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Optical Resolution by Replacing Crystallization of (*RS*)-4-Thiazolidinecarboxylic Acid with L-Amino Acids as Cosolute

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The optical resolution by replacing crystallization of (*RS*)-4-thiazolidinecarboxylic acid [(*RS*)-THC] was carried out with coexisting eight L-amino acids and the effect of the latter was examined. L-Isoleucine [L-Ile] and L-cysteine [L-Cys] as the cosolute led to preferential crystallization of (*R*)-THC from an aqueous solution of (*RS*)-THC, whereas the other L-amino acids gave (*S*)-THC. L-Cys, L-threonine, L-4-hydroxyproline [L-Hyp], and L-serine as the cosolute seemed to give better selectivity for crystallization of one enantiomer than L-Ile, L-valine L-proline, and L-alanine. The optical resolution with L-Hyp was successfully achieved and suggested a possibility of successive optical resolution to give both (*R*)- and (*S*)-THC.

Racemates existing in a conglomerate have been optically resolved by preferential crystallization. Optical resolution by replacing crystallization is another procedure for obtaining one enantiomer from a conglomerate and can be achieved by adding an optically active cosolute in a racemic supersaturated solution without formation of diastereomers. This technique, however, has not thoroughly been studied.[1-6] Since replacing crystallization is based on interactions between an enantiomer and the optically active cosolute, crystallization of one enantiomer is inhibited by an attractive interaction with the cosolute while the other enantiomer crystallizes. Further, replacing crystallization may give a clue to elucidate the chiral interaction between different optically active modifications in a solution. Such attractive interaction, however, may make it difficult to achieve a successive optical resolution for obtaining both enantiomers. One of the major problems in the optical resolution by replacing crystallization, therefore, is the selection of an optically active cosolute. In view of this, we examined the effect of cosolutes for the replacing crystallization of (*RS*)-4-thiazolidinecarboxylic acid [abbreviated as (*RS*)-THC] which was found to exist in a conglomerate.[7]

(*RS*)-THC has been obtained by reaction of DL-cysteine with formaldehyde[8,9] and can be regarded as a cyclic amino acid. We attempted to use L-amino acids as optically active cosolutes because a cosolute whose structure was similar to that of the racemate seemed to result in good optical resolution by replacing crystallization. In addition, the cosolute must be more soluble than the racemate because crystallization of the cosolute makes it difficult to separate the crystallized mixture into the cosolute and the resolved enantiomer. L-Isoleucine [L-Ile], L-cysteine [L-Cys], L-valine [L-Val], L-proline [L-Pro], L-alanine [L-Ala], L-threonine [L-Thr], L-4-hydroxyproline [L-Hyp], and L-serine [L-Ser] seem to be more soluble in water than THC.[7,10] Based on these considerations we explored the conditions, such as the nature and the amount of a cosolute, for successive optical resolution by replacing crystallization of (*RS*)-THC with these amino acids as cosolute to obtain (*R*)- and (*S*)-THC.

Experimental

Materials. L-Ile, DL- and L-Cys, L-Val, L-Pro, L-Ala, L-Thr, L-Hyp, and L-Ser were purchased from Kanto Chemical Co., Ltd, Wako Pure Chemicals Ind., Kokusan Chemical Works, Ltd., or Sigma Chemicals Co.

(*RS*)- and (*R*)-THC were obtained, respectively, by allowing DL- and L-Cys to react with formaldehyde in acetic acid.[9] (*S*)-THC was obtained by asymmetric transformation of (*R*)-THC via salt formation with (2*R*, 3*R*)-tartaric acid.[9]

Replacing Crystallization. (*RS*)-THC (1.900 g, 14.27 mmol) and 7.13 mmol of L-Ile, L-Cys, L-Val, L-Pro, L-Ala, L-Thr, L-Hyp, or L-Ser were dissolved in 25 cm^3 of water at 40 °C and then the solution was slowly cooled to 10 °C. After stirring the mixture for 0.5—8 h at 10 °C, the precipitated THC was collected by filtration, washed with a small amount of methanol, and dried. The optical purity (*OP*/%) of the obtained (*R*)- or (*S*)-THC was determined on the basis of the specific rotation of (*R*)-THC ($[\alpha]_D^{20}$ −141 ° (*c* 0.500, water)).[8] The yield of optically pure modification (*YOPM*/g), degree of resolution (*DR*/%), and degrees of crystallization of (*R*)- and (*S*)-THC ($DC_{(R)}$ and $DC_{(S)}$/%) were calculated from Eqs. 1—4, respectively,

$$YOPM/\mathrm{g} = [\mathrm{Yield/g} \times OP/\%]/100, \quad (1)$$

$$DR/\% = [YOPM/\mathrm{g} \times 100]/[0.950 - S_{(R)\text{ or }(S)}/\mathrm{g}], \quad (2)$$

$$DC_{(S)}/\% = (1/2)[\mathrm{Yield/g} - YOPM/\mathrm{g}] \times 100/[0.950 - S_{(S)}/\mathrm{g}], \quad (3)$$

$$DC_{(R)}/\% = (1/2)[\mathrm{Yield/g} + YOPM/\mathrm{g}] \times 100/[0.950 - S_{(R)}/\mathrm{g}], \quad (4)$$

