Optical Resolution by Preferential Crystallization and Replacing Crystallization of DL-Allothreonine

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The ternary solubility diagram, X-ray diffraction pattern, and infrared spectra have suggested that DL-allothreonine [DL-aThr] exists as a conglomerate. The optical resolution by successive preferential crystallization gave both D- and L-aThr of 95% optical purities in 9—13% degrees of resolution. The successive replacing crystallization was more successfully achieved by the coexisting 4-hydroxy-L-proline, as an optically active cosolute, in a racemic supersaturated solution. D-aThr of 94% optical purity was allowed to crystallize preferentially without seeding D-aThr in 24% degree of resolution, whereas L-aThr of 81% optical purity from the mother liquor was allowed to crystallize by seeding L-aThr in 22% degree of resolution. Recystallization of the obtained D- and L-aThr from water gave an optically pure form.

D- and L-Allothreonine [abbreviated as D- and L-aThr] are useful chiral reagents in asymmetric syntheses, similar to threonine [Thr], because they have two asymmetric centers and supply a target compound with two chiral centers.[1] Nonproteine amino acids, D- and L-aThr, are very expensive because they must be prepared by synthetic transformations from D- and L-Thr.[2] On the other hand, DL-aThr is obtained by the ammonolysis of (2*RS*,3*SR*)-2,3-epoxybutanoic acid[3] [(*RS*)-EBA] and (2*RS*,3*SR*)-2-bromo-3-hydroxybutanoic acid[4] [(*RS*)-BHA]. (*RS*)-EBA is synthesized by the oxidation of inexpensive (*E*)-2-butenoic acid [(*E*)-BA] with hydrogen peroxide,[5,6] and (*RS*)-BHA is obtained by a reaction (*E*)-BA with bromine in the presence of water.[4] We therefore examined the possibility for the optical resolution of DL-aThr to obtain both D- and L-aThr.

Since DL-aThr does not form a salt with ordinary resolving agents, such as optically active carboxylic acids and amines, *N*-formyl-*O*-methyl-DL-allothreonine[7] and *N*-phthaloyl-DL-allothreonine[8] were subjected to optical resolution by using brucine as a resolving agent; regeneration of the resolved derivatives gives D- and L-aThr. However, such processes are tedious, and may result in a partial racemization.[9] We therefore attempted an optical resolution of DL-aThr without a transformation into its derivatives.

An optical resolution by preferential crystallization is a simple procedure for obtaining enantiomers from a racemic modification, and is achieved by providing a small amount of one enantiomer, as seed crystals, in a supersaturated racemic solution. This procedure, however, requires that the racemic modification exists as a conglomerate.[10] We attempted the optical resolution by a preferential crystallization of DL-aThr, because DL-aThr seemed to be a conglomerate in view of the melting point,[11] infrared spectrum,[12,13] solubility,[11] ternary solubility diagram,[14] and X-ray diffraction pattern.[15]

The optical resolution by replacing crystallization is another procedure for obtaining an enantiomer from a conglomerate, and is achieved by allowing an optically active cosolute to coexist in a racemic solution without providing seed crystals.[16] This procedure seems to give a better result than that of preferential crystallization, because the supersaturated solution remains more stable during crystallization of one enantiomer.[16] The optical resolution by preferential crystallization has a possibility for obtaining both enantiomers by successive optical resolution. Successive optical resolution by replacing crystallization, however, seems to be difficult because of an attractive interaction acting between one enantiomer and an optically active cosolute.[16] We therefore explored favorable cosolute and optimal conditions for successive replacing crystallization of DL-aThr by using L-alanine [L-Ala], L-serine [L-Ser], L-proline [L-Pro], and 4-hydroxy-L-proline [L-Hyp] as optically active cosolutes. Based on the results, the successive optical resolution by replacing crystallization of DL-aThr was carried out to obtain both D- and L-aThr.

