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Optical Resolution by Replacing Crystallization of DL-Threonine

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Optical resolution by replacing crystallization of DL-threonine (DL-Thr) was achieved in the presence of an equimolar amount of L-proline as an optically active cosolute. This optical resolution gave D-Thr with 91% optical purity in 78% degree of resolution, and optically pure L-Thr was obtained from the mother liquor.

L-Threonine (abbreviated as L-Thr) as an essential amino acid has been obtained from natural products and used as a material for medicines and food additives. Moreover, both L-and D-Thr are useful as chiral reagents in asymmetric syntheses because they are capable of providing two chiral centers in a target substance. DL-Thr exists in a conglomerate, which can be optically resolved by preferential crystallization.[1,2] Optical resolution by replacing crystallization is also a procedure for obtaining one enantiomer from a conglomerate.[3] This optical resolution can be achieved by allowing an optically active cosolute to exist in a racemic solution without formation of diastereomers. This paper describes an attempt of optical resolution by replacing crystallization of DL-Thr.

Although the optical resolution by replacing crystallization has not fully been studied,[3—6] this procedure seems to give a good result when we employ an optically active cosolute whose structure is similar to that of the racemate. For the replacing crystallization of DL-Thr, we chose L-proline (L-Pro) as an optically active cosolute though the structure of L-Pro, as a cyclic amino acid, is different from that of DL-Thr. Since L-Pro dissolves in methanol and Thr does not, it is possible to separate Thr and L-Pro from their mixture. This suggests a possibility for, after crystallization of one enantiomer, obtaining the other enantiomer from the mother liquor. The optical resolution by replacing crystallization of DL-Thr, therefore, was tried by allowing L-Pro as an optically active cosolute to coexist in an aqueous supersaturated solution of DL-Thr.

Experimental

Amino Acids. DL-Thr was purchased from Sigma Chemicals Co. and L-Thr ($[\alpha]_D^{20}$ −28.1° (c 1.00, water)) and L-Pro ($[\alpha]_D^{20}$ −85.9° (c 1.00, water)) from Kokusan Chemical Works, Ltd. The D-Thr obtained by optical resolution was recrystallized from water: $[\alpha]_D^{20}$ +28.4° (c 1.00, water).

Optical Resolution. Crystallization by Seeding: DL-Thr (5.173 g, 43.43 mmol) was dissolved in 25 cm^3 of water at 40 °C. The solution was slowly cooled to 10 °C and seeded with 0.050 g of D-Thr. After stirring the mixture for 0.5—7 h at 100 rpm and 10 °C, the precipitated D-Thr was collected by filtration, washed with a small amount of methanol, and dried.

The optical resolution was also performed similarly by making 5.000 g (43.43 mmol) of L-Pro to coexist.

The optical purity of the obtained D-Thr was determined on the basis of the specific rotation ($[\alpha]_D^{26}$ +28.4° (water))[7] of D-Thr. The yield of optically pure modification (YOPM) and the amounts of crystallization of D- and L-Thr (W_D and W_L/g) were calculated by

$$\text{YOPM/g} = [\text{Yield/g} \times \text{Optical purity/\%}]/100 - S, \quad (1)$$

$$W_L/\text{g} = (1/2)[\text{Yield/g} - \text{YOPM/g} - S], \quad (2)$$

$$W_D/\text{g} = \text{YOPM/g} + W_L/\text{g}, \quad (3)$$

where S is the amount of the seed crystals (0.050 g in the above optical resolutions).

Replacing Crystallization: DL-Thr (6.897 g, 57.90 mmol) and 2.000—7.333 g (17.37—63.69 mmol) of L-Pro were dissolved in 25 cm^3 of water. The solution was stirred for 3 h at 100 rpm and 10 °C; the solution containing 6.666 g (57.90 mmol) of L-Pro was stirred for 1.5—4.5 h. The precipitated D-Thr was collected by filtration, washed with a small amount of methanol, and dried. The YOPM, W_L, and W_D were calculated by Eqs. 1—3, respectively; the S value in Eqs. 1 and 2 is zero. The degree of resolution of the D-Thr obtained from a solution containing 6.666 g of L-Pro was calculated by

$$\text{Degree of resolution/\%} = \text{YOPM/g} \times 100/1.567, \quad (4)$$

where the difference between the half initial amount of DL-Thr and the solubility of D-Thr in this system is 1.567 g.

