

Note

Optical Resolution by the Replacing Crystallization of DL-Threonine with L-Alanine as an Optically Active Cosolute

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DL-Threonine (DL-Thr) was optically resolved by replacing crystallization with L-alanine (L-Ala) as an optically active cosolute. D-Thr was preferentially crystallized from a supersaturated aqueous solution of DL-Thr in the presence of L-Ala. Optical resolution was successfully achieved to afford D-Thr with an optical purity of 96–98% and L-Thr of 91–95%. The partially resolved D- and L-Thr were recrystallized from water, taking account of the solubility of DL-Thr, to efficiently yield both enantiomers in an optically pure form.

Key words: threonine; optical resolution; replacing crystallization; L-alanine

D- and L-Threonine (D- and L-Thr) are useful as chiral reagents in asymmetric syntheses because they are capable of providing two chiral centers in a target substance.¹⁾ D-Thr, however, is more expensive than L-Thr because of difficulty in obtaining it from natural products in a large quantity. We, therefore, attempted to obtain both D- and L-Thr from DL-Thr by optical resolution.

Racemates exist in the forms of racemic compounds, racemic solid solutions, and conglomerates. DL-Thr is known to be a conglomerate, defined as a mechanical mixture of crystals of both enantiomers.²⁾ Therefore, DL-Thr has been optically resolved by preferential crystallization³⁾ and replacing crystallization.^{4–6)} Optical resolution by replacing crystallization is a procedure for allowing the preferential crystallization of one enantiomer from a supersaturated racemic solution in the presence of an optically active cosolute. Optical resolution is achieved on the basis of interaction between each of the enantiomers and the cosolute. This interaction produces a different solubility for each enantiomer. Therefore, optical resolution by replacing crystallization seems to be a good procedure for obtaining one enantiomer, although it is difficult to obtain the other enantiomer whose solubility is increased in the presence of the cosolute.^{4,7)} When an optically active compound with a structure similar to that of the conglomerate is used as the cosolute, optical resolution seems to give good results. We have already reported the optical resolution of DL-Thr by using L-serine (L-Ser) which has a hydroxyl group in the side chain, as with DL-Thr.⁶⁾ Although this optical resolution afforded D- and L-Thr with an optical purity of 90%, they could not be obtained in a high yield.⁶⁾ The

solubility of both D- and L-Thr was increased in the presence of L-Ser because of the marked structural similarity between Thr and L-Ser, and hence crystallization of both D- and L-Thr from the racemic solution seemed to be inhibited.⁶⁾ We thus attempted the optical resolution of DL-Thr by using L-alanine (L-Ala), which does not contain hydroxyl groups, as the optically active cosolute, to more efficiently obtain D- and L-Thr.

First, the solubility of DL-, D-, and L-Thr was measured in the presences of 12.0 mmol and 24.0 mmol of L-Ala in 100 cm³ of water at 10°C, the values being summarized in Table 1.

When L-Ala (12.0 mmol) was present in aqueous solutions of DL-, D-, and L-Thr, D-Thr was less soluble than L-Thr, and hence was estimated to preferentially crystallize from a supersaturated solution of DL-Thr. DL- and L-Thr were each more soluble in the presence than in the absence of L-Ala, whereas the solubility of D-Thr was approximately equivalent to that in the absence of L-Ala. When L-Ala (24.0 mmol) was present, DL-Thr was more soluble than in the presence of 12.0 mmol of L-Ala, although the solubility of D-Thr in the solution of DL-Thr was slightly higher than that in the absence of L-Ala.

