  Bull. Chem. Soc. Jpn., 57, 1328—1330 (1984)

The Preparation of DL-Isoleucine by Asymmetric Transformation

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(Received December 2, 1983)

The chemical synthesis of isoleucine leads to a mixture of DL-isoleucine and DL-alloisoleucine. The preparation of DL-isoleucine from the mixture was studied. When the mixture was reacted with 1,5-naphthalenedisulfonic acid in water, DL-isoleucine formed the less soluble 1,5-naphthalenedisulfonate and could be separated in the form of the salt from the mixture. The soluble DL-alloisoleucine salt was easily epimerized by heating in acetic acid in the presence of salicylaldehyde and free isoleucine mixture. Asymmetric transformation between the two diastereoisomeric racemates was carried out by the simultaneous combination of the fractional crystallization of the less soluble DL-isoleucine salt and the epimerization of the soluble DL-alloisoleucine salt. DL-Isoleucine salt was obtained in a yield of up to 95% from a DL-isoleucine–DL-alloisoleucine mixture or even from DL-alloisoleucine.

L-Isoleucine (L-Ile) is an essential amino acid useful as a nutrient or a food additive. The chemical synthesis of isoleucine leads to a mixture (Ile-mixture) of DL-Ile and DL-alloisoleucine (DL-allo-Ile), since the amino acid has two asymmetric carbon atoms. In order to obtain L-Ile from the mixture by a conventional optical-resolution method, such as the asymmetric hydrolysis of *N*-acyl-DL-amino acids by aminoacylase, it is necessary to separate DL-Ile from the Ile-mixture before the optical resolution.[1,2] Several methods for the separation of DL-Ile from the Ile-mixture are known. For example, DL-Ile or the sodium salt can be separated from the mixture by fractional crystallization with an appropriate solvent in which DL-Ile or the salt is less soluble than DL-allo-Ile or its sodium salt.[3,4] Also, *N*-acetyl-DL-Ile may be obtained as the more soluble fraction in a similar way.[2,5,6] In these methods, however, the crystallization must be systematically repeated several times to isolate the pure product; this results in low yields and is time-consuming.[7] Generally, the separation of the diastereoisomers of isoleucine is so difficult and tedious that no method to meet industrial demands is found in literatures. To develop practical and convenient methods for the production of DL-Ile, we have studied the separation of DL-Ile from the Ile-mixture by fractional crystallization and the preparation of DL-Ile by asymmetric transformation, *i.e.*, by a combination of fractional crystallization and simultaneous epimerization.

It has been well known that some aromatic sulfonic acids have been used as specific precipitating reagents for the separation of amino acids.[8] We have ourselves previously developed a technique using aromatic sulfonic acids for the optical resolution of DL-amino acid by means of a preferential crystallization procedure.[9–12] Aromatic sulfonic acids were chosen because they vary greatly in properties and easily form salts with any kind of amino acid; also, their salts tend to form racemic mixtures suitable for optical resolution by means of the preferential crystallization procedure. In the present study, with a similar idea, the separation of DL-Ile from the Ile-mixture was investigated using aromatic sulfonic acids. We prepared a wide variety of aromatic sulfonates of the Ile-mixture and screened the salts suitable for the separation. As a result, the salt of DL-Ile with 1,5-naphthalenedisulfonic acid (NDS) was found to form easy handling crystals from water in a good yield and to be very suitable for the separation of the two diastereoisomeric racemates. When the Ile-mixture was reacted with NDS in water, DL-Ile selectively crystallized as the less soluble salt, which was composed of two moles of DL-Ile and one mole of NDS, while soluble DL-allo-Ile·NDS remained almost quantitatively in the mother liquor. The DL-Ile·NDS obtained by the above procedure was contaminated with approx. 7% of DL-allo-Ile·NDS, but the sulfonate once recrystallized from water had an allo-Ile content as low as 0.3%.

From an economic point of view, it is wasteful to discard the DL-allo-Ile remaining in the mother liquor because the epimerization of DL-allo-Ile leads to a mixture of DL-Ile and DL-allo-Ile, from which DL-Ile can be separated in the form of the salt with NDS, as has been described above. Therefore, we investigated the epimerization of the DL-allo-Ile·NDS remaining in the filtrate after the separation of the DL-Ile·

Table 1. Effects of salicylaldehyde and Ile-mixture on the epimerization of L-Ile·NDS[a]

Additive	Molar equiv	Epimerization degree/%
None	—	3
Salicylaldehyde	1.0	7
Ile-mixture	1.0	5
	2.0	7
Salicylaldehyde/	1.0/1.0	39
Ile-mixture	1.0/2.0	43
	1.0/5.0	45
	1.0/10.0	44

a) The reaction was carried out at 100 °C for 3 h in acetic acid containing 5% water.