Here, $S_{(S)}$ and $S_{(R)}$ denote, respectively, the solubilities of (*S*)- and (*R*)-THC in (*RS*)-THC in 25 cm^3 of water at 10 °C when L-Ile and L-Cys were used as cosolute, and the subscripts (*R*) and (*S*) in Eqs. 3 and 4 are interchanged when the other L- amino acids were used.

Optical Resolution. Optical Resolution by Use of L-Proline: (*RS*)-THC (1.900 g, 14.27 mmol) and 2.300 g (19.98 mmol) of L-Pro were dissolved in 25 cm^3 of water. The solution was stirred for 3 h at 10 °C to give 0.220 g of (*S*)-THC

($[\alpha]_D^{20}$ +139° (*c* 0.500, water)). The mother liquor was dried under reduced pressure. After stirring a suspension of the residue in 100 cm³ of methanol for 1 h at room temperature, (*R*)-THC (1.570 g, $[\alpha]_D^{20}$ −21.2° (*c* 0.500, water)) was filtered off; the methanol filtrate was dried to recover 2.208 g of L-Pro. The (*R*)-THC was recrystallized from 21 cm³ of water to give 0.203 g of optically pure (*R*)-THC ($[\alpha]_D^{20}$ −141° (*c* 0.500, water)). Drying of the filtrate gave 1.176 g of (*RS*)-THC.

Optical Resolution by Use of L-Cysteine: (*RS*)-THC (2.663 g, 20.00 mmol) and 2.423 g (20.00 mmol) of L-Cys were dissolved in 25 cm³ of water at 50 °C. The solution was stirred for 5—40 min at 5 °C. The precipitated (*R*)-THC was collected by filtration, washed with methanol, and dried.

Successive Optical Resolution by Use of L-Threonine: (*RS*)-THC (1.900 g, 14.27 mmol) and 0.510 g (4.28 mmol) of L-Thr were dissolved in 25 cm³ of water. After stirring the solution for 2.5 h at 10 °C, the precipitated (*S*)-THC (0.339 g) was obtained. The mother liquor was stirred for 6 h at 10 °C to give 0.128 g of (*R*)-THC. (*RS*)-THC (0.467 g) was dissolved in the filtrate and then the solution was treated similarly to the case where L-Hyp was used as cosolute, as described below. The degrees of resolution of the obtained (*R*)- and (*S*)-THC were calculated from Eq. 5, as described below, by using 0.623 g as the *S* value for (*R*)-THC and 0.606 g as that for (*S*)-THC.

Successive Optical Resolution by Use of L-4-Hydroxyproline: (*RS*)-THC (3.800 g, 28.54 mmol) and 1.871 g (14.27 mmol) of L-Hyp were dissolved in 50 cm³ of water. After stirring the solution for 3 h at 10 °C, the precipitated (*S*)-THC (0.584 g) was filtered off, washed with methanol, and dried. The mother liquor was stirred for 10 h at 10 °C to give 0.243 g of (*R*)-THC. After dissolving (*RS*)-THC (0.827 g) in the filtrate, followed by stirring for 3 h at 10 °C, the precipitated (*S*)-THC (0.443 g) was obtained. The filtrate was treated in a manner similar to the above. The degrees of resolution of the obtained (*R*)- or (*S*)-THC were calculated from

$$DR/\% = [YOPM/\mathrm{g} \times 100]/[\text{Operation amount/g} - S], \quad (5)$$

where *S* was the solubility of (*R*)-THC (1.259 g) or (*S*)-THC (1.251 g) in (*RS*)-THC in 50 cm³ of water at 10 °C.