The activity coefficient is useful to examine the interaction between D- and L-Thr and L-Ala.^{5,6)} Therefore, the activity coefficients (γ) of D- and L-Thr were calculated on the basis of their solubility in the presence of L-Ala from eq. (1),⁵⁾ as shown in Table 1:

$$\ln x = -1750/T + 1.60 - \ln \gamma \quad (1)$$

where x is the mole fraction of D- or L-Thr in the saturated solution at temperature T (K). The activity coefficient of L-Thr in the presence of L-Ala was smaller than that in the absence of L-Ala, and that in the solution of DL-Thr tended to become smaller with increasing amount of L-Ala. On the other hand, the activity coefficient of D-Thr in the presence of 12.0 mmol and 24.0 mmol of L-Ala was approximately equivalent to that in the absence of L-Ala. Therefore, although attractive interaction was estimated to occur only between L-Thr and L-Ala, there is neither an attractive nor repulsive interaction between D-Thr and L-Ala. These results seemed to favor the use of L-Ala as the optically active cosolute for the selective crystallization of D-Thr in the first stage from the supersaturated racemic solution, because the crystallization

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Abbreviations: L-Ala, L-alanine; Thr, threonine; L-Ser, L-serine

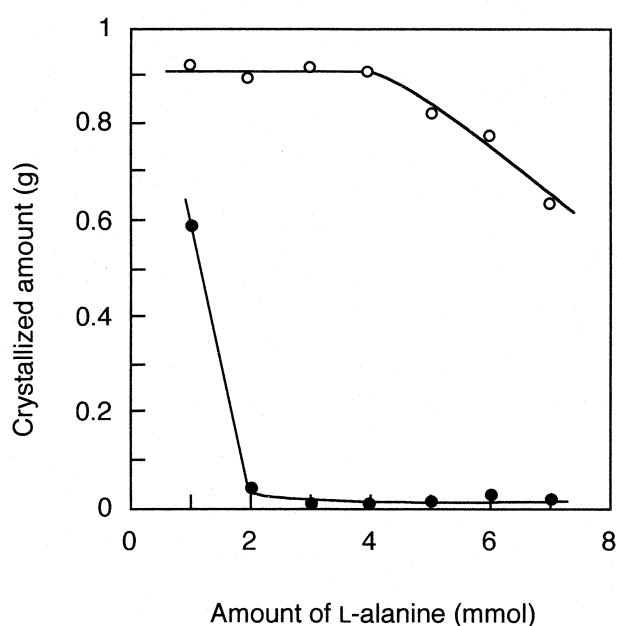
Table 1. Solubility of DL-, D-, and L-Threonine^{a)}

Threonine	L-Ala as optically active cosolute (mmol)	Solubility [g (100 cm ³ of water) ⁻¹]	Activity coefficient
DL-Thr ^{b)}	— ^{c)}	14.96 {D-Thr 7.48 L-Thr 7.48}	0.93
L-Thr ^{b)}	— ^{c)}	6.95	0.99
DL-Thr	12.0	15.44 {D-Thr 7.45 L-Thr 7.99}	0.93
D-Thr	12.0	6.91	0.87
L-Thr	12.0	7.67	0.99
DL-Thr	24.0	16.08 {D-Thr 7.65 L-Thr 8.43}	0.89
			0.92
			0.83

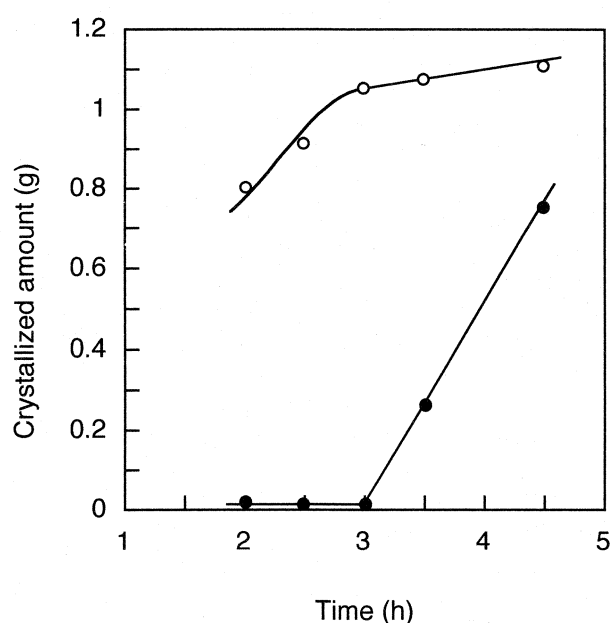
^{a)} Conditions: water, 100 cm³; temperature, 10°C.

