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Note

Optical Resolution by Preferential Crystallization of (*RS*)-2-Amino-3-(2-carboxyethylthio)propanoic Acid

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Electrophilic additions of DL- and L-Cys to propenoic acid afforded (*RS*)- and (*R*)-2-amino-3-(2-carboxyethylthio)propanoic acids [(*RS*)- and (*R*)-ACE], respectively. (*RS*)-ACE was found to exist as a conglomerate based on its melting point, solubility, and infrared spectrum. (*RS*)-ACE was optically resolved by preferential crystallization to yield (*R*)- and (*S*)-ACE. The obtained (*R*)- and (*S*)-ACE were efficiently recrystallized from water, taking account of the solubility of (*RS*)-ACE, to give them in optically pure form.

Key words: optically active 2-amino-3-(2-carboxyethylthio)propanoic acid; conglomerate; optical resolution; preferential crystallization

Optical resolution by preferential crystallization is a simple and useful method for the large-scale separation of enantiomers from racemates.[1-3] Optical resolution is achieved by providing a small amount of one enantiomer as seed crystals in a supersaturated racemic solution. Although racemates exist in the form of racemic compounds, conglomerates, and racemic solid solutions, only conglomerates, which are defined as a mechanical mixture of crystals of both enantiomers, can be optically resolved by preferential crystallization. However, conglomerates are much less common than racemic compounds.[1] Therefore, we have been screening for racemic amino acids that exist as conglomerates[4,5] and found another example.

Many L-*S*-(carboxyalkyl)cysteine forms have been found in plants.[6-9] Of the racemates of such amino acids, only (*RS*)-2-amino-3-(carboxymethylthio)-propanoic acid[10] and its ammonium salt[11] are reported to exist as conglomerates and have been optically resolved by preferential crystallization. (*R*)-2-Amino-3-(2-carboxyethylthio)propanoic acid [(*R*)-ACE; L-*S*-(2-carboxyethyl)cysteine] has been isolated from the seeds of *Acasia*[6,7] and *Minosaceae* species[8] and determined in young leaves of *Calloandra rubescens*.[9] We attempted to optically resolve (*RS*)-ACE by preferential crystallization. To our knowledge, this is the first attempt to optically resolve (*RS*)-ACE.

Although (*R*)-ACE has been synthesized by nucleophilic substitution of 3-chloropropanoic acid with L-Cys,[12] we tried more convenient syntheses of (*RS*)- and (*R*)-ACE by electrophilic additions of DL- and L-Cys to propenoic acid, respectively. The (*RS*)- and (*R*)-ACE samples obtained were determined from their ^{1}H-NMR spectra and elemental analyses.

The racemic structure was examined by comparing the melting point, solubility and infrared spectrum of the racemate with those of the enantiomer.[1-3] Conglomerates melt at a lower temperature than their enantiomers.[1] Although the (*RS*)-ACE sample obtained here was decomposed by heating, (*RS*)-ACE had a lower decomposition temperature than (*R*)-ACE. (*RS*)-ACE was more soluble in water than (*R*)-ACE and showed an infrared spectrum identical to that of (*R*)-ACE. Racemates that exist as a conglomerates are known to have such characteristics.[1,4,5] Therefore, (*RS*)-ACE was subjected to optical resolution by preferential crystallization.

To optimize the conditions, the optical resolution was conducted by stirring aqueous solutions with 111, 120, 133, 140, and 155% degrees of supersaturation for 10 min at 10°C (Fig. 1). (*R*)-ACE was employed as the seed crystals. Although the specific rotation of (*R*)-ACE in 1 M hydrochloric acid has been reported,[13] its absolute value is smaller than that in 0.05 M aqueous sodium hydroxide. Therefore, the optical purity [*OP* (%)] of the obtained (*R*)-ACE was calculated on the basis of the specific rotation ($[\alpha]_D^{20}-32.8°$ (*c* 1.00, 0.05 M NaOH)) of optically pure (*R*)-ACE prepared as just described. The yield of the enantiomer [*YE* (g)] in Fig. 1 was calculated from

$$YE(\mathrm{g}) = [Yield(\mathrm{g}) \times OP(\%)]/100 - 0.100$$

where *Yield* indicates the sum of the seed crystals (0.100 g of (*R*)-ACE) and crystallized ACE. The yield of the enantiomer is the amount of crystallized optically pure (*R*)-ACE and corresponds to the theoretical yield of optically pure (*R*)-ACE obtained by separating from partially resolved (*R*)-ACE.