Solubility. (*RS*)-, (*R*)-, or (*S*)-THC (1.900 g, 14.27 mmol) was dissolved in a solution containing 7.13 mmol of an L-amino acid in 25 cm³ of water at 40 °C. After vigorously stirring the solution for 10 h at 10 °C, the precipitated THC was rapidly collected by filtration, washed with a small amount of methanol, and thoroughly dried. The solubility (g (100 cm³ water)$^{-1}$) at 10 °C was calculated on the basis of the weight of the THC. In the case of dissolving (*RS*)-THC, the solubilities of (*R*)- and (*S*)-THC were estimated on the basis of the optical purity of the THC obtained by filtration and its weight; optical rotation was measured at 589 nm with a Union Giken PM-101 digital polarimeter with a quarz cell of 5.00 cm path length.

Results and Discussion

Solubility of 4-Thiazolidinecarboxylic Acid in the Presence of L-Amino Acid. Statistical mechanical theory has shown a possibility for preferential crystallization of one enantiomer from a racemic supersaturated solution containing an optically active cosolute because the solubilities of enantiomers differ from each other.[11]

Table 1. Solubility of 4-Thiazolidinecarboxylic Acid[a]

L-Amino acid as cosolute	Solubility/g (100 cm³ water)$^{-1}$			
	(*RS*)-THC		(*R*)-THC	(*S*)-THC
	(*R*)-THC	(*S*)-THC		
L-Ile	2.40	2.43	2.32	2.36
L-Cys	2.49	2.52	2.45	2.49
L-Val	2.43	2.42	2.38	2.36
L-Pro	2.47	2.46	2.42	2.36
L-Ala	2.46	2.43	2.44	2.38
L-Thr	2.53	2.42	2.49	2.45
	2.49[b]	2.42[b]	2.44[b]	2.41[b]
L-Hyp	2.52	2.50	2.51	2.48
L-Ser	2.60	2.47	2.57	2.47

a) L-Amino acid (28.54 mmol) as cosolute was dissolved in 100 cm³ of water; temperature 10 °C. b) L-Thr (17.12 mmol) as the cosolute was dissolved in 100 cm³ of water.

The solubilities of (*RS*)-, (*R*)-, and (*S*)-THC, therefore, were measured in an aqueous solution containing L-Ile, L-Cys, L-Val, L-Ala, L-Pro, L-Thr, L-Hyp, or L-Ser as the optically active cosolute at 10 °C and are listed in Table 1.

Although the solubilities of (*R*)- and (*S*)-THC in aqueous solutions containing these L-amino acids are comparable to each other, (*S*)-THC was more soluble than (*R*)-THC in solutions containing L-Ile and L-Cys, whereas (*R*)-THC was more soluble than (*S*)-THC in solutions containing the other L-amino acids. These above results suggested that (*R*)-THC crystallizes preferentially from a solution containing (*RS*)-THC and L-Ile or L-Cys, and (*S*)-THC from solutions containing the other L-amino acids.

When different amino acids coexist in an aqueous solution, a hydrophobic interaction takes place between the amino acids and one amino acid could influence the solubility of other amino acids.[5,11] Therefore, a relationship between the solubilities and their hydrophobic substituent parameters (π)[12-14] of the side chains on the L-amino acid as cosolute has been examined and is shown in Fig. 1.

The solubilities of (*RS*)-, (*R*)- and (*S*)-THC tend to increase with a decrease in the π value, that is, in the hydrophobic property of the side chain. Since the π value of THC is estimated to be 0.01,[14] THC may be more soluble in solutions containing an L-amino acid with a weakly hydrophobic side chain.

The π value of L-Cys (1.54) suggests that L-Cys has a strongly hydrophobic side chain.[12] The solubility of THC in a solution containing L-Cys, however, was higher than those in solutions containing L-Ile, L-Val, L-Pro, or L-Ala and comparable to those in solutions containing L-Thr and L-Hyp, as seen in Fig. 1. Although Fauchere et al.[12] and Nozaki et al.[15] reported that the side chain of L-Cys is strongly hydrophobic, Wolfenden et al.[16] regarded it as a hydrophilic side chain. The side chain of L-Cys, therefore, may be weakly hydrophobic of magnitude