^{b)} See ref. 5.

^{c)} None.

**Fig. 1.** Influence of the Amount of L-Alanine as the Optically Active Cosolute on the Replacing Crystallization of DL-Threonine.

Conditions: DL-Thr, 5.956 g (50.0 mmol); L-Ala, 0.0891–0.624 g (1.00–7.00 mmol); solvent, 25 cm³ of water; temperature, 10°C; resolution time, 2.5 h. Crystallized amount: ○, D-Thr; ●, L-Thr.

**Fig. 2.** Influence of Resolution Time on the Replacing Crystallization of DL-Threonine.

Conditions: DL-Thr, 5.956 g (50.0 mmol); L-Ala, 0.267 g (3.00 mmol); solvent, 25 cm³ of water; temperature, 10°C. Crystallized amount: ○, D-Thr; ●, L-Thr.

of L-Thr would be prevented by the attractive interaction with L-Ala during the crystallization of D-Thr.

Based on these results, the optical resolution of DL-Thr was attempted by using L-Ala as the optically active cosolute. To optimize the conditions, optical resolution was conducted by stirring solutions containing 50.0 mmol (5.956 g) of DL-Thr and 1.00–7.00 mmol of L-Ala in 25 cm³ of water for 2.5 h at 10°C. The results are shown in Fig. 1.

As expected from the solubility, D-Thr was preferentially crystallized from the racemic solution. D-Thr thus obtained was determined by its ¹H-NMR spectrum to be free from L-Ala. The crystallized amounts [*CA_L* and *CA_D* (g)] of L- and D-Thr in Fig. 1 were calculated from

$$CA_L (g) = (1/2)[Yield (g) \times (100 - Op (\%))/100] \quad (2)$$

and

$$CA_D (g) = Yield (g) - CA_L (g) \quad (3)$$

where *Op* (%) is the optical purity of D-Thr obtained and was calculated on the basis of the specific rotation of authentic D-Thr: $[\alpha]_D^{25} + 28.4^\circ$ (water).⁸⁾

In the presence of 1.00–4.00 mmol of L-Ala, the crystallized amount of D-Thr was approximately constant (0.89–0.92 g). When a larger amount (5.00–7.00 mmol) of L-Ala was added to the racemic solution, the amount of D-Thr (0.64–0.82 g) decreased with increasing amount of L-Ala. This result suggests that the solubility of D-Thr was slightly increased with increasing amount of L-Ala, as shown in Table 1, and hence that the crystallization of D-Thr was inhibited by reducing the degree of supersaturation. On the other hand, L-Thr (0.59 g) was crystallized from the solution containing 1.00 mmol of L-Ala, but hardly any L-Thr was (0.01–0.04 g) crystal-

Table 2. Successive Optical Resolution by Replacing Crystallization of DL-Threonine^{a)}

Run	Added amount of DL-Thr (g)	Operation amount of D- and L-Thr ^{b)} (g)		Resolution time (h)	Thr obtained		
		D-Thr	L-Thr		Yield (g)	Optical purity ^{c)} (%)	Degree of resolution (%)
1	5.956	2.978	2.978	3	D 1.073	96.8	93.2
2	1.073	2.459	3.498	3.5	L 1.102	94.5	69.4
3	1.102	2.979	2.977	2	D 0.989	97.0	85.9
4	0.990	2.500	3.457	2	L 1.214	91.4	76.0
5	1.214	3.055	2.902	2.5	D 0.973	98.1	80.1
6	0.970	2.576	3.378	1	L 1.001	95.2	69.0

^{a)} Conditions: optically active cosolute, 0.267 g (3.00 mmol) of L-Ala; solvent, 25 cm³ of water; temperature, 10°C.