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NDS. Our previous report[13] showed that various optically active α-amino acids were easily racemized by heating them moderately in an acetic acid solution in the presence of a catalytic amount of aldehyde. Although the NDS salt of DL-allo-Ile was epimerized only a little in the same way, the epimerization of DL-allo-Ile·NDS was found to be greatly accelerated by heating it in an acetic acid solution in the presence of large amounts (one molar equivalent or above) of salicylaldehyde and free Ile-mixture. The effects of salicylaldehyde and the free Ile-mixture on the epimerization of L-Ile·NDS are shown in Table 1.

Then, using the epimerization method described above, we studied the asymmetric transformation between two diastereoisomeric racemates by the simultaneous combination of the fractional crystallization of the less soluble DL-Ile·NDS and the epimerization of the soluble DL-allo-Ile·NDS. DL-Ile·NDS was fractionally precipitated even under the epimerizing conditions, while the precipitated crystals were nevermore epimerized. In the liquid phase, the epimerization of DL-allo-Ile·NDS proceeded simultaneously, and the equilibrium shifted in favor of DL-Ile·NDS as a whole by the continuous precipitation of DL-Ile·NDS from the epimerizing solution, so that a direct conversion of DL-allo-Ile·NDS into DL-Ile·NDS could be achieved by a so-called second-order asymmetric transformation. Starting with the Ile-mixture (Ile/allo-Ile ratio=56.4/43.6), DL-Ile·NDS (Ile/allo-Ile ratio=89.8/10.2) was obtained in a yield of 90% based on the original weight of the Ile-mixture by means of a reaction at 100 °C for 16 h. Also, starting with DL-allo-Ile, crystals of DL-Ile·NDS (Ile/allo-Ile ratio=93.1/6.9) were precipitated in a yield of up to 94.5% based on the original weight of DL-allo-Ile·NDS by means of a reaction at 100 °C for 30 h. The change in the composition of both diastereoisomeric racemates during the reaction is shown in Fig. 1. When the DL-Ile·NDS obtained above was once recrystallized from water, the ratio of Ile/allo-Ile was larger than 1000/2.

Although second-order asymmetric transformation has been reported for numerous examples of the diastereoisomeric salt of a racemic modification, including some amino acids with an optically active resolving agent,[14-16] no report has described the asymmetric transformation between two optically stable diastereoisomeric racemates in the absence of any auxiliary chiral agent. The present one-step asymmetric transformation method for the preparation of DL-Ile from a synthetic mixture of DL-Ile and DL-allo-Ile has a definite advantage and is suitable for industrial application.

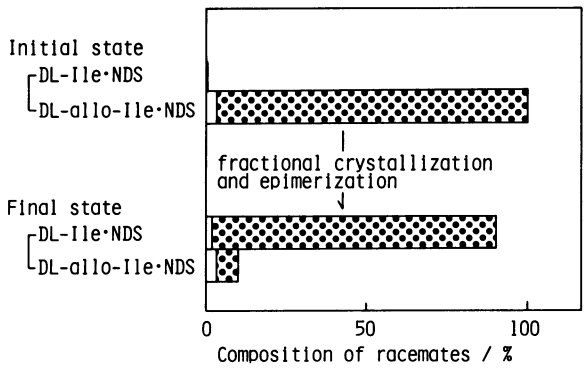

Fig. 1. The change of the composition by asymmetric transformation.

▩, crystals of NDS salt; □, NDS salt in solution.

Experimental

Materials. The diastereoisomeric mixture (Ile-mixture), consisting of 56.4% DL-Ile and 43.6% DL-allo-Ile, and L-Ile manufactured by our company, Tanabe Seiyaku Co., Ltd., were used. The DL-allo-Ile was prepared by the method of Greenstein *et al.*[2] The 1,5-naphthalenedisulfonic acid, salicylaldehyde, and other chemicals were obtained from the Tokyo Kasei Kogyo Co., Ltd.

Analyses. All samples were air-dried overnight at 50 °C. Elemental analyses were performed with a Perkin-Elmer 240 elemental analyzer. Amino acid analyses were performed by means of a Hitachi high-speed amino-acid analyzer, Model 835 [resin, Hitachi Custom Resin 2619; eluting buffer, 0.2 M sodium citrate buffer (1 M=1 mol dm^{-3}, pH 4.25); column temperature, 55 °C]. Under the conditions employed, the lower limit of the detection of allo-Ile was approximately 0.05%. The optical rotation was measured with a Perkin-Elmer 141 automatic polarimeter.

Separation of DL-Ile·NDS by Fractional Crystallization. A mixture of the Ile-mixture (10.0 g) and NDS (13.6 g; water content, 19.3%) was dissolved in water (120 ml) by heating at 80 °C and then allowed to stand in a refrigerator overnight. The crystals thus precipitated were collected by filtration, washed with a small amount of cold water, and dried to give crude DL-Ile·NDS (10.8 g); apparent yield, 91.3% (based on the DL-Ile in the starting Ile-mixture); DL-Ile/DL-allo-Ile ratio=93.5/6.5. The crude DL-Ile·NDS (10.8 g) was recrystallized twice from water (110 ml) to give pure DL-Ile·NDS (6.3 g); yield, 53.2% (based on the DL-Ile in the starting Ile-mixture); DL-allo-Ile, undetected; mp 240—241 °C (decomp). Found: C, 48.07; H, 6.27; N, 4.85; S, 11.71%. Calcd for $C_{22}H_{34}N_2O_{10}S_2$: C, 47.99; H, 6.22; N, 5.09; S, 11.64%. Solubility in water (g/100 ml): 1.84 (20 °C), 2.22 (30 °C).

