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; conglomerase; 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.†4 Therefore, we have been screening for racemic amino acids that exist as conglomerates†5-7 and found another example. Many L-S-(carboxyalkyl)cysteine forms have been found in plants.†8-9 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-carboxyethylthio)cysteine] has been isolated from the seeds of Acacia†12-13 and Minosaceae species†14 and determined in young leaves of Callitroa rubescens.†15 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,†20 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 1H-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 enantiomers.†21-23 Conglomeras melt at a lower temperature than their enantiomers.†24 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 conglomerates are known to have such characteristics.†24 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,†25 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 ([a]β-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

\[ YE (g) = \frac{[Yield (g) \times OP (%)]}{100 - 0.1} \]

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.
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 1). (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 4000–4000 cm⁻¹ with a Perkin-Elmer model 1600 FT-IR spectrometer by the KBr disk method. ¹H-NMR spectra were recorded with a JNM-FX270 FT NMR SYSTEM, sodium 3-(trimethylsilyl)propanesulphonate (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 Abbé 3L refractometer at 25°C.

(S)-ACP·HCl was synthesized from D-Ser;¹⁶ mp 193–194°C; [α]_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 propenolic acid (300 mmol, 21.6 g) in 300 cm³ 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 cm³ at 55°C under reduced pressure, the solution was stood for 3 days at 5°C to further precipitate (RS)- or (R)-ACE. (S)-ACE: Yield 41.2 g (70.8%); mp 198–200°C (decomp). ¹H-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, SCH₂CH₃), 2.75 (2H, t, J = 6.6 Hz, SCH₂CH₃). Anal. Found: C, 37.25; H, 5.76; N, 7.22%. Calcd. for C₇H₁₀O₃N:S: 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); [α]_D^20 = -9.35° (c 2.00, 1 M HCl); [α]_D^19 = -32.8° (c 1.00, 0.05 M NaOH) (lit., [α]_D^19 = -8.1° (c 5.25, 1 M HCl), [α]_D^19 = -10.0° (1 M HCl), [α]_D^19 = -9.33° (c 3.0, 1 M HCl)). Anal. Found: C, 37.47; H, 5.78; N, 7.25%. The ¹H-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 cm³ 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 cm³). The solu-
tion was then adjusted to pH 3.5 with 5 mL 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 cm³ 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; [α]_	ext{D}^{20} +9.35 ° (c 2.00, 1 M HCl); [α]_	ext{D}^{20} +32.8 ° (c 1.00, 0.05 M NaOH). Anal. Found: C, 37.30; H, 5.80; N, 7.25%. The 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 cm³ 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; [α]_	ext{D}^{20} -30.7 ° (c 1.00, 0.05 M NaOH). (R)-ACE obtained from the 120% supersaturated solution: yield 0.207 g; [α]_	ext{D}^{20} -31.2 ° (c 1.00, 0.05 M NaOH). (R)-ACE obtained from the 133% supersaturated solution: yield 0.251 g; [α]_	ext{D}^{20} -31.8 ° (c 1.00, 0.05 M NaOH). (R)-ACE obtained from the 140% supersaturated solution: yield 0.323 g; [α]_	ext{D}^{20} -19.9 ° (c 1.00, 0.05 M NaOH). (R)-ACE obtained from the 155% supersaturated solution: yield 0.538 g; [α]_	ext{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; [α]_	ext{D}^{20} -21.5 ° (c 1.00, 0.05 M NaOH). (R)-ACE obtained at 30 min: yield 0.482 g; [α]_	ext{D}^{20} -10.4 ° (c 1.00, 0.05 M NaOH). (R)-ACE obtained at 40 min: yield 0.724 g; [α]_	ext{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 cm³ 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 cm³ 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 cm³ of water in a manner similar to that for (R)-ACE. (R)-ACE: Yield 1.59 g; mp 219–221°C; [α]_	ext{D}^{20} -32.8 ° (c 1.00, 0.05 M NaOH). (S)-ACE: Yield 1.65 g; mp 219–220°C; [α]_	ext{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 (3.00 g) or (R)-ACE (1.500 g) was dissolved in 50 cm³ 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 cm³ water)⁻¹; (R)-ACE, 1.903 g (100 cm³ water)⁻¹.

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**References**