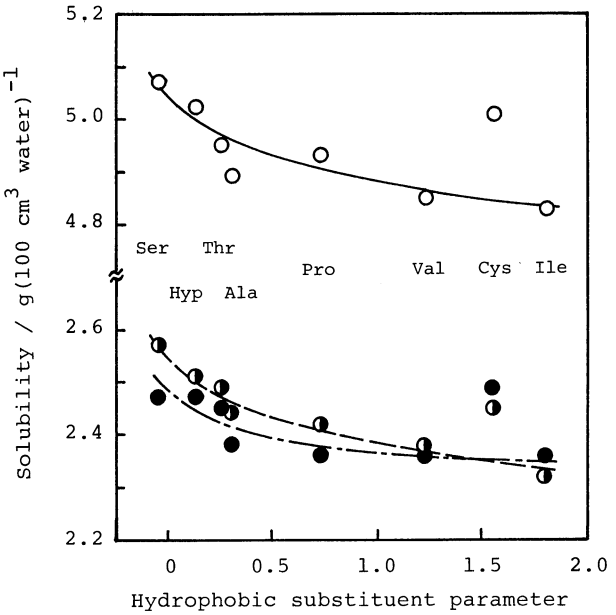

Fig. 1. Solubility of 4-thiazolidinecarboxylic acid in the presence of L-amino acid. Conditions: L-Amino acid 28.54 mmol; water 100 cm^3; temperature 10 °C. 4-Thiazolidinecarboxylic acid [THC]: ○ (*RS*)-THC; ◑ (*R*)-THC; ● (*S*)-THC.

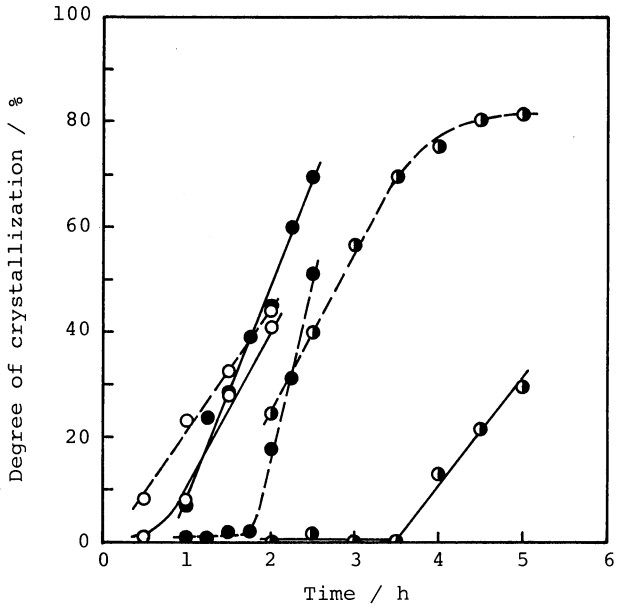

Fig. 2. Replacing crystallizations by use of L-isoleucine, L-cysteine, and L-proline as cosolute. Conditions: (*RS*)-THC 14.27 mmol; L-amino acid 7.13 mmol; water 25 cm^3; temperature 10 °C. L-Amino acid: ○ L-isoleucine; ◑ L-cysteine; ● L-proline. —: (*S*)-THC. ---: (*R*)-THC.

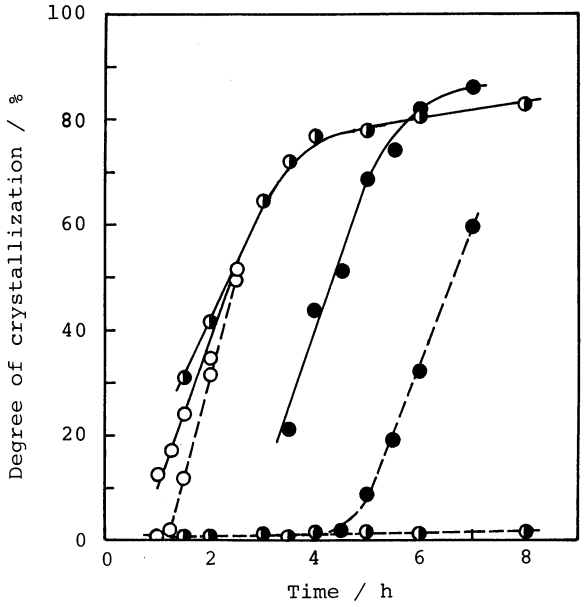

Fig. 3. Replacing crystallizations by use of L-alanine, L-threonine, and L-serine as cosolute. Conditions: (*RS*)-THC 14.27 mmol; L-amino acid 7.13 mmol; water 25 cm^3; temperature 10 °C. L-Amino acid: ○ L-alanine; ◑ L-threonine; ● L-serine. —: (*S*)-THC. ---: (*R*)-THC.

comparable with those of L-Thr and L-Hyp.

Replacing Crystallization of (*RS*)-4-Thiazolidinecarboxylic Acid. The replacing crystallization of (*RS*)-THC was carried out by allowing a half molar amount of an L-amino acid for (*RS*)-THC to coexist in an aqueous solution at 10 °C. The results are shown in Figs. 2 and 3 and listed in Table 2; the result obtained with L-Hyp is given in Fig. 6 and that by L-Val not in these figures.