Epimerization of Ile·NDS. The experiments aimed at the epimerization of Ile·NDS were carried out by using L-Ile·NDS,** since the optical rotations of L-Ile and D-allo-Ile are nearly equal in magnitude and opposite in sign. A mixture of L-Ile·NDS (0.1 g), salicylaldehyde (0.018 ml, 1.0 mol per 1.0 mol of L-Ile·NDS), an Ile-mixture (0.023—0.23 g, 1.0—10.0 mol per 1.0 mol of L-Ile·NDS), and 95% acetic acid*** (10 ml) was heated in a sealed tube at 100 °C for 3 h. The reaction mixture was diluted with 1 M-hydrochloric acid (5 ml), and the optical rotation was measured. The epimeriza-

** L-Ile·NDS was prepared as follows. A mixture of L-Ile (13.1 g) and NDS (14.5 g) was dissolved in water (50 ml) at an elevated temperature, and then the solution was allowed to stand in a refrigerator overnight. The resulting precipitates were collected, washed with cold water, and air-dried at 50 °C to give L-Ile·NDS (22.5 g); mp 253—254 °C (decomp), $[\alpha]_D^{25}$ +13.4° (c=1, water). The product was composed of 2 mol of L-Ile, 1 mol of NDS, and 1 mol of water.

*** Although the extent of epimerization decreased with the increase in the water content of acetic acid, as has been described in our previous report,[13] acetic acid containing 5% water was used in order to dissolve the L-Ile·NDS.

tion degree was calculated as follows:

$$\frac{\text{initial optical rotation} - \text{final optical rotation}}{\text{initial optical rotation}} \times 100.$$

The effects of the salicylaldehyde and the Ile-mixture on the epimerization of L-Ile·NDS are shown in Table 1.

Production of DL-*Ile·NDS by Asymmetric Transformation. From Ile-Mixture*: An Ile-mixture (13.1 g), NDS (17.9 g, water content, 19.3%; equivalent amount with Ile-mixture, 13.1 g), acetic anhydride (18.0 ml; equimolar amount with water contained in NDS, 17.9 g), and a combination of an Ile-mixture (2.2 g) and salicylaldehyde (0.22 ml) to accelerate the epimerization of DL-allo-Ile·NDS were added to glacial acetic acid (30 ml). The mixture once completely dissolved, and soon most of the Ile·NDS precipitated. The mixture was then stirred at 100 °C for 16 h. The crystals thus precipitated were quickly collected by filtration, washed with a small amount of acetic acid, and dried to give crude DL-Ile·NDS (24.8 g); yield, 90.1% (based on Ile-mixture·NDS), Ile/allo-Ile=89.8/10.2. The Ile/allo-Ile ratio of the mother liquor was 41.5/58.5. The crude DL-Ile·NDS (24.8 g) was recrystallized from water (300 ml) to give DL-Ile·NDS (17.6 g); Ile/allo-Ile ratio=1000/2.

From DL-*Allo-Ile*: DL-Allo-Ile (13.1 g, 100 mmol), NDS (14.3 g, water content 19.3%, 40 mmol), salicylaldehyde (0.26 ml, 2.5 mmol), and acetic anhydride (14.4 ml; equimolar amount with water contained in NDS, 14.3 g) were added to glacial acetic acid (60 ml). The mixture was stirred at 100 °C, seeded with finely pulverized crystals of DL-Ile·NDS (0.01 g) after 3 h, and further stirred for 30 h at the same temperature. The reaction mixture was then cooled to room temperature and stirred for 1 h. The crystals thus precipitated were collected by filtration, washed with a small amount of acetic acid, and dried to give DL-Ile·NDS (20.8 g); yield, 94.5% (based on DL-allo-Ile·NDS); Ile/allo-Ile=93.1/6.9. The Ile/allo-Ile ratio of the mother liquor was 40.7/59.3. The change in the composition of both diastereoisomeric racemates during the reaction is shown in Fig. 1.

Preparation of Pure DL-*Ile.* Recrystallized DL-Ile·NDS (16.0 g) was dissolved in water (800 ml). The solution was passed through a column of Amberlite IR-45 (70 ml, OH-form), and the column was washed with water. The effluent and washed water were concentrated, and methanol (45 ml) was added to the concentrated solution. The crystals thus precipitated were collected by filtration, washed with a small amount of cold water, and dried to give DL-Ile (7.3 g); DL-allo-Ile, undetected.

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