When the 111–133% supersaturated solutions were employed, (*R*)-ACE with an optical purity of 93–97% was obtained and no undesired rapid crystallization of

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Abbreviations: ACE, 2-amino-3-(2-carboxyethylthio)propanoic acid; ACP·HCl, 2-amino-3-chloropropanoic acid hydrochloride; *OP*, optical purity; *YE*, yield of enantiomer.

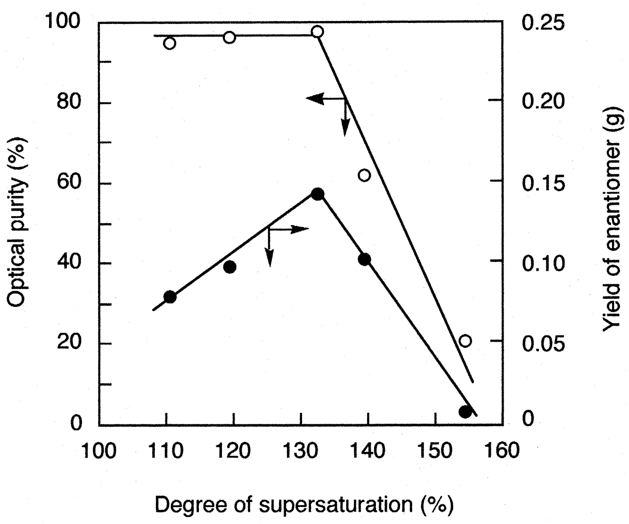

Fig. 1. Relationship between the Optical Purity or Yield of the Enantiomer and the Degree of Supersaturation in the Preferential Crystallization of (*RS*)-2-Amino-3-(2-carboxyethylthio)propanoic Acid.

Conditions: (*RS*)-ACE, 2.502, 2.705, 2.998, 3.156, and 3.494 g; seed crystals, 0.100 g of (*R*)-ACE; solvent, 50 cm^3 of water; temperature, 10°C. ○ Optical purity. ● Yield of the enantiomer of crystallized (*R*)-ACE.

Table I. Successive Optical Resolution by Preferential Crystallization of (*RS*)-2-Amino-3-(2-carboxyethylthio)propanoic Acid[a]

Run	Added amount of (*RS*)-ACE (g)	Resolution time (min)	ACE obtained	
			Yield[b] (g)	*OP*[c] (%)
1	2.998	10	(*R*) 0.251	96.8
2	0.151	20	(*S*) 0.375	64.9
3	0.275	5	(*R*) 0.342	70.3
4	0.242	5	(*S*) 0.312	80.5

[a] Conditions: seed crystals, 0.100 g of (*R*)- or (*S*)-ACE; solvent, 50 cm^3 of water; temperature, 10°C.

[b] *Yield* indicates the sum of the amounts of crystallized ACE and seed crystals.

[c] *OP*: optical purity.

unseeded (*S*)-ACE was apparent. Therefore, (*RS*)-ACE was confirmed to exist as a conglomerate. When the 140 and 155% supersaturated solutions were employed, the optical resolution gave poor results because of rapid crystallization of unseeded (*S*)-ACE, as shown in Fig. 1 and described in the Experimental section. Therefore, optical resolution using the 133% supersaturated solution gave (*R*)-ACE of 97% optical purity with the highest yield of the enantiomer, as shown in Fig. 1. Optical resolution was carried out by using the 133% supersaturated solution at a resolution time of 20–40 min. However, unseeded (*S*)-ACE began to crystallize rapidly even at 20 min. Based on these results, successive optical resolution was performed by employing the 133% supersaturated solution as the initial solution, and by stirring for 10 min (Table I). (*S*)-ACE employed as the seed crystals was synthesized in a 49% yield by the reaction of 3-mercaptopropanoic acid with (*S*)-2-amino-3-chloropropanoic acid hydrochloride [(*S*)-ACE·HCl], which in turn was prepared from D-Ser.

The successive optical resolution yielded (*R*)- and (*S*)-ACE with an optical purity of 65–97%. Although the obtained (*R*)- and (*S*)-ACE did not have very high optical purity, they could be efficiently recrystallized from water, taking account of the solubility of (*RS*)-ACE, as described in the Experimental section. For example, optically pure (*R*)-ACE (1.59 g) was obtained from 2.00 g of (*R*)-ACE with an optical purity of 82%, and optically pure (*S*)-ACE (1.65 g) from 2.20 g of (*S*)-ACE with an optical purity of 77%.

