Enantiomeric Resolution of *p*-Toluenesulfonate of Valine Benzyl Ester by Preferential Crystallizaion

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ABSTRACT Preferential crystallization of amino acid derivatives by seeding a pure enantiomer into racemic amino acid solutions has been studied for many years. However, few examples of valine derivatives have been reported so far. Although there have been some reports using valine hydrogen chloride with preferential crystallization, it is difficult to obtain optical isomers for valine derivatives using preferential crystallization. In this study, repeated preferential crystallization of *p*-toluenesulfonate valine benzyl ester with a 20% e.e. in 2-propanol gave a 94% e.e. on sonication. Sonication accelerated crystallization rate, but there was not a big difference in e.e. between with and without sonication. However, this research demonstrates the first preferential crystallization of *p*-toluenesulfonate of valine benzyl esters with an acceleration of crystallization using sonication. *Chirality 24:188–192, 2012.*

KEY WORDS: preferential crystallization; valine benzyl ester; optical resolution; *p*-toluenesulfonate; sonication

INTRODUCTION

Resolution using preferential crystallization, which is also called as "resolution by entrainment," often includes a seeding process of an enantiomer to a racemic solution.[1–5] Successive resolution of an enantiomer depends on the crystal features of the compound used. Compounds where the crystal is classified as a "conglomerate" may be applicable to preferential crystallization, and many different methods[1–5] for preferential crystallization have been performed, after Gernez[3,4] observed the effect of entrainment.

Collecting mother liquids, which is called as "preferential enrichment,"[6] is sometimes effective for obtaining optical pure enantiomers. Although seeding enantiomeric pure crystals to a racemic solution is a common technique, only about 250 examples have been reported in the published reports,[3] and these were acquired via a "trial and error" process. In these examples, the preferential crystallization of amino acids has been investigated to elucidate the features of the derivatives of amino acids that are applicable to this method. There are very few accounts of the preferential crystallization of valine derivatives, and only valine hydrochloride[7,8] and *p*-xylenesulfonate[9] are known examples.[2,3,7–9]

In this article, the authors have reported on the optical resolution of the substituted benzenesulfonates [**(L)-4** and **(D)-4**, **(L)-5** and **(D)-5**, and **(L)-6** and **(D)-6**) of valine benzyl esters shown in Figure 1. This research included the preferential crystallization by entrainment and a study of the effect of sonication during the preferential crystallization of substituted benzenesulfonates of valine benzyl esters. Three pairs of substituted benzenesulfonates shown in Figure 1 were prepared by the azeotropic water removing from valine [**(L)-1** or **(D)-1**), benzyl alcohol (**2**), and substituted benzenesulfonic acid (**3a-c**).

RESULTS AND DISCUSSION

Relationship Between the Melting Point and the Ratio of Enantiomer

Figure 2 shows a diagram of the relationship between the melting point and the DL-ratio of the benzenesulfonate of valine benzyl esters [**(L)-4, (D)-4**]. The pure benzenesulfonates of **(L)-4** and **(D)-4** show higher melting points than those of the corresponding enantiomer mixtures. The lowest melting point was recorded for a mixture containing 50% D and 50% L of the enantiomers. This result suggests that the benzenesulfonate of benzyl valinate [**(L)-4, (D)-4**] may form a conglomerate.

Figure 3 is a diagram of the relationship between the melting point and the DL-ratio of *p*-toluenesulfonate valine benzyl esters [**(L)-5, (D)-5**]. The pure *p*-toluenesulfonate of **(L)-5** and **(D)-5** shows a higher melting point (154–157°C) than that of the corresponding enantiomer mixture. The lowest melting point (128–131°C) was recorded for a mixture containing 50% of each enantiomer. This result suggests that *p*-toluenesulfonate [**(L)-5, (D)-5**] valine benzyl esters may also form a conglomerate, similar to **(L)-4** and **(D)-4**. The difference in the melting points was about 25°C. However, because 2,5-dimethylbenzenesulfonate benzyl valinate (**(L)-6, (D)-6**) did not form crystals at room temperature, recrystallization experiments were not performed in this case.