^{b)} The operation amounts in runs 2–6 were calculated from the results in 1–5, respectively.

^{c)} The optical purities of D- and L-Thr obtained were calculated on the basis of the specific rotation of authentic D-Thr; lit.⁸⁾ $[\alpha]_D^{25} + 28.4^\circ$ (water).

lized from solutions containing a larger amount of L-Ala. Therefore, optical resolution with 2.00–4.00 mmol of L-Ala gave D-Thr with an optical purity of 91–98% in a yield of 0.92–0.94 g. Based on these results, optical resolution with 3.00 mmol of L-Ala was carried out for resolution times of 2–4.5 h, as shown in Fig. 2.

The crystallized amount of D-Thr rapidly increased with resolution time during the first 2–3 h and then more gradually increased with longer resolution time. On the other hand, L-Thr began to crystallize rapidly at 3.5 h, but not during the first 3 h. Therefore, optical resolution at 3 h gave D-Thr with an optical purity of 97% in a yield of 1.07 g. Based on these results, successive optical resolution was attempted by stirring the racemic solution containing 3.00 mmol of L-Ala as the initial solution for 3 h. These results are summarized in Table 2. The degrees of resolution of D- and L-Thr [*DR* (%)] in Table 2 were calculated from

$$DR (\%) = [Yield (g) \times Op (\%) / 100] / [\text{operation amount of D- or L-Thr (g)} - S] \quad (4)$$

where *S* (g) is the solubility of D-Thr (1.863 g) or L-Thr (1.997 g) in DL-Thr in 25 cm³ of water at 10°C. The operation amount (g) is the amount of D- or L-Thr in the racemic solution, and the values in runs 2–6 being calculated on the basis of the results from runs 1–5, respectively.

Successive optical resolution was successfully achieved to afford D-Thr with an optical purity of 96–98% in a degree of resolution of 80–93%, and L-Thr with an optical purity of 91–95% in a degree of resolution of 69–76%.

Partially resolved D- and L-Thr could be recrystallized from water, taking account of the solubility of DL-Thr,⁵⁾ to give both optically pure Thr enantiomers, as described in the Experimental section; for example, optically pure D-Thr (18.9 g) was obtained from 26.6 g of D-Thr with an optical purity of 73%, and optically pure L-Thr (6.78 g) was obtained from 9.84 g of L-Thr with an optical purity of 69%.

Experimental

Specific rotation values were measured at 589 nm with a Horiba Seisakusho SEPA-300 auto-polarimeter

equipped with a quartz cell with a 5.00-cm path length. ¹H-NMR spectra were recorded with a JNM-FX270 FT NMR system with sodium 3-(trimethylsilyl)propanesulfonate (DSS) as an internal standard. Chemical shift values were reported in δ units downfield from DSS.

DL-, D-, and L-Thr and L-Ala were purchased from Wako Pure Chemical Ind.

Replacing crystallization. DL-Thr (5.956 g, 50.0 mmol) and L-Ala (0.0891–0.624 g, 1.00–7.00 mmol) were dissolved in 25 cm³ of water at 50°C. After cooling the solution to 10°C over 30 min and then stirring for 2.5 h with a blade (0.80 cm width; 2.5 cm length) at 100 rpm and 10°C, precipitated D-Thr was collected by filtration, washed with a small amount of methanol, and dried.