The mother liquor after the optical resolution for 3 h using a solution containing 6.666 g (57.90 mmol) of L-Pro was dried under reduced pressure. After stirring a suspension of the residue in 200 cm^3 of methanol for 2 h at room temperature, 5.469 g of Thr ($[\alpha]_D^{20}$ −5.9° (c 1.00, water)) was filtered off; the methanol filtrate was dried to recover 6.451 g of L-Pro ($[\alpha]_D^{20}$ −84.7° (c 1.00, water)) as the residue. A solution of the Thr in 20 cm^3 of water was stirred for 5 h in an ice bath to give 1.309 g of optically pure L-Thr ($[\alpha]_D^{20}$ −28.4° (c 1.00, water)). Drying of the filtrate gave 4.074 g of DL-Thr ($[\alpha]_D^{20}$ +1.0° (c 1.00, water)).

Solubility. DL-, D-, or L-Thr (6.000 g) was dissolved in 25 cm^3 of water or in a solution of 5.000 or 6.666 g of L-Pro in 25 cm^3 of water at 40 °C. After vigorously stirring the solution for 10 h at 10 °C, the precipitated Thr was rapidly collected by filtration, washed with a small amount of methanol, and thoroughly dried. The solubility at 10 °C was calculated on

Table 1. Solubility of Threonine

Temperature	Solvent	Solubility/g (100 cm^3 water)$^{-1}$			
		DL-Thr		D-Thr	L-Thr
°C		D-Thr	L-Thr		
10	Water	7.474	7.474	—	6.949
10	L-Pro–Water[a]	7.521	8.557	7.108	7.223
10	L-Pro–Water[b]	7.526	8.887	7.140	7.334
15	Water	8.175	8.175	—	7.711
20	Water	9.190	9.190	—	8.548

a) The solvent contained 20.000 g of L-Pro in 100 cm^3 of water. b) The solvent contained 26.664 g of L-Pro in 100 cm^3 of water.

the basis of the weight of Thr. In the case of dissolving DL-Thr in a solution containing L-Pro, the solubilities of D- and L-Thr were estimated on the basis of the optical purity of the Thr obtained by filtration and its weight.

Measurement. Optical rotations were measured at 589 nm with a Union Giken PM-101 digital polarimeter with a quarz cell of 0.50 dm path length.

Results and Discussion

Thermodynamic Properties of Saturated Solution. Solubility: Statistical mechanical theory has shown that solubilities of enantiomers differ from each other in an optically active solvent or in a solution containing an optically active cosolute.[8] If a racemic modification is a conglomerate, one enantiomer has a possibility for cyrstallizing preferentially from a solution containing the conglomerate and optically active cosolute.[8] The solubilities of DL-, D-, and L-Thr were measured both in water and in an aqueous solution containing L-Pro at 10 °C (Table 1).

DL-, D-, and L-Thr were more soluble in the aqueous solution containing L-Pro than in water, and their solubilities increased with an increase in amount of L-Pro. Since L-Thr was more soluble than D-Thr in the presence of L-Pro, Thr at eutectic point was no longer racemate and slightly had an abundance of L-Thr; at the eutectic point, the mole fraction of D-Thr was estimated to be 0.0107, that of L-Thr 0.0126, that of L-Pro 0.0391, and that of water 0.938.