Infrared Absorption Spectra of Compounds 4 and 5

Infrared absorption spectrum of solid **(L)-4** was compared with the racemic one [**(DL)-4**] to show that these spectra are identical as well as **(D)-5** with its racemic solid [**(DL)-5**]. The authors could not find any difference between the enantiomeric solid and racemic solid as shown in Figure 4. The authors conclude that these compounds (**4** and **5**) make conglomerate.

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Received for publication 31 October 2010; Accepted 29 September 2011
DOI: 10.1002/chir.21982
Published online 16 December 2011 in Wiley Online Library (wileyonlinelibrary.com).
Contribution to the Proceedings of the 22nd International Symposium on Chirality [ISCD22]

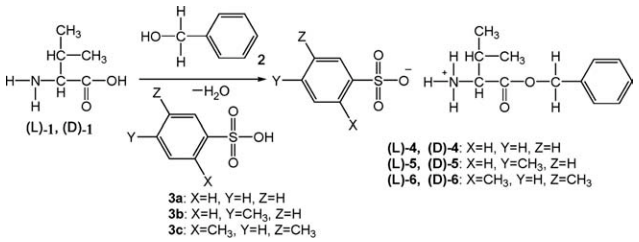

Fig. 1. Preparation of substituted benzenesulfonates of valine benzyl ester.

Resolution of Enantiomers of Benzyl Valinate by Means of HPLC

The ratio of the L to D enantiomers was determined by comparing the integrated area of one enantiomer with that of another after resolving the enantiomers using high-performance liquid chromatography equipped with a chiral stationary phase (OA-8000). A typical chromatogram of an enantiomer mixture of the benzyl valinates is shown in Figure 5. Benzyl D-valinate and benzyl L-valinate corresponded to the first and second peak, respectively, with the third peak corresponding to *p*-toluenesulfonic acid. These peaks were fully separated on the chromatogram.

Solvent for Recrystallization

Recrystallization of the salts of valine benzyl esters **(L)-4–(D)-4** and **(L)-5–(D)-5** were examined using methanol, ethanol, 2-propanol, and ethanol/diethyl ether. Methanol and ethanol were found not to be suitable for this purpose, because of their very high solubility. Although ethanol/diethyl ether solvent system was examined for crystallization, it was very difficult to control the crystallization speed. In this case, both the enantiomeric purity and the melting point of the resulting crystals were almost same as those of the racemic mixture. However, 2-propanol was found to be suitable for this purpose and so was used in all subsequent experiments.

Effect of Sonication. Seeding conventionally leads crystallization to be initiated nonuniformly and sometimes results in crystal growth at different rates at different nucleating

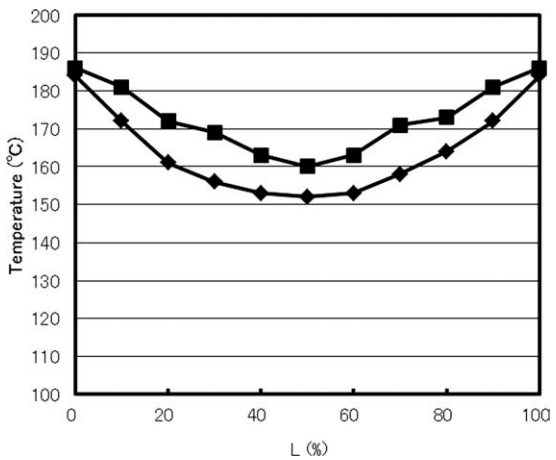

Fig. 2. Melting point of enantiomer mixture of benzenesulfonates of valine benzyl esters [**(L)-4, (D)-4**]. The upper line connects upper points of melting range and the lower line connects lower points of melting range.