Experimental

Specific rotation values were measured at 589 nm with a Horiba Seisakusho SEPA-300 auto-polarimeter equipped with a quartz cell of 5.00-cm path length. Infrared spectra were obtained in the range of 4000–400 cm^{-1} with a Perkin-Elmer model 1600 FT-IR spectrometer by the KBr disk method. ^{1}H-NMR spectra were recorded with a JNM-FX270 FT NMR SYSTEM, sodium 3-(trimethylsilyl)propanesulfonate (DSS) being used as an internal standard. Chemical shift values are reported in δ units downfield from DSS. Refractive index values were measured with a Shimadzu Abbe 3L refractometer at 25°C.

(*S*)-ACP·HCl was synthesized from D-Ser;[14] mp 193–194°C; $[\alpha]_D^{20}$ $-10.4°$ (*c* 2.00, methanol). D-Ser was purchased from Wako Pure Chemical Ind., L-Cys was purchased from Kokusan Chemical Works, and DL-Cys was prepared from DL-Cys hydrochloride purchased from Tokyo Kasei Kogyo Co.

(RS)- and (R)-2-Amino-3-(2-carboxyethylthio)-propanoic Acid. A solution of DL- or L-Cys (300 mmol, 36.4 g) and propenoic acid (300 mmol, 21.6 g) in 300 cm^3 of water was allowed to stand for 6 h at room temperature and then overnight at 5°C. The precipitated (*RS*)- or (*R*)-ACE was collected by filtration, washed thoroughly with methanol, and dried. After concentrating the filtrate to 150 cm^3 at 55°C under reduced pressure, the solution was stood for 3 days at 5°C to further precipitate (*RS*)- or (*R*)-ACE. (*RS*)-ACE: Yield 41.2 g (70.8%); mp 198–200°C (decomp). ^{1}H-NMR (1 M DCl) δ: 4.39 (1H, dd, J=4.5, 7.1 Hz, 2-CH), 3.28 (1H, dd, J=4.5, 15.0 Hz, 3-CH-H), 3.18 (1H, dd, J=7.1, 15.0 Hz, 3-CH-H), 2.88 (2H, t, J=6.6 Hz, SCH_2CH_2), 2.75 (2H, t, J=6.6 Hz, SCH_2CH_2). *Anal.* Found: C, 37.25; H, 5.76; N, 7.22%. Calcd. for $C_6H_{11}O_4NS$: C, 37.30; H, 5.74; N, 7.25%. (*R*)-ACE: Yield 43.8 g (75.3%); mp 219–221°C (decomp) (lit., 218°C[13]); $[\alpha]_D^{20}$ $-9.35°$ (*c* 2.00, 1 M HCl), $[\alpha]_D^{20}$ $-32.8°$ (*c* 1.00, 0.05 M NaOH) (lit., $[\alpha]_D^{22}$ $-8.1°$ (*c* 5.25, 1 M HCl),[6] $[\alpha]_D^{24}$ $-7.0°$ (1 M HCl),[12] $[\alpha]_D^{20}$ $-9.33°$ (*c* 3.0, 1 M HCl)[13]). *Anal.* Found: C, 37.47; H, 5.78; N, 7.25%. The ^{1}H-NMR spectrum of (*R*)-ACE was virtually identical with that of (*RS*)-ACE.

(S)-2-Amino-3-(2-carboxyethylthio)propanoic Acid. A solution of (*S*)-ACP·HCl (30.0 mmol, 4.80 g) and 3-mercaptopropanoic acid (35.0 mmol, 3.72 g) in 20 cm^3 of water was stirred for 3 days at room temperature while being maintained at pH 12 to 13 by gradually adding 5 M aqueous sodium hydroxide (15 cm^3). The solu-

tion was then adjusted to pH 3.5 with 5 M hydrochloric acid. After stirring the solution for 3 h in an ice bath, the precipitated (*S*)-ACE was collected by filtration. A suspension of crude (*S*)-ACE in 10 cm^3 of water was stirred for 2 h at 45 °C and then for 1 h in an ice bath. The purified (*S*)-ACE was filtered off, washed with a small amount of water, and dried. (*S*)-ACE: Yield 2.79 g (49.4%); mp 218–220°C; $[\alpha]_D^{20}+9.35°$ (*c* 2.00, 1 M HCl); $[\alpha]_D^{20}+32.8°$ (*c* 1.00, 0.05 M NaOH). *Anal.* Found: C, 37.30; H, 5.80; N, 7.25%. The ^{1}H-NMR spectrum of (*S*)-ACE was virtually identical with that of (*RS*)-ACE.