Experimental

Apparatus. The specific rotation was measured at 589 nm with a Horiba Seisakusho SEPA-200 auto polarimeter equipped with a quartz cell of 5.00 cm path length. The infrared spectra were obtained in the 4000—400 cm^{-1} range with a Perkin-Elmer (Model 1600 FT-IR) spectrometer by the KBr disk method. The ^1H NMR spectra were recorded on a JEOL (JNM-PMX 60 NMR) spectrometer in deuterium oxide with DSS used as an internal standard substance. Refractive indexes were measured with a Shimadzu refractometer (Abbe 3L). X-Ray diffraction patterns were obtained with a Material Analysis and Characterization Science Co., Ltd. MXP^3 X-Ray diffractometer.

Materials. Amino Acids. L-Hyp ($[\alpha]_D^{20}$ −72.9° (*c* 1.00, water)) and L-Ala ($[\alpha]_D^{20}$ +14.0° (*c* 1.00, 5 mol dm^{-3} HCl)) were purchased from Wako Pure Chemicals Ind. and L-Pro ($[\alpha]_D^{20}$ −83.5° (*c* 1.00, water)), L-Ser ($[\alpha]_D^{20}$ −7.0° (*c* 2.00, water)), and L-Thr $[\alpha]_D^{20}$ −28.3° (*c* 1.00, water)) from Kokusan Chemical Works, Ltd. DL-Thr was purchased from Sigma Chemicals Co.

DL-, D-, and L-Allothreonine: The L-aThr employed as seed crystals was prepared by a synthetic procedure from L-Thr;[2] mp 269—270 °C (decomp) (lit,[18] 273—274 °C (decomp)); R_f value 0.12 (lit,[4,17] 0.13—0.14); $[\alpha]_D^{20}$ +32.8° (*c* 1.00, 1 mol dm^{-3} HCl) (lit,[8] $[\alpha]_D^{20}$ +32.8° (*c* 1.00,

1 mol dm^{-3} HCl). Found: C, 40.33; H, 7.55; N, 11.75%. Calcd for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%.

The D-aThr obtained by optical resolution was recrystallized from water: $[\alpha]_D^{20}$ −32.8° (*c* 1.00, 1 mol dm^{-3} HCl) (lit,[8] $[\alpha]_D^{20}$ −32.4° (*c* 1.00, 1 mol dm^{-3} HCl)).

DL-aThr was prepared according to a procedure reported by Carter et al.:[4] mp 240—242 °C (decomp) (lit,[4] 242—243 °C (decomp); R_f value 0.12 (lit,[4,17] 0.13—0.14; lit,[17] of DL-Thr 0.18—0.19). Found: C, 40.06; H, 7.55; N, 11.86%.

Paper Chromatography of DL- and L-Allothreonine: Paper chromatography of DL- and L-aThr were carried out using an ascending technique on Toyo Roshi Co., Ltd. No. 50 chromatography filter paper; the upper water-poor layer from a mixture of 1-butanol (200 cm^3), water (150 cm^3), acetone (25 cm^3), and 25% ammonium hydroxide (25 cm^3) was used as the developing solvent.[4,17] The ascent was carried out for 5 h at room temperature. After evaporating the developing solvent at 80 °C, the filter paper was treated with a 0.2% (wt/vol) ninhydrin solution in 1-butanol saturated with water and dried at 80 °C. A violet spot was obtained. For a comparision with the R_f values of aThr, the papergrams of DL- and L-Thr were prepared as described above: R_f values 0.17, respectively (lit,[17] of DL-Thr 0.18—0.19).

Optical Resolution by Successive Preferential Crystallization. DL-aThr (6.669 g) was dissolved in 50 cm^3 of water at 40 °C to give a racemic solution with 130% degrees of supersaturation at 10 °C. After gradually cooling the solution to 10 °C, followed by seeding with 0.050 g of L-aThr, the mixture was slowly stirred for 2 h at 10 °C. The precipitated L-aThr was collected by filtration, washed with a small amount of methanol, and dried. DL-aThr (0.503 g) was dissolved in the filtrate at 40 °C. The solution was cooled to 10 °C and then seeded with 0.050 g of D-aThr. After stirring the mixture for 2 h at 10 °C, the precipitated D-aThr was collected by filtration. The filtrate was treated in similar manner to that mentioned above. The degrees of resolution (*DR*/%) of the D- and L-aThr obtained were calculated from

$$DR/\% = [100 \times YOPM/\mathrm{g}]/(\text{Amount of D- or L-aThr/g})$$

and

$$YOPM/\mathrm{g} = [Yield/\mathrm{g} \times OP/\%]/100 - 0.050,$$

where the amounts of D- or L-aThr are those in the supersaturated solution, *YOPM* is the yield of an optically pure modification of the obtained D- or L-aThr, and *OP* is the optical purity.