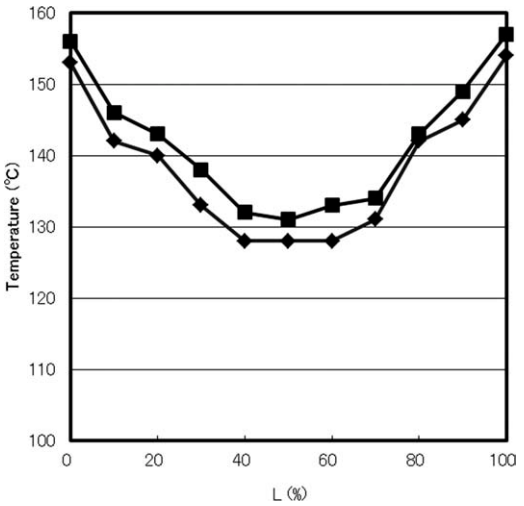

Fig. 3. Melting point of enantiomer mixture of *p*-toluenesulfonates of valine benzyl esters [**(L)-5, (D)-5**]. The upper line connects upper points of melting range and the lower line connects lower points of melting range.

sites.[10] In this case, the crystal size may be distributed with broadness and inequality. Crystallization is often caused by a slight change in external factors, such as temperature or pressure fluctuations.[10]

Ultrasound gives pressure fluctuation as an external factor in crystallization processes to initiate seeding and control subsequent crystal growth. Cavitation bubbles by ultrasound act as nuclei for crystal growth disrupting seeds and nuclei of crystals. Thus ultrasound increases the number of nuclei present in the medium. Correct choice of sonication conditions may help to produce crystals with a uniform and designated size.[10] However, there are not many examples of application to enantiomeric resolution using sonication.

A comparison of crystallization under sonication to that without sonication is shown in Figure 6. The *p*-toluenesulfonate of DL valine benzyl esters [**(L)-5** and **(D)-5**, total mass = 400 mg) was dissolved in 2-propanol (2.7 ml) by warming and seeded with the D- or L-enantiomer (1 mg) at room temperature. Crystals were obtained using filtration just after the samples were subjected to different sonication periods (1, 2, 3, 4, 5, and 6 min) at 45 kHz. While no crystals were recovered without sonication, even after a period of 6 min, the recovery of crystals was 60% after sonication for only 2 min. However, the recovery did not improve on further sonication. Therefore, the authors have used a sonication period of 2 min, followed by standing at room temperature for a period of 4 min as our preferential crystallization procedure. Because the crystallization rate without sonication was much slower than that with sonication, it took 20 min's standing without sonication to obtain the almost same recovery as that in case of 2 min's sonication plus 4 min's standing. The frequencies of the ultrasound irradiation used were 28, 45, and 100 Hz. Figure 7 shows the dependency of the crystal recovery on the frequency of sonication used for different initial DL ratios of the enantiomers.

Preferential Crystallization on Sonication[2,10–13]

Sonication at a frequency of 28 kHz gave the highest recovery compared with that at obtained for other sonication frequencies, except of an initial DL mix of 50%. The samples possessing a higher e.e. tended to show a higher recovery, except for the racemic mixture (i.e., D = 50% and L = 50%).