Optical Resolution by Preferential Crystallization. (*RS*)-ACE (2.502, 2.705, 2.998, 3.156, and 3.494 g) was dissolved in 50 cm^3 of water at 40°C to prepare solutions with 111, 120, 133, 140, and 155% degrees of supersaturation at 10°C, respectively. The solution was gradually cooled to 10°C over a period of 1 h and then seeded with 0.100 g of (*R*)-ACE. After stirring the mixture for 10 min with a blade (0.70 cm width; 2.0 cm length) at 100 rpm and 10°C, the crystallized (*R*)-ACE was quickly collected by filtration, washed with methanol, and thoroughly dried. (*R*)-ACE obtained from the 111% supersaturated solution: yield 0.191 g; $[\alpha]_D^{20}-30.7°$ (*c* 1.00, 0.05 M NaOH). (*R*)-ACE obtained from the 120% supersaturated solution: yield 0.207 g; $[\alpha]_D^{20}-31.2°$ (*c* 1.00, 0.05 M NaOH). (*R*)-ACE obtained from the 133% supersaturated solution: yield 0.251 g; $[\alpha]_D^{20}-31.8°$ (*c* 1.00, 0.05 M NaOH). (*R*)-ACE obtained from the 140% supersaturated solution: yield 0.332 g; $[\alpha]_D^{20}-19.9°$ (*c* 1.00, 0.05 M NaOH). (*R*)-ACE obtained from the 155% supersaturated solution: yield 0.538 g; $[\alpha]_D^{20}-6.46°$ (*c* 1.00, 0.05 M NaOH).

Optical resolution was carried out for the solution with 133% degree of supersaturation by stirring for 20, 30, and 40 min at 10°C in a manner similar to that already mentioned. (*R*)-ACE obtained at 20 min: yield 0.311 g; $[\alpha]_D^{20}-21.5°$ (*c* 1.00, 0.05 M NaOH). (*R*)-ACE obtained at 30 min: yield 0.482 g; $[\alpha]_D^{20}-10.4°$ (*c* 1.00, 0.05 M NaOH). (*R*)-ACE obtained at 40 min: yield 0.724 g; $[\alpha]_D^{20}-5.02°$ (*c* 1.00, 0.05 M NaOH).

Successive Optical Resolution by Preferential Crystallization. (*RS*)-ACE (2.998 g) was dissolved in 50 cm^3 of water at 40°C. The solution was gradually cooled to 10° C over a period of 1 h and then seeded with 0.100 g of (*R*)-ACE. After stirring the mixture for 10 min at 10°C, (*R*)-ACE (0.251 g) was quickly collected by filtration, washed with methanol, and thoroughly dried (run 1 in Table I). (*RS*)-ACE (0.151 g) was dissolved in the filtrate at 40°C and then the resulting solution was gradually cooled to 10°C. (*S*)-ACE (0.100 g) was added as seed crystals and then the mixture was stirred for 20 min. (*S*)-ACE (0.375 g) was collected by filtration, washed with a small amount of methanol, and dried (run 2 in Table I). The filtrate was treated in a manner similar to that just described; the detailed conditions for runs 3 and 4 are given in Table I.

Purification of Partially Resolved (R)- and (S)-2-Amino-3-(2-carboxyethylthio)propanoic Acid. (*R*)-ACE (2.00 g) with an optical purity of 82% was dissolved in 8 cm^3 of water at 40°C. The mixture was vigorously stirred for 8 h at 10°C, before the purified (*R*)-ACE was collected by filtration and dried. (*S*)-ACE (2.20 g) with an optical purity of 77% was recrystallized from 11 cm^3 of water in a manner similar to that for (*R*)-ACE. (*R*)-ACE: Yield 1.59 g; mp 219–221°C; $[\alpha]_D^{20}-32.8°$ (*c* 1.00, 0.05 M NaOH). (*S*)-ACE: Yield 1.65 g; mp 219–220°C; $[\alpha]_D^{20}+32.8°$ (*c* 1.00, 0.05 M NaOH).

Determination of the Solubility of (RS)- and (R)-2-Amino-3-(2-carboxyethylthio)propanoic Acid. (*RS*)-ACE (3.000 g) or (*R*)-ACE (1.500 g) was dissolved in 50 cm^3 of water at 40°C. The solution was vigorously stirred at 10°C, an appropriate portion of the solution was pipetted from the mixture, avoiding contamination with solid ACE, and the refractive index was measured at 20°C. The mixture was stirred at 10°C until the refractive index had reached a constant value. The solubility was determined according to calibration curves prepared previously. Solubility at 10°C: (*RS*)-ACE, 4.508 g (100 cm^3 water)$^{-1}$; (*R*)-ACE, 1.903 g (100 cm^3 water)$^{-1}$.

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