3	60.86	6.41	7.38	80—87	−0.59	−15.11	92.0	+14.53	88.5
		(60.72	6.85	7.33)[f]						
9	92.9	60.45	6.44	7.33	88—105	−2.98	−20.00	91.4	+16.58	75.8
		(60.72	6.85	7.32)[f]						
10	88.2	61.47	6.98	7.13	86—95	+0.50	−13.41	89.8	—[e]	—[e]
		(61.32	7.03	7.15)[f]						
11	41.4	61.44	5.72	6.55	77—90	−1.69	−7.00	53.8	—[e]	—[e]
		(61.63	5.98	6.74)[f]						
12	67.4	61.84	5.97	6.57	111—123	−1.29	−3.38	40.1	—[e]	—[e]
		(62.16	6.16	6.59)[f]						

a) Recovered from salts. b) Recovered from filtrates. c) c=1, MeOH, 25°C. d) Authentic hydrochloride salts of L-amino acid esters had $[\alpha]_D^{25}$; **6**: +14.64°; **7**: +24.45°; **8**: +16.42°; **9**: +21.88°; **10**: +14.94°; **11**: +13.00°; **12**: +8.42°. e) Not crystallized (oily residue). f) Calculated.

Leu–OMe (**9**), H–Leu–OEt (**10**), H–Tyr–OMe (**11**), and H–Tyr–OEt (**12**) were similarly studied in AcOEt using **1** as the resolving agent. The results for the formation of the salts between **1** and amino acid esters are summarized in Table 2, which shows that **1** formed salts with most of amino acid esters in good yields, except for Tyr ester. The salt were decomposed into **1** and amino acid esters, and then the hydrochloride salts of the latters were recovered.

It can be seen from Tables 1 and 2 that **1** forms the salts preferentially with D-amino acid esters, and that high optical purities can be achieved for the hydrophobic amino acid esters such as Phe, Val, and Leu, while esters of Ala and Tyr showed rather poorer optical resolutions. Better selectivity in the former group may suggest that hydrophobic interaction of side-chain substituents with the phenyl group of Phe in **1** is important in selectivity during salt formation. Such interaction was also suggested in ligand-exchange chromatography and high-voltage capillary zone electrophoresis to separate amino acid enantiomers by using copper(II) complex of L-α-aspartyl-L-phenylalanine methyl ester as chiral mobile phase.[6-8] In the case of Tyr esters, poorer resolutions may be attributable to the hydrophilic interaction or hydrogen bonding of OH group, which may hamper the hydrophobic interaction of its phenyl group.

The fact that the aspartame precursors form the salts preferentially with D-amino acid esters also suggests that one could recover L-amino acid esters from the filtrate after separation of the deposited salt by filtration. Thus, the filtrates were treated by the procedures as shown in Scheme 1, and L-rich amino acid esters were indeed recovered.

In addition to the procedure to form salts in organic solvents as shown in Scheme 1, it was also found that salt formation could be effected in an aqueous system by treating Na salt of **1** with hydrochloride salts of amino acid esters. Thus **1** was dissolved in water containing 2 equivalents of NaOH, followed by mixing with 2 equivalents of DL-**4**·HCl to cause deposition of the salt. In this case, both components are highly soluble in H_2O, and yet the resulting salt is poorly soluble, thus with a very simple procedure almost quantitative yield (ca. 98%) can be attained. From the salt and the filtrate, D-**4** and L-**4** with optical purity of 97 and 95%, respectively, could be recovered as the hydrochloride salts (see Table 1, Entry 4).

To summarize, the method is attractive, since many amino acid esters can be resolved by **1**, an important precursor to sweetener aspartame.[3] It is also of great advantage that the optical resolution can be effected in aqueous system in high yield and high optical purity by using the Na salt of **1** as the resolving agent. From the present results, it may be suggested that these dipeptides be applicable to separation of optical isomers of amino acids in chromatography when they are covalently bonded to suitable supporting materials and then used as column-packing materials.

Experimental

General. *N*-Benzyloxycarbonyl-L-α-aspartyl-L-phenylalanine methyl ester (**1**), *N*-(*p*-methoxybenzyloxycarbonyl)-L-α-aspartyl-L-phenylalanine methyl ester (**2**), and *N*-benzyloxylcarbonyl-L-α-aspartyl-L-phenylalanine ethyl ester (**3**) were prepared enzymatically as described previously.[2] Amino acid esters were synthesized by the Fisher esterification method.[9] Optical rotations were recorded on a Union Giken PM-101 digital polarimeter.

Procedure for Optical Resolution in AcOEt (Scheme 1). Racemic amino acid ester hydrochloride (2—3 g) was dissolved in 20 mL of H_2O, and neutralized by 1.1 mol equivalent of $NaHCO_3$. The liberated amino acid ester was extracted into CH_2Cl_2. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed with rotary evaporator. The oily residue was dissolved in 20 mL of hot AcOEt, and the solution was added with agitation into 20 mL of hot AcOEt containing an *N*-protected aspartylphenylalanine ester at the mol ratio of the ester and the dipeptide being 2 : 1. The mixture was kept in a refrigerator, and the deposited salt was collected by filtration.

The decomposition of the salt and recovery of hydrochloride of the ester were carried out as follows. The salt was suspended in 20—30 mL of aq HCl (HCl/salt=1.5 mol ratio), stirred for 1 h, and then the dipeptide component was collected by filtration. The filtrate was neutralized by $NaHCO_3$, the liberated amino acid ester was extracted into CH_2Cl_2. After the solution was dried, HCl gas was introduced, and the precipitate was collected by filtration. The optical rotation of the hydrochloride was measured and the optical purity was calculated by comparison with the $[\alpha]_D^{25}$ of the authentic L-isomer.

Isolation of amino acid ester hydrochloride from the filtrate after separation of the deposited salt was carried out as follows. The filtrate was evaporated on a rotary evaporator, the residue was dissolved in CH_2Cl_2, and HCl gas was introduced into it to cause deposition of the amino acid ester hydrochloride.

Procedure for Optical Resolutions Using Other Solvent Systeems. Resolution in EDC was carried out essentially by the same procedure. In the case MeOH–Et_2O solvent system, Et_2O was added into the MeOH solution containing the dipeptide and the amino acid ester. Resolution in H_2O was carried out by dissolving 2 mmol of **1** in 20 mL of 0.2 mol dm^{-3} aq NaOH, followed by addition of 4 mmol hydrochlorides of amino acid esters in 10 mL of H_2O. The deposited salt was treated as before.

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3) The product can be easily converted to L-α-aspartyl-L-phenylalanine methyl ester, the synthetic sweetener aspartame, thus this reaction provides a unique method to prepare the sweetener.[4,5]

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