As expected from the solubilities, (*R*)-THC was crystallized preferentially from solutions containing L-Ile and L-Cys, whereas (*S*)-THC from those containing the other L-amino acids.

When L-Thr was used as a cosolute, (*R*)-THC did not crystallize even in 8 h. Since DL-Thr exists in a conglomerate, strongly attractive interaction seems to be in force between L-Thr and (*R*)-THC in the aqueous solution; (*R*)-THC corresponds to an L-amino acid in configuration.

By use of L-Hyp as a cosolute, the replacing crystallization seems to give the best result. Supersaturated (*S*)-THC crystallized nearly quantitatively in 3—4 h, then (*R*)-THC began to crystallize rapidly; the $DC_{(S)}$ and $DC_{(R)}$ values at 3 h were 98 and 2%, respectively.

One of the major problems in optical resolution by replacing crystallization is a difference between the beginning time for crystallization of one enantiomer and the retardation time for other enantiomer. The yield of the enantiomer with high optical purity increases with an increase in the difference, and hence the optical resolution is carried out efficiently. Then the beginning time for crystallization of (*S*)-THC ($t_{(S)}$/h) was estimated by extrapolating the linear portion in Figs. 2, 3, and 6 to zero degree of crystallization. The retardation time for crystallization of (*R*)-THC ($t_{(R)}$/h) was defined as the time at which (*R*)-THC began to crystallize rapidly; when

Table 2. Replacing Crystallization of (*RS*)-4-Thiazolidinecarboxylic Acid[a)]

L-Amino acid as cosolute	Stirring time	THC obtained					Δt[d)]
		Configuration	Yield	Optical purity	*YOPM*[b)]	DR_{max}[c)]	
	h		g	%	g	%	h
L-Ile	0.5	(*R*)	0.036	77.8	0.028	8.0	−0.25
L-Cys	3.5	(*R*)	0.228	100	0.228	69.7	−2.25
L-Val	0.75	(*S*)	0.031	87.1	0.027	7.8	0.30
L-Pro	1.75	(*S*)	0.137	91.2	0.125	37.2	1.00
L-Ala	1.25	(*S*)	0.064	81.3	0.052	15.2	0.68
L-Thr	8	(*S*)	0.290	96.9	0.281	81.4	>8
L-Hyp	3	(*S*)	0.324	96.3	0.312	96.0	2.38
L-Ser	5	(*S*)	0.254	79.8	0.202	60.8	2.00

a) (*RS*)-THC 1.900 g (14.27 mmol); L-amino acid 7.13 mmol; water 25 cm³; temperature 10°C. b) *YOPM*: Yield of optically pure modification. c) DR_{max}: Maximal value of degree of resolution. d) $\Delta t = t_{(R)} - t_{(S)}$; $t_{(R)}$ and $t_{(S)}$ denote retardation times for crystallization of (*R*)-THC and (*S*)-THC, respectively.

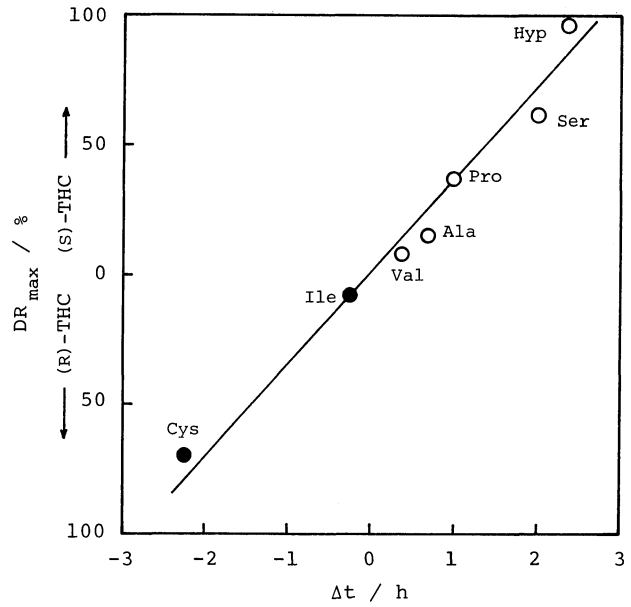

Fig. 4. Maximal degree of resolution in replacing crystallization. Conditions for maximal degree of resolution: See Table 2. ○: (*S*)-THC. ●: (*R*)-THC.