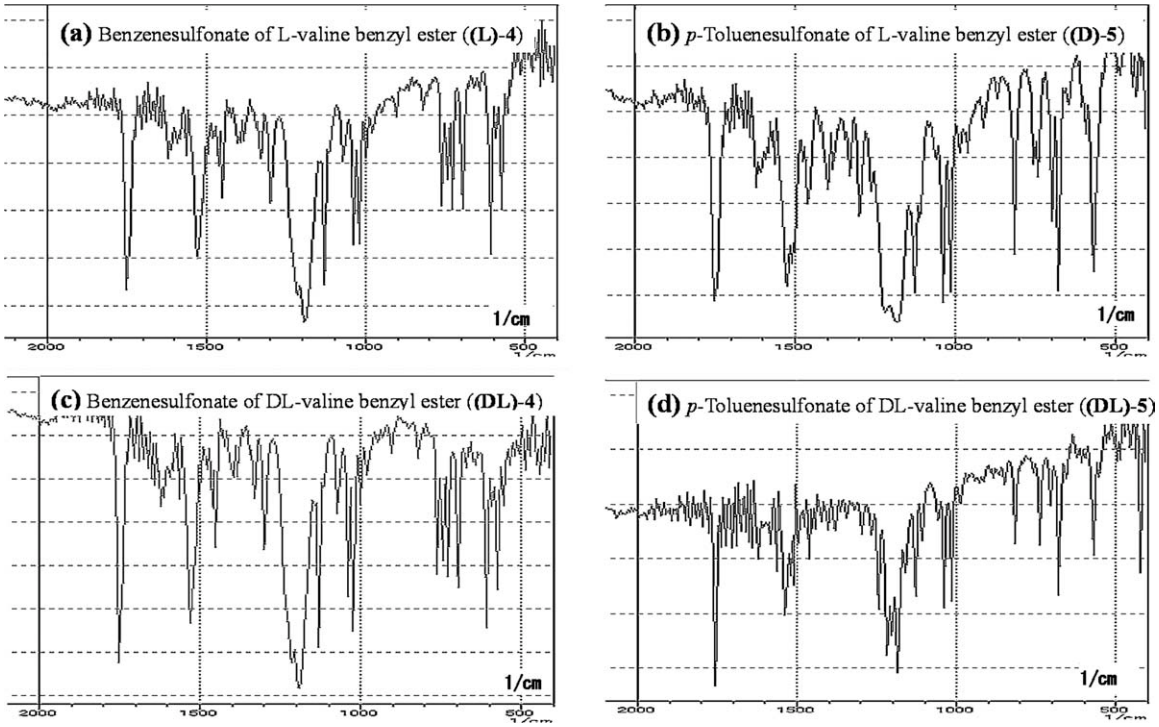

Fig. 4. Infrared absorption spectra of benzenesulfonates of valine benzyl ester [**(a)**: (L)-4, **(c)**: (DL)-4] and *p*-toluenesulfonates of valine benzyl ester [**(b)**: (D)-5, **(d)**: (DL)-5].

Consideration of the recovery is not the only important concern but also the e.e. of the crystal is as important.

Figure 8 shows the dependency of the e.e. of the crystals on the frequency of sonication for different initial DL ratios of the enantiomers. Sonication at a frequency of 45 kHz gave the highest e.e. compared with the e.e. obtained after sonication at other frequencies. The samples possessing a higher e.e. also showed a higher e.e. after recrystallization with sonication. The racemic mixture showed the lowest e.e., which was almost 0%.

The enantiomeric excess after crystallization was compared with that before crystallization to observe the effect of sonication on the increase in the enantiomeric excess, as shown in Figure 9. All the samples obtained after crystallization showed a higher e.e. than the samples before crystallization. The increase in e.e. after recrystallization using sonication at 45 kHz reached 23%. However, the preferential crystallization without sonication gave the similar increase in e.e. (from the initial crystal: L 70%, D 30%) to that with sonication. This means sonication accelerated the crystallization rate without lowering the e.e. The sonication at 45 kHz was more effective on gaining higher e.e. and the sonication at 28 kHz was effective on higher recovery in the same experimental

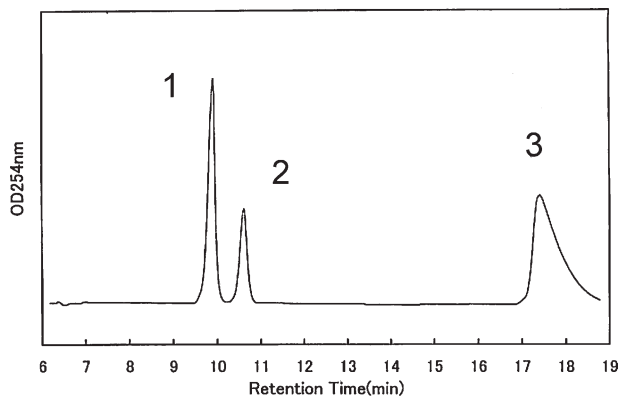

Fig. 5. A typical chromatogram of *p*-toluenesulfonate of valine benzyl esters (peak 1: D-valine benzyl ester; peak 2: L-valine benzyl ester; peak 3: *p*-toluenesulfonic acid). Column: OA-8000 (250 mm × 4.6 mm); Detection: UV 254 nm; Eluant: $HClO_4$ aq. (pH_2)-CH_3CN 70:30; Flow rate 0.6 ml/min.