Optical Resolution by Replacing Crystallization of DL-Allothreonine. Replacing Crystallization: DL-aThr (8.531 g, 71.6 mmol) and 50.0 mmol of L-Ala (4.455 g), L-Ser (5.255 g), L-Pro (5.757 g), or L-Hyp (6.557 g) were dissolved in 50 cm^3 of water at 50 °C. After gradually cooling the solution to 10 °C, followed by slowly stirring for 4—10 h at 10 °C, the precipitated D-aThr was collected by filtration, washed with a small amount of methanol, and dried.

Successive Optical Resolution of DL-Allothreonine: DL-aThr (8.531 g, 71.6 mmol) and 6.557 g of L-Hyp (50.0 mmol) were dissolved in 50 cm^3 of water at 50 °C. After cooling the solution slowly to 10 °C, followed by stirring for 7 h at 10 °C, the precipitated D-aThr was collected by filtration, washed with a small amount of methanol, and dried. DL-aThr (1.463 g) was dissolved in the filtrate at 50 °C. After cooling to 10 °C, followed by seeding with 0.050 g of L-aThr, the mixture was stirred for 3.5 h at 10 °C; the precipitated L-aThr was then collected by filtration. The filtrate was treated in a similar manner to that mentioned above. The degree of resolution was calculated on the basis of the amount of D- or L-aThr in the supersaturated solution, similarly to that for successive preferential crystallization.

Purification: The obtained D- and L-aThr were purified by considering the solubility of DL-aThr. For example, a suspension of D-aThr (4.13 g) of 71% optical purity in 12 cm^3 of water was stirred for 6 h at 10 °C. The purified D-aThr was collected by filtration, washed with a small amount of cold water and methanol, and dried. The optically pure D-aThr was obtained in a 2.89 g yield. L-aThr (4.25 g) of 75% optical purity was similarly treated with 11 cm^3 of water to give optically pure L-aThr in a 3.15 g yield.

Solubility. DL-, D-, or L-aThr (3.50 g) was dissolved in water (20 cm^3) or a solution containing 20.0 mmol of L-Ala, L-Ser, L-Pro, or L-Hyp in 20 cm^3 of water at 40 °C. After vigorous stirring of the solution at 10 °C, an appropriate portion of the solution was pipetted out from the mixture, while avoiding any contamination of solid aThr; the refractive index was measured at 25 °C. The mixture was stirred at 10 °C until the refractive index showed a constant value. The solubility was determined on the basis of the calibration of a previously prepared curve.

In preparing the ternary solubility diagram of aThr in water, the solubilities of mixtures of DL- and L-aThr were measured at 10 °C, similarly as mentioned above. After the refractive index of the solution showed a constant value, the solid aThr was filtered and thoroughly dried; the specific rotation was then measured. The amounts of D- and L-aThr in the solution were calculated on the basis of the solubility of aThr and the specific rotation of the solid aThr.

Results and Discussion

Racemic Structure of DL-Allothreonine and Optical Resolution by Successive Preferential Crystallization. The racemic structure of DL-aThr was examined because any optical resolution by preferential crystallization requires that DL-aThr exists in a conglomerate.[10] The physical properties of DL- and L-aThr are summarized in Table 1.

The infrared spectrum[12,13] and X-ray diffraction pattern[15] of DL-aThr are identical with those of L-aThr, respectively. DL-aThr was decomposed at a lower temperature during heating than was L-aThr.[11] In addition, DL-aThr is more soluble than L-aThr, and the ternary solubility diagram (Fig. 1) illustrates a simple ternary mixed pattern, namely, that of a conglomerate. The above facts suggested that DL-aThr exists in a conglomerate.[12—15] Therefore, the optical resolution by successive preferential crystallization of DL-aThr was attempted in order to obtain both D- and L-aThr. The results are summarized in Table 2.