Activity Coefficient: If the difference in heat capacity of a solute between liquid and solid states is assumed to be zero, the solubility of a solute at T K is approximated by

$$\ln x = -\Delta H^{f}/(RT) + \Delta H^{f}/(RT^{f}) - \ln \gamma, \qquad (5)$$

where x is mole fraction, ΔH^{f} enthaply of fusion, T^{f} melting point, γ activity coefficient of the solute, and R the gas constant.

The solubilities of DL-Thr in water at 10 and 20 °C in Table 1 and that at 25 °C[9] give by the least squares method from

$$\ln x = -1750/T + 1.67, \qquad (6)$$

where x is the mole fraction of D- or L-Thr for a saturated solution of DL-Thr at T K (correlation coefficient 0.998). The γ value of D- or L-Thr for a saturated solution of DL-Thr in water is estimated to be 0.928 at 25 °C[10] and is assumed to be constant at 10—25 °C because the range of temperature is narrow. A comparison of Eq. 6 with Eq. 5 leads to

$$\ln x = -1750/T + 1.60 - \ln \gamma. \qquad (7)$$

Table 2. Activity Coefficient of Threonine in Saturated Solution[a]

Solvent	Activity coefficient			
	DL-Thr		D-Thr	L-Thr
	D-Thr	L-Thr		
Water	0.927	0.927	0.986	0.986
L-Pro–Water[b]	0.951	0.836	0.994	0.978
L-Pro–Water[c]	0.960	0.813	0.999	0.994

a) Temperature: 10 °C. b) The solvent contained 20.000 g of L-Pro in 100 cm^3 of water. b) The solvent contained 26.664 g of L-Pro in 100 cm^3 of water.

The γ values of D- and L-Thr in aqueous solutions at 10 °C were calculated on the basis of the solubilities in Table 1 by Eq. 7 and are listed in Table 2.

The γ values of D- and L-Thr in a solution of DL-Thr were smaller than those in solutions of D- and L-Thr, respectively. This result shows that an attractive interaction is acting between D- and L-Thr in the solution of DL-Thr. The γ values of L-Thr decreased with an increase in the amount of L-Pro, whereas those of D-Thr tended to increase. The above results show that the attractive interaction between L-Thr and L-Pro is stronger than that between D- and L-Thr and that a repulsive interaction is acting between D-Thr and L-Pro. Although a supersaturated solution may not necessarily be regarded as the same as a saturated solution, the primary nucleation of D-Thr in the supersaturated solution of DL-Thr is considered, on the basis of the phenomena mentioned above, to be accelerated in the presence of L-Pro but that of L-Thr to be prevented.[11]

Optical Resolution by Seeding. The solubilities suggested that D-Thr was preferentially crystallized from the supersaturated solution of DL-Thr in the presence of L-Pro. The optical resolution, therefore, was tried by seeding with D-Thr to compare replacing crystallization with ordinary preferential crystallization. These results are shown in Fig. 1.

Rapid crystallization of L-Thr has not been observed in either optical resolution, because the crystallized D-Thr had a 90—99% optical purity and the W_L value was not more than 0.02 g. DL-Thr is more soluble in aqueous solution in the presence of L-Pro than in its absence. Therefore, when the amount of DL-Thr dissolved in aqueous solution containing L-Pro is equal to that in water, the degree of supersaturation of the former solution is less than that of the latter solution. This made us presume that crystallization of D-Thr from the supersaturated solution in the presence of L-Pro would be slower than in the absence of L-Pro. Figure 1, however, shows that the W_D value in the presence of L-Pro is 1.9—2.6 times as large as that in the absence of L-Pro. This result supports that D-Thr is rapidly crystallized in the presence of L-Pro because the primary nucleation of D-Thr is accelerated by the repulsive interaction between D-Thr and L-Pro.

Optical Resolution by Replacing Crystallization. Optimization of the amount of L-Pro was conducted by stirring a solution of 57.90 mmol of DL-Thr and 17.37—63.69 mmol of L-Pro in 25 cm³ of water for 3 h at 10 °C. This result is shown in Fig. 2.