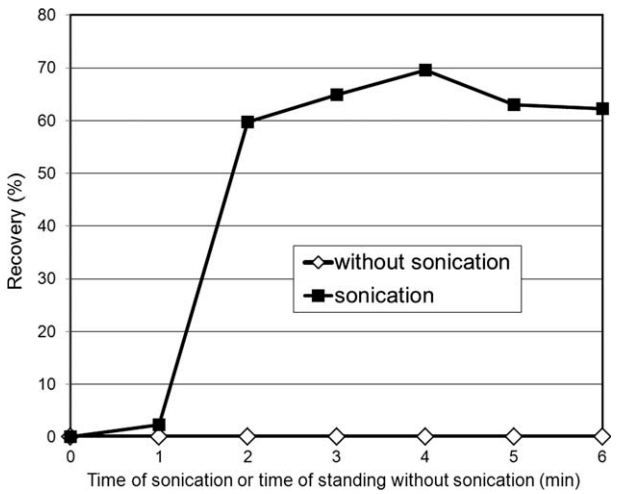

Fig. 6. Recovery of crystal of compounds (L)-5 and (D)-5 in the different sonication period at 45 kHz. Sonication was carried out at room temperature. The *p*-toluenesulfonate of DL valine benzyl esters [(L)-5 and (D)-5, total mass = 400 mg] was dissolved in 2-propanol (2.7 ml) upon warming and seeded with the D- or L-enantiomer (1 mg) at room temperature. Crystals were obtained using filtration just after the samples were subjected to different sonication periods (1, 2, 3, 4, 5, and 6 min) at 45 kHz.

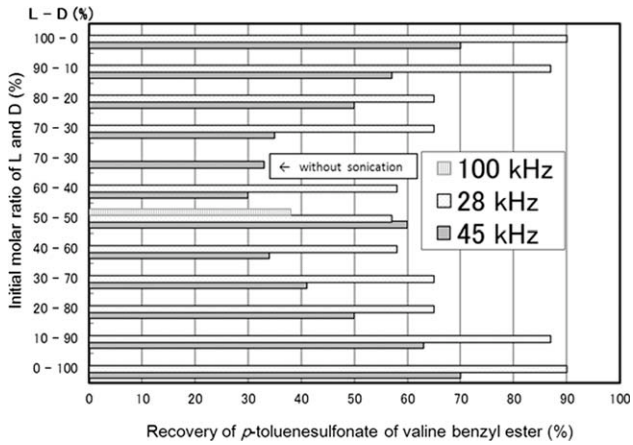

Fig. 7. Frequency-dependence of recovery of *p*-toluenesulfonate of valine benzyl esters [**(L)-5** and **(D)-5**]. The mixture (total: 400 mg) of (L)-5 and (D)-5 with the ratio of L% – D% were dissolved in 2-propanol (2.7 ml) upon warming and seeded with the D- or L-enantiomer (1 mg) at room temperature. D- and L-enantiomer was seeded to (D)- and (L)-rich solution, respectively. Crystals were obtained using filtration just after the samples were subjected to sonication periods 2 min at 45, 28, 100 kHz and then 4 min's standing.

condition (2 min's sonication after seeding and 4 min's standing). This may suggest that the sonication at 28 kHz makes crystallization faster to afford higher recovery and lower e.e. of one enantiomer because of crystallization of another enantiomer. On the other hand, the sonication at 45 kHz results slower crystallization to give higher e.e. and lower recovery. Therefore, choices in frequencies of sonication and crystallization time and so on may control e.e. and recovery of crystals.

Increase of E.e. of Benzyl Valinate by Repeating Recrystallization

Recrystallization using D-rich **5** of 20% e.e. was repeated four times. Each recrystallization was carried out in 2-propanol, seeding with 1 mg of (D)-**5** crystal, using a 2-min sonication period to afford a final e.e. of 94%, as shown in Figure