L- and D-aThr of 93—98% optical purities were obtained in about 11% degrees of resolution; the degree

Table 1. Physical Properties of DL- and L-Allothreonine

aThr	Mp[a] °C	Solubility at 10 °C g/100 cm^3 of water	Specific rotation[b] °	Spectrum IR[c]	Spectrum X-Ray[d]
DL-aThr	240—242	10.3	—	}Identical	}Identical
L-aThr	269—270	5.28	+32.8		

a) Decomposition. b) Specific rotation: $[\alpha]_D^{20}$ (c 1.00, 1 mol dm $^{-3}$ HCl). c) IR spectrum. d) X-Ray diffraction pattern.

Table 2. Optical Resolution by Successive Preferential Crystallization of DL-Allothreonine[a]

Run	Added amount of DL-aThr g	Resolution time h	aThr obtained Yield g	*OP*[b] %	*YOPM*[c] g	*DR*[d] %
1	6.669	2	0.553 (L)	93.0	0.464	11.7
2	0.503	2	0.457 (D)	95.7	0.387	9.2
3	0.407	2	0.508 (L)	95.6	0.436	11.1
4	0.458	3	0.554 (D)	97.3	0.489	11.6
5	0.504	1.5	0.513 (L)	97.8	0.452	11.4
6	0.463	3	0.554 (D)	94.0	0.471	11.2
7	0.504	2	0.622 (L)	94.0	0.533	13.4
8	0.572	2	0.522 (D)	95.2	0.447	10.6

a) Conditions: Water 50 cm^3; initial degree of supersaturation 130%; seed crystals 0.050 g; temperature 10 °C. b) *OP*: Optical purity. c) *YOPM*: The yield of optically pure modification. d) *DR*: Degree of resolution.

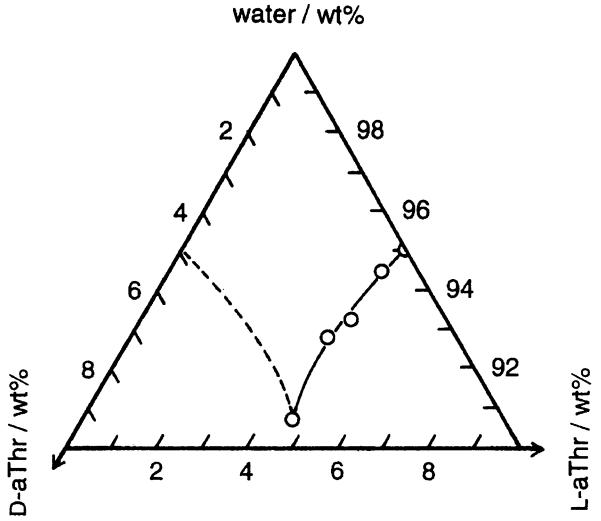

Fig. 1. Ternary phase diagram of solubility of allothreonine. Solvent: Water. Temperature: 10 °C.

of resolution was calculated based on the amount of L- and D-aThr in the supersaturated solution. This result supports the above suggestion that DL-aThr exists in a conglomerate.

Solubility in the Presence of L-Amino Acids as Cosolutes. The optical resolution of DL-aThr was achieved by preferential crystallization to give both D- and L-aThr, as described above. Further, we attempted an optical resolution by replacing crystallization, which is another procedure to give an enantiomer from a conglomerate, in order to obtain more efficiently optically active aThr.

For a conglomerate such as DL-aThr, statistical mechanical theory has shown that the solubilities of the enantiomers differ from each other in an optically active solvent and in a solution containing an optically active cosolute, such as L-amino acid.[16] The optical resolution upon replacing crystallization of DL-Thr was studied using L-Pro as an optically active cosolute.[19] The solubility of DL-Thr in an aqueous solution containing L-Pro suggested that an attractive interaction take place between L-Thr and L-Pro, and that there is a repulsive interaction between D-Thr and L-Pro.[19] The solubilities of DL-, D-, and L-aThr were measured at 10 °C in water and in an aqueous solution containing L-Ala, L-Ser, L-Pro, or L-Hyp (Table 3).