L-Ile and L-Cys were used as cosolute, the beginning and retardation times are denoted as $t_{(R)}$ and $t_{(S)}$ h, respectively. The difference between $t_{(S)}$ and $t_{(R)}$ h is represented as Δt/h; when L-Ile and L-Cys were used as cosolute, their Δt were negative values. The degree of resolution was used as a measure of the optical resolution efficiency, and the maximal value (DR_{max}/%) was attained at around the retardation time for crystallization of (*R*)- or (*S*)-THC; the DR_{max} values are listed in Table 2. A relationship between the Δt and DR_{max} values is depicted in Fig. 4.

The DR_{max} value tends to increase with a decrease in hydrophobicity of the side chain on the L-amino acids used as cosolute. Since the replacing crystallization by use of L-Thr gave (*S*)-THC with 97% optical purity and 81% degree of resolution even in 8 h (Table 2 and Fig. 3), the Δt value was estimated to be more than 8 h. This replacing crystallization, therefore, seems to require a longer time for giving (*S*)-THC in over 90% degree of resolution.

When L-Ser with a hydrophilic side chain was used as cosolute, (*S*)-THC with 80% optical purity was obtained in a maximal degree of resolution of 61% in 5 h. The replacing crystallization at a longer time, however, gave (*S*)-THC with lower optical purity because (*R*)-THC also began to crystallize rapidly at 5 h.

Optical Resolution of (*RS*)-4-Thiazolidinecarboxylic Acid. Optimization of the Amount of L-Amino Acids as Cosolute: A preferential crystallization has a possibility of successive optical resolution for obtaining both enantiomers.[7] However, successive optical resolution by replacing crystallization seems to be difficult because an attractive interaction is acting between one enantiomer and the optically active cosolute in a solution. We therefore attempted to find out a favorable cosolute and optimal conditions for successive optical resolution by replacing crystallization. Based on the results of replacing crystallization, optimization of the amount of L-Cys, L-Thr, or L-Hyp was done by stirring a solution of 14.27 mmol of (*RS*)-THC and 1.43—15.70 mmol of these amino acids; the amount of L-Pro was varied in a range of 8.56—22.82 mmol of L-Pro. The results are shown in Fig. 5.

When L-Thr and L-Hyp were used as cosolutes, replacing crystallization in the presence of 1.43—7.13 mmol of these amino acids gave (*S*)-THC with low optical purity because a fairly large amount of (*R*)-THC crystallized concomitantly. Although the amount of crystallization of (*S*)-THC was approximately constant over a range of 1.43—4.28 mmol of L-Thr, the amount tended to increase with an increase in the amount of coexisting L-Hyp (2.85—7.13 mmol). On the other hand, the amount of crystallization of (*R*)-THC decreased with an increase in the amount of L-Thr and L-Hyp. When a larger amount of L-Thr or L-Hyp coexisted, the amount of crystallization of (*S*)-THC decreased and (*R*)-THC hardly crystallized. These replacing crystallizations gave (*S*)-THC with approxi-

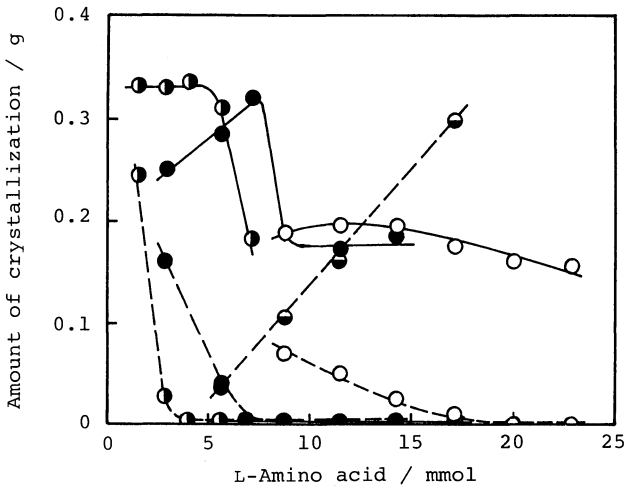

Fig. 5. Optimization of the amount of L-amino acid as cosolute. Conditions: (*RS*)-THC 14.27 mmol; L-amino acid 1.43—22.82 mmol; water 25 cm^3; temperature 10 °C. Stirring time: L-Proline (○) 2 h; L-cysteine (◒) 2 h; L-threonine (◑) 2.5 h; L-4-hydroxyproline (●) 3 h. —: (*S*)-THC. – – –: (*R*)-THC.