Since L-Thr was not rapidly crystallized under these conditions and the W_L values ranged from 0.02 to 0.11 g, the obtained D-Thr had a 73—99% optical purity. Although the W_D values tended to increase with an increase in the amount of L-Pro by the repulsive interaction between D-Thr and L-Pro, the values seemed to be constant in the range of 52.11 to 63.69 mmol of L-Pro. The optical resolution, therefore, was performed with a coexisting equimolar L-Pro (57.90 mmol) for DL-Thr. The results are summarized in Table 3.

D-Thr with 91% optical purity was obtained in 78% degree of resolution after 3 h. Drying of its mother liquor, followed by treatment of the residue with methanol, gave Thr and L-Pro (97% recovery). Optically pure L-Thr was obtained by recrystallizing the Thr from water by the procedure described in the experimental section. DL-Thr was recovered by drying

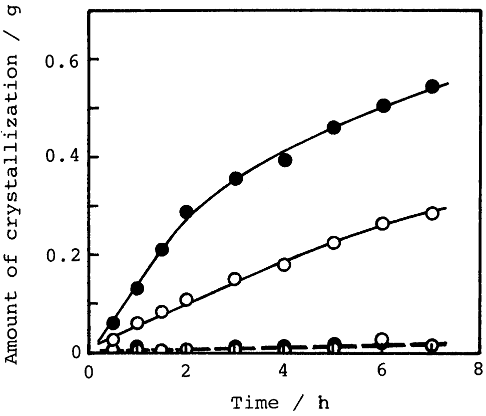

Fig. 1. Comparison of replacing crystallization with preferential crystallization. DL-Thr: 5.173 g. Seed crystals: 0.050 g of D-Thr. Water: 25 cm³. Temperature: 10 °C. —: D-Thr. ---: L-Thr. ○: Preferential crystallization. ●: Replacing crystallization; 5.000 g of L-Pro was contained in the aqueous solution.

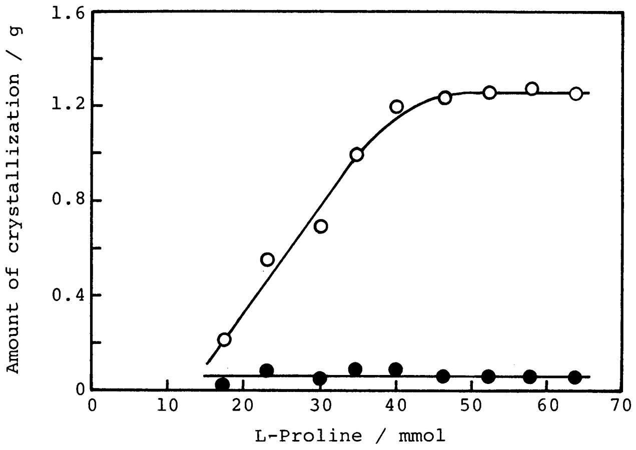

Fig. 2. Influence of amount of L-proline on replacing crystallization. DL-Thr: 6.897 g (57.90 mmol). Water: 25 cm³. Temperature: 10 °C. ○: D-Thr. ●: L-Thr.

Table 3. Optical Resolution by Replacing Crystallization of DL-Threonine[a)]

Resolution time h	Yield g	Optical purity %	YOPM[b)] g	Degree of resolution %
1.5	0.389	94.0	0.366	23.4
2.0	0.491	95.1	0.467	29.8
2.5	1.095	94.4	1.034	66.0
3.0	1.338	91.1	1.219	77.8
3.5	1.377	82.4	1.135	72.4
4.0	1.758	57.4	1.009	64.4
4.5	1.981	40.5	0.802	51.2

a) The supersaturated solution consisted of 6.897 g of DL-Thr and 6.666 g of L-Pro in 25 cm³ of water; temperature 10 °C. b) YOPM: Yield of optically pure modification.

the aqueous filtrate.

The optical resolution by replacing crystallization of DL-Thr can give D- and L-Thr by using L-Pro as an optically active cosolute.

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