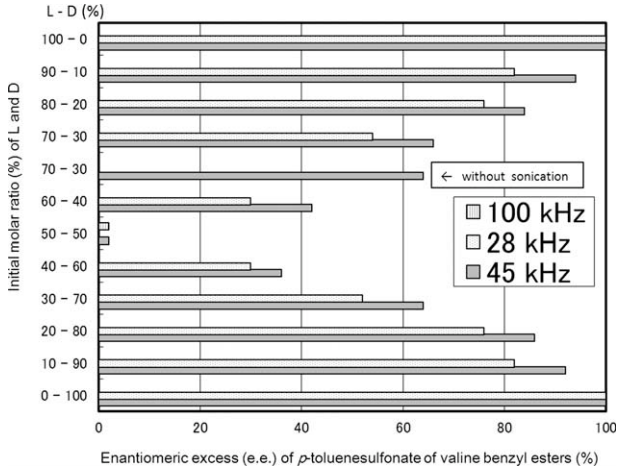

Fig. 8. Frequency-dependence of e.e. of *p*-toluenesulfonate of valine benzyl esters [(L)-5 and (D)-5]. The mixture (total: 400 mg) of (L)-5 and (D)-5 with the ratio of L% – D% were dissolved in 2-propanol (2.7 ml) upon warming and seeded with the D- or L-enantiomer (1 mg) at room temperature. D- and L-enantiomer was seeded to (D)- and (L)-rich solution, respectively. Crystals were obtained using filtration just after the samples were subjected to sonication periods 2 min at 45, 28, 100 kHz and then 4 min's standing.

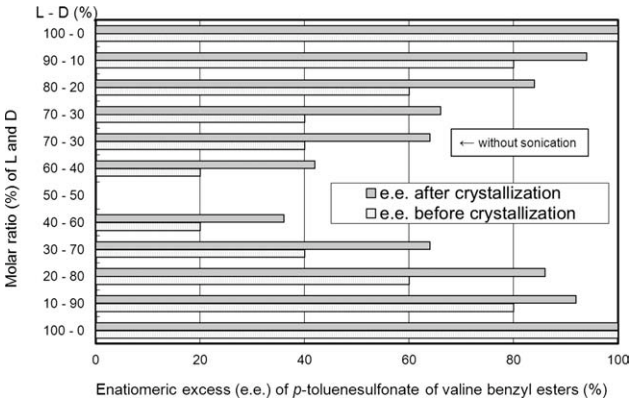

Fig. 9. E.e. comparison of *p*-toluenesulfonate of valine benzyl ester [**(L)-5** and **(D)-5**] after crystallization with that before crystallization using sonication at 45 Hz.

10. With regard to the effect of sonication on the preferential crystallization, the author has to say that both recovery and e.e. were almost similar between crystals after sonication and those without sonication at 45 kHz. In this case, the effect of sonication was not found. To consider the effect of sonication on e.e. increase, racemic crystal (DL total 1.0 mg) was seeded in the nonracemic solutions containing compound 5 (D 70% plus L 30%, total 400 mg) and 2-propanol (2.7 ml). After 2 min's sonication plus 4 min's standing, the formed crystal was filtered to give 100 mg. And 6 min's standing without sonication gave 50 mg. The enantiomeric excess of the resulted crystals was the same as the initial crystals (e.e. 40%: D 70% plus L 30%) in regardless of sonication or nonsonication.

Recrystallization using L-rich **4** of 20% e.e. was repeated thrice in 2-propanol, seeding with 1 mg of (L)-**4** crystal on 2 min's sonication plus 4 min's standing to afford a final e.e. of 24%. The increase in e.e. was very smaller compared with the preferential crystallization of compound **5**.

CONCLUSIONS

p-Toluenesulfonate of valine benzyl ester (**5**) was optically resolved using preferential crystallization. Sonication accelerated the crystallization of *p*-toluenesulfonate of valine benzyl ester. The enantiomeric excess and the recovery of the

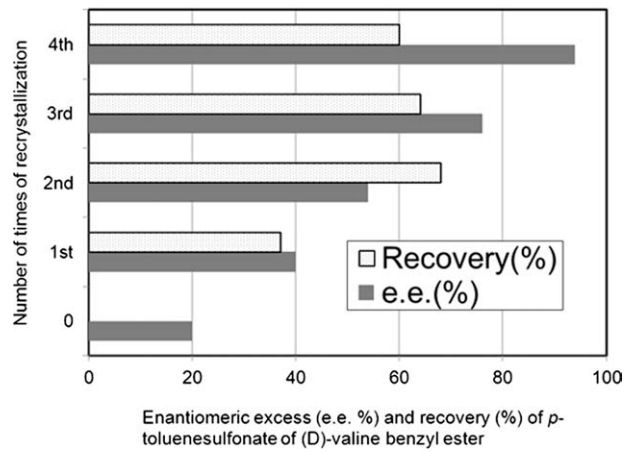

Fig. 10. Increase of e.e. by repeating recrystallization of *p*-toluenesulfonate of (D)-rich valine benzyl ester [(**D**)-rich 5].