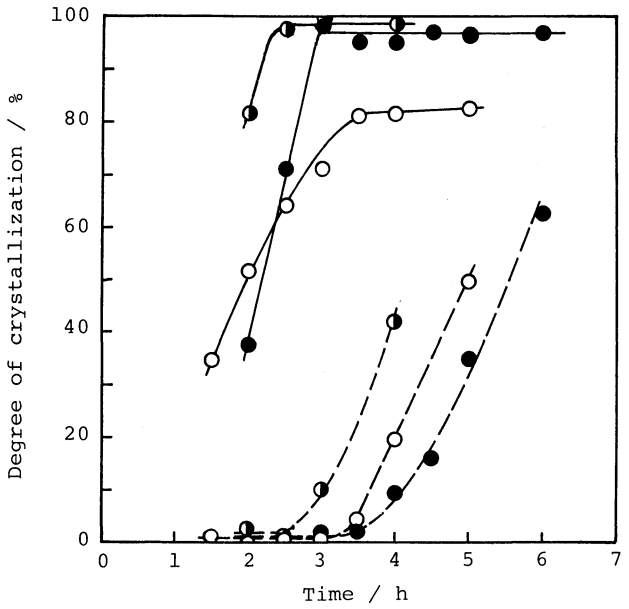

Fig. 6. Optimization of the stirring time by use of L-proline, L-threonine, and L-4-hydroxyproline. Conditions: (*RS*)-THC 14.27 mmol; L-proline (○) 19.98 mmol; L-threonine (◑) 4.28 mmol; L-4-hydroxyproline (●) 7.13 mmol; water 25 cm^3; temperature 10 °C. —: (*S*)-THC. – – –: (*R*)-THC.

mately 100% optical purity; the weights were 0.339 g with 4.28 mmol of L-Thr and 0.329 g with 7.13 mmol of L-Hyp. Further, the replacing crystallization with these levels of L-Thr and L-Hyp were carried out by stirring for 2—6 h (Fig. 6), and the optimal conditions were estimated from these results.

When L-Cys was used as cosolute, the amount of crystallization of (*R*)-THC increased with an increase in the amount of L-Cys, whereas rapid crystallization of (*S*)-THC did not take place. This result suggests that a strongly replusive interaction occurs between (*R*)-THC and L-Cys and an attractive one between (*S*)-THC and L-Cys.

Optical Resolution by Use of L-Proline as Cosolute: Although the replacing crystallization by use of L-Pro as cosolute gave (*S*)-THC with 91% optical purity, the maximal degree of resolution was low (37%), as seen in Table 2. However, it seems possible to separate THC and L-Pro from their mixture, which is prepared by drying a mother liquor, because L-Pro dissolves in methanol but THC does not. The replacing crystallization with 19.98 mmol of L-Pro gave (*S*)-THC with 96% optical purity in 0.159 g yield by stirring the solution for 2 h, as shown in Fig. 5, and (*S*)-THC with 99% optical purity in 0.220 g yield by stirring for 3 h, as shown in Fig. 6. After collecting the latter (*S*)-THC by filtration, drying of its mother liquor, and subsequent treatment of the residue with methanol, we obtained (*R*)-THC with 15% optical purity in 1.570 g yield and L-Pro in 96% (2.208 g) recovery. Optically pure (*R*)-THC (0.203 g) was obtained by recrystallizing the (*R*)-THC from water, and 1.176 g of (*RS*)-THC was recovered by drying the aqueous filtrate. Although this optical resolution gives both (*R*)- and (*S*)-THC with approximately 100% optical purity and allows to recover (*RS*)-THC and L-Pro efficiently, the procedure may be tedious. The successive optical resolution by use of L-Cys, L-Thr, and L-Hyp as cosolute, therefore, were attempted to obtain both (*R*)- and (*S*)-THC.

Optical Resolution by Use of L-Cysteine as Cosolute: The replacing crystallization with 14.27 mmol of (*RS*)-THC and 7.13 mmol of L-Cys gave optically pure (*R*)-THC in 70% degree of resolution in 3.5 h, as seen in Table 2. (*S*)-THC, however, did not crystallize from the mother liquor. Since the use of a smaller amount of L-Cys lowered extremely the yield of (*R*)-THC, as seen in Fig. 5, the replacing crystallization with equimolar amounts (20.00 mmol) of (*RS*)-THC and L-Cys was attempted by stirring for 5—40 min at 5 °C. The results are summarized in Table 3. The replacing crystallization in 5—20 min gave (*R*)-THC with 91—99% optical purity in 80% degree of resolution based on the half amount of the starting (*RS*)-THC. Although these results are comparable to that attained by a diastereomeric procedure,[17] the successive optical resolution was not achieved because (*S*)-THC did not crystallize even by allowing the mother liquor to stand overnight at 5 °C.