DL-, D-, and L-aThr were less soluble in an aqueous so-

Table 3. Solubilities of Allothreonine in Aqueou Solution Containing L-Amino Acid[a]

Cosolute	Solubility (g/100 cm^3 of water) DL-aThr	D-aThr	L-aThr
None	10.3	—	5.28
L-Ala	9.98	4.86	5.01
L-Ser	11.3	5.44	5.70
L-Pro	9.42	4.65	4.76
L-Hyp	10.0	4.91	5.07

a) Solubilities were measured in the presence of 100 mmol of cosolutes at 10 °C, respectively.

Table 4. Optical Resolution by Replacing Crystallization of DL-Allothreonine[a)]

Cosolute (g)	Added amount of DL-aThr	D-aThr obtained			
		Yield	*OP*[b)]	*YOPM*[c)]	*DR*[d)]
	g	g	%	g	%
L-Ala (4.455)	8.481	0.237	76.2	0.181	4.3
L-Ser (5.255)	9.570	0.772	90.0	0.695	14.5
L-Pro (5.757)	8.009	1.369	49.4	0.676	16.9
L-Hyp (6.557)	8.531	1.402	82.3	1.154	27.1

a) Conditions: Water 50 cm^3; cosolute 50.0 mmol; resolution time 7 h; temperature 10 °C; degree of supersaturation of DL-aThr 170%. b) *OP*: Optical purity. c) *YOPM*: The yield of optically pure modification. d) *DR*: Degree of resolution.

Table 5. Optical Resolution by Successive Replacing Crystallization of DL-Allothreonine[a)]

Run	Added amount of DL-aThr	Resolution time	aThr obtained			
			Yield	*OP*[b)]	*YOPM*[c)]	*DR*[d)]
	g	h	g	%	g	%
1[e)]	8.531	7	1.308 (D)	83.4	1.091	25.7
2[e)]	1.299	—[f)]	0.910 (L)	14.9	0.136	2.8
1[e)]	8.531	7	1.480 (D)	79.6	1.178	27.6
2[g)]	1.463	3.5	1.859 (L)	78.6	1.411	29.1
3[e)]	1.715	7	2.142 (D)	53.4	1.144	26.1
4[g)]	2.137	2.5	1.301 (L)	80.6	0.999	21.7
5[e)]	1.248	8	1.087 (D)	93.6	1.017	24.2
6[g)]	1.485	2.5	1.792 (L)	65.7	1.127	23.4

a) Conditions: Water 50 cm^3; L-Hyp as a cosolute 6.557 g (50.0 mmol); temperature 10 °C. b) *OP*: Optical purity. c) *YOPM*: The yield of optically pure modification. d) *DR*: Degree of resolution. e) Not seeing. f) After stirring for 10 h, the solution was stood for 16 h at 5 °C. g) Seeding: 0.050 g of L-aThr as seed crystals at 10 °C.

lution containing L-Ala, L-Pro, or L-Hyp than in water, though aThr were more soluble in an aqueous solution of L-Ser. In addition, the solubilities tended to be more soluble in aqueous solutions of L-Ser and L-Hyp with a hydroxyl group at the side chain in those of L-Ala and L-Pro with a hydrophobic side chain. Since these results show that D-aThr is less soluble than L-aThr in an aqueous solution containing L-amino acids, D-aThr is estimated to crystallize preferentially from supersaturated solutions of DL-aThr in the presence of these L-amino acids, than is L-aThr.

Optical Resolution by Replacing Crystallization. Replacing crystallization of DL-aThr was attempted at a resolution time of 7 h for solutions with 170% degree of supersaturation in order to explore a favorable L-amino acid as an optically active cosolute; L-Ala, L-Ser, L-Pro, and L-Hyp were employed as cosolutes. The results are summarized in Table 4.