obtained crystals depended on the frequency of the ultrasound irradiation used. Crystallization of *p*-toluenesulfonate of valine benzyl esters (**D-rich 5**) with a 20% e.e. in 2-propanol gave a final e.e. of 94%. However, preferential crystallization of **4** and **6** did not succeed. Sonication increased the rate of preferential crystallization. This research demonstrates the first preferential crystallization of *p*-toluenesulfonate of valine benzyl esters and an acceleration of crystallization using sonication.

EXPERIMENTAL

Chemicals

The D-valine and L-valine used were purchased from Nippon Kayaku and Nippon Rika, respectively. The benzyl alcohol, substituted benzene sulfonic acids, and benzene used were purchased from Kanto Chemical.

Preparation of Substituted Benzenesulfonates (4, 5, 6) of Valine Benzyl Ester

D-Valine (150 mmol, 17.6 g), benzyl alcohol (450 mmol), and *p*-toluenesulfonic acid monohydrate (165 mmol, 31.4 g) were mixed in benzene (80 ml). The resulting mixture was refluxed for a period of 23 h to remove the evolved water using azeotropic condensation from the benzyl alcohol and valine (D- or L-) to give *p*-toluenesulfonate of valine benzyl ester (44.8 g, 79%). After several recrystallization stages, pure crystals were obtained (13.9 g, yield = 31%), mp = 154–157°C, $[\alpha]_D^{24} = +3.3$, C = 1.0, ethanol, and mp = 153–156°C, $[\alpha]_D^{24} = -3.6$, C = 1.0, ethanol for *p*-toluenesulfonates of D- and L-valine benzyl esters [**(D)-5** and **(L)-5**], respectively.

Benzenesulfonates [**(D)-4** and **(L)-4]** of valine benzyl esters were prepared by the similar manner in the yield of 73%, mp = 184–186°C, $[\alpha]_D^{21} = +2.5$, C = 1.0, ethanol, and 77%, mp = 184–186°C, $[\alpha]_D^{21} = -2.8$, C = 1.0, ethanol, respectively.

2,5-Dimethyl-benzenesulfonates [**(D)-6** and **(L)-6)**] of valine benzyl esters were prepared by neutralization of free valine benzyl esters extracted from (D)-5 and (L)-5 with 2,5-dimethyl-benzenesulfonate (**3c**). Because the products were pure but oils, further purification was not carried out.

Apparatus

The melting point of the benzyl valinate crystals was measured using a Buchi 530. Separation of diastereomers was performed using a high-performance liquid chromatograph system, comprising a Hitachi L-6000 pump, an L-4200 detector, and a D-2500 chromato-integrator. A Sumichiral OA-8000 chiral analytical column was used to resolve the enantiomers. Sonication was carried out using a Yamato Bransonic B-1200 sonicator at 45 kHz and a Velvo-clear VS-100 III sonicator at 28 and 100 kHz. A Jusco DIP-181 digital polarimeter was used to measure the optical rotation of the samples. Infrared absorption spectra were measured with a Shimadzu FTIR-8400S.

Preferential Crystallization

The substituted benzenesulfonates [**(L)-4, (D)-4, (L)-5,** and **(D)-5**] of the L- and D-valine benzyl esters were mixed at different molar ratios (total mass = 400 mg) and dissolved in 2-propanol as the solvent. This solution was seeded with a 1.0-mg enantiomer crystal at 25°C. After seeding, the solution in a glass Erlenmeyer's flask (10 ml) surrounded by water bath at 25°C was irradiated for 2 min form the bottom of the flask by ultrasound. The obtained crystal after 4 min's standing was filtered.

Determination of DL-ratio in Benzyl Valinate Crystal

Crystal of *p*-toluenesulfonate of valine benzyl ester dissolved in a solution of $HClO_4$ aq. (pH 2) – CH_3CN 70:30 was injected into a high-performance liquid chromatograph equipped with a chiral column (OA-8000, Sumika Analytical Center).

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