Optical Resolution by Use of L-Threonine as Cosolute: The successive optical resolution was attempted with 14.27 mmol of (*RS*)-THC and 4.28 mmol of L-Thr in 25 cm^3 of water at 10 °C. The result is given in Table 4.

Although (*S*)-THC with 98% optical purity was obtained in 96% degree of resolution from the initial solution (Run 1 in Table 4) and optically pure (*R*)-THC

Table 3. Optical Resolution of (*RS*)-4-Thiazolidinecarboxylic Acid by Use of L-Cysteine as Cosolute[a)]

Stirring time	(*R*)-THC obtained		
	Yield	Optical purity	Degree of resolution[b)]
min	g	%	%
5	1.127	97.6	82.6
10	1.095	99.3	81.6
15	1.109	96.1	80.0
20	1.148	90.9	78.3
30	1.194	81.2	72.8
40	1.393	58.5	61.2

a) (*RS*)-THC 2.663 g (20.00 mmol); L-Cys 2.423 g (20.00 mmol); water 25 cm^3; temperature 5 °C.
b) The degree of resolution was calculated on the basis of 1.332 g (10.00 mmol) of THC.

Table 4. Successive Optical Resolution of (*RS*)-4-Thiazolidinecarboxylic Acid[a)]

L-Amino acid as cosolute	Run	Added amount of (*RS*)-THC	Operation amount[b)]/g		Stirring time	THC obtained			
			(*R*)-THC	(*S*)-THC		Configuration	Yield	Optical purity	Degree of resolution
		g			h		g	%	%
L-Thr[c)]	1	1.900	0.950	0.950	2.5	(*S*)	0.339	97.7	96.3
	2	0	0.946	0.615	6	(*R*)	0.128	100	39.6
	3	0.467	1.052	0.849	3	(*R*)	0.324	47.0	35.5
	4	0	0.814	0.763	8[d)]	(*S*)	0.461	5.3	—
L-Hyp[e)]	1	3.800	1.900	1.900	3	(*S*)	0.584	98.3	88.5
	2	0	1.895	1.321	10	(*R*)	0.243	100	38.2
	3	0.827	2.066	1.735	3	(*S*)	0.443	50.3	46.0
	4	0	1.956	1.402	9	(*R*)	0.531	100	76.2
	5	0.974	1.912	1.889	3	(*S*)	0.558	96.9	84.7
	6	0	1.903	1.340	8	(*R*)	0.390	100	60.6

a) Temperature: 10 °C. b) The operation amounts of (*R*)- and (*S*)-THC in Runs 2—4 and 2—6 were calculated on the basis of the results in Runs 1—3 and 1—5, respectively. c) (*RS*)-THC 1.900 g (14.27 mmol); L-Thr 0.510 g (4.28 mmol); water 25 cm^3. d) After being stirred for 8 h at 10 °C, the mixture was allowed to stand overnight at 5 °C. e) (*RS*)-THC 3.800 g (28.54 mmol); L-Hyp 1.871 g (14.27 mmol); water 50 cm^3.

crystallized from the mother liquor, the yield of (*R*)-THC (0.128 g) was low (Run 2); the degree of resolution was calculated to be 40% on the basis of its solubility. (*R*)-THC with 47% optical purity, therefore, crystallized from a solution, which dissolved (*RS*)-THC in the filtrate (Run 3), and the subsequent procedure gave nearly racemic (*S*)-THC (Run 4). The poor results in Runs 3 and 4 appear to be due to poor crystallization of (*R*)-THC in Run 2.

Successive Optical Resolution by Use of L-4-Hydroxyproline as Cosolute: Figure 6 shows that the replacing crystallization by use of 7.13 mmol of L-Hyp in 3 h gave (*S*)-THC with 96% optical purity in 96% degree of resolution and (*R*)-THC crystallized rapidly at longer time than 3 h. After collecting the (*S*)-THC by filtration, optically pure (*R*)-THC was obtained in 0.207 g yield by stirring the filtrate for 6 h at 10 °C. The degree of resolution of (*R*)-THC was calculated to be 61% on the basis of its solubility. These results would suggest a possibility of successive optical resolution. The successive optical resolution was attempted with 28.54 mmol of (*RS*)-THC and 14.27 mmol of L-Hyp in 50 cm^3 of water (Table 4).

(*S*)-THC and (*R*)-THC with approximately 100% optical purity were obtained in 89 and 38% degrees of resolution in Runs 1 and 2 in Table 4, respectively. Although the (*S*)-THC obtained in Run 3 had low optical purity (50%), (*R*)- and (*S*)-THC with approximately 100% optical purity were obtained in 60—85% degree of resolution in Runs 4—6.

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