As suggested from the solubilities in the presence of the L-amino acids, replacing crystallization caused it to preferentially crystallize D-aThr from solutions of DL-aThr. When L-Ala and L-Pro were used as the cosolutes, D-aThr of 76 and 49% optical purities were obtained in 4 and 17% degrees of resolution, respectively. On the other hand, replacing crystallization in the presence of L-Ser and L-Hyp gave D-aThr of 90 and 83% optical purities in 15 and 27% degrees of resolution, respectively. These results suggested that L-Ser and L-Hyp with a hydroxyl group at the side chain are favorable as cosolutes for replacing crystallization. Therefore, optical resolutions by using L-Ser and L-Hyp were carried out for resolution times of 4—10 h. The relationships between the resolution time and degree of crystallization are shown in Fig. 2.

When L-Hyp was used as the cosolute, D-aThr crystallized rapidly at a resolution time greater than 4 h; however, a rapid crystallization of L-aThr was not observed up to 7—8 h. Therefore, D-aThr of 68 and 38% optical purities was obtained in 26 and 20% degrees of resolution at 8 and 9 h. When L-Ser was used, the crystallization of L-aThr was not observed within 7 h. Although the optical resolution at 10 h gave D-aThr of 84% optical purity in 22% degree of resolution, a longer resolution time requires one to obtain D-aThr at a higher degree of resolution. The above results suggest that using L-Hyp as a cosolute is more efficient for a relatively short resolution time.

Optical Resolution by Successive Replacing Crystallization. We next attempted a successive optical resolution of DL-aThr by using L-Hyp as cosolutes. These results are listed in Table 5. When seed crystals were not provided, D-aThr with 83% optical pu-

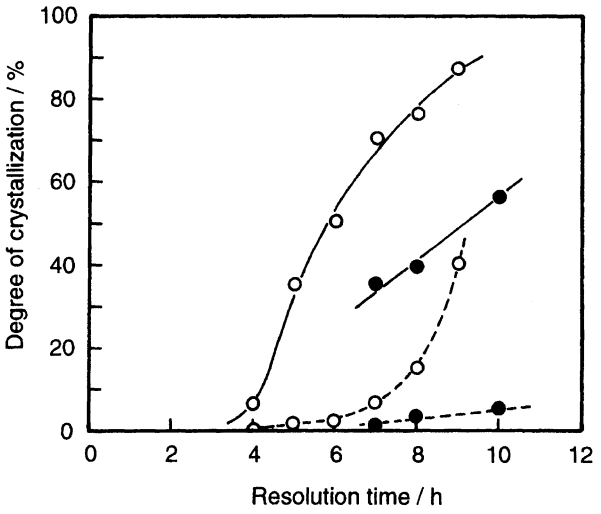

Fig. 2. Relationship between resolution time and degree of crystallization. Conditions: DL-aThr 8.531 g; L-Hyp 6.557 g; water 50 cm^3; temperature 10 °C, plot mark ○. —: D-aThr. ---: L-aThr. Conditions: DL-aThr 9.570 g; L-Ser 5.255 g; water 50 cm^3; temperature 10 °C, plot mark ●. —: D-aThr. ---: L-aThr.

rity was obtained at a degree of resolution of 26% from the initial solution. After filtering D-aThr, 1.299 g of DL-aThr was dissolved in the filtrate; the solution was then stirred for 10 h at 10 °C. L-aThr, however, was not crystallized. The solution was therefore allowed to stand for 16 h at 5 °C in order to crystallize L-aThr. However, both the optical purity (15%) and degree of resolution (3%) had extremely low values. To induce the crystallization of L-aThr from the filtrate, after filtrating D-aThr replacing crystallization was attempted by combining with preferential crystallization, namely, seeding of L-aThr to the filtrate. The optical resolution gave D- and L-aThr with optical purities of 53—94% in degrees of resolution of 22—29%. These degrees of resolution were approximately twice those by preferential crystallization (Table 2). Recrystallization of the obtained D- and L-aThr from water gave optically pure D- and L-aThr, as described in the Experimental section.

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