D-Thr obtained by using 1.00 mmol of L-Ala: yield, 1.508 g; $[\alpha]_D^{20} + 6.28^\circ$ (*c* 1.00, water). D-Thr obtained by using 2.00 mmol of L-Ala: yield, 0.936 g; $[\alpha]_D^{20} + 25.8^\circ$ (*c* 1.00, water). D-Thr obtained by using 3.00 mmol of L-Ala: yield, 0.922 g; $[\alpha]_D^{20} + 27.8^\circ$ (*c* 1.00, water). D-Thr obtained by using 4.00 mmol of L-Ala: yield, 0.919 g; $[\alpha]_D^{20} + 27.8^\circ$ (*c* 1.00, water). D-Thr obtained by using 5.00 mmol of L-Ala: yield, 0.835 g; $[\alpha]_D^{20} + 27.3^\circ$ (*c* 1.00, water). D-Thr obtained by using 6.00 mmol of L-Ala: yield, 0.806 g; $[\alpha]_D^{20} + 26.1^\circ$ (*c* 1.00, water). D-Thr obtained by using 7.00 mmol of L-Ala: yield, 0.660 g; $[\alpha]_D^{20} + 26.3^\circ$ (*c* 1.00, water).

Optical resolution was carried out by using 3.00 mmol of L-Ala and stirring for 2–4.5 h at 10°C in a manner similar to that already described.

D-Thr obtained at 2 h: yield, 0.814 g; $[\alpha]_D^{20} + 27.4^\circ$ (*c* 1.00, water). D-Thr obtained at 3 h: yield, 1.073 g; $[\alpha]_D^{20} + 27.5^\circ$ (*c* 1.00, water). D-Thr obtained at 3.5 h: yield, 1.338 g; $[\alpha]_D^{20} + 17.1^\circ$ (*c* 1.00, water). D-Thr obtained at 4.5 h: yield, 1.856 g; $[\alpha]_D^{20} + 5.45^\circ$ (*c* 1.00, water).

Successive optical resolution. DL-Thr (5.956 g, 50.0 mmol) and L-Ala (0.267 g, 3.00 mmol) were dissolved in 25 cm³ of water at 50°C. After cooling the solution to 10°C and then stirring for 3 h at 10°C, precipitated D-Thr (1.073 g) was collected by filtration and dried (run 1 in Table 2). DL-Thr (1.073 g) was dissolved in the filtrate at 50°C. After cooling the solution to 10°C and then stir-

ring for 3.5 h, precipitated L-Thr (1.102 g) was collected by filtration (run 2 in Table 2). Optical resolution was carried out at 10°C by adding more DL-Thr to the filtrate in a manner similar to that just described; the detailed conditions are given for runs 3–6 in Table 2.

Purification of the partially resolved D- and L-threonine. D-Thr (26.6 g) ($[\alpha]_D^{20} + 20.8^\circ$ (c 1.00, water)) was added to water (50 cm³). The mixture was vigorously stirred for 10 h at 10°C, before the purified D-Thr was collected by filtration and dried. L-Thr (9.84 g) ($[\alpha]_D^{20} - 19.6^\circ$ (c 1.00, water)) was purified by using 22 cm³ of water in a manner similar to that described for D-Thr.

D-Thr: yield, 18.9 g; $[\alpha]_D^{20} + 28.4^\circ$ (c 1.00, water). ¹H-NMR (270 MHz, D₂O, DSS) δ : 4.25 (1H, qd, $J=6.5$, 4.9 Hz, 3-CH), 3.58 (1H, d, $J=4.9$ Hz, 2-CH), 1.32 (3H, d, $J=6.6$ Hz, 4-CH₃).

L-Thr: yield, 6.78 g; $[\alpha]_D^{20} - 28.4^\circ$ (c 1.00, water). The ¹H-NMR spectrum was virtually identical to that of D-Thr.

Solubility. DL-, D-, or L-Thr (5.956 g, 50.0 mmol) was dissolved in a solution containing 0.267 (3.00) or 0.535 g (6.00 mmol) of L-Ala in 25 cm³ of water at 60°C. After vigorously stirring the solution for 10 h at 10°C, the precipitated Thr was rapidly collected by filtration and thoroughly dried. The solubility at 10°C was calculated

on the basis of the weight of Thr. For the dissolution of DL-Thr, the solubility of D- and L-Thr was estimated on the basis of the optical purity of Thr obtained by filtration and its weight.

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