November, 1981] © *1981 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, ***54,** 3291—3295 (1981)* 3291

Asymmetric Transformation of *N*-Acetyl-DL-leucine

Chikara Hongo,* Shigeki Yamada, and Ichiro Chibata
Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd.,
16-89, Kashima-3-chome, Yodogawa-ku, Osaka 532

(Received March 25, 1981)

The asymmetric transformation of *N*-acetyl-DL-leucine by a combination of preferential crystallization of a desired isomer and simultaneous racemization of the opposite isomer was studied. The reaction was analyzed in detail and a practical method for the asymmetric transformation was established. A supersaturated solution of *N*-Ac-DL-Leu in acetic acid containing catalytic amounts of acetic anhydride was seeded with the crystals of *N*-Ac-L-Leu and cooled at a rate of 10 °C/h from 100 °C to 40 °C. As a result, almost optically pure *N*-Ac-L-Leu was obtained in a yield of 70%, based on the original weight of *N*-Ac-DL-Leu.

Our previous paper[1)] reported some experimental facts demonstrating the asymmetric transformation of certain *N*-acylamino acids, including *N*-acetyl-DL-leucine (*N*-Ac-DL-Leu), *N*-butyryl-DL-proline, and *N*-benzoyl-DL-phenylglycine. The asymmetric transformation was achieved by a combination of an optical resolution process and racemization process. Among these experiments, a combination of the preferential crystallization of the seeded *N*-Ac-L-Leu from the acetic acid solution supersaturated with *N*-Ac-DL-Leu and racemization of the unseeded *N*-Ac-D-Leu remaining in the liquid phase was considered to be most promising for practical application. However, the details were not investigated and the yields were low. Subsequently to that work, the reaction was analyzed and a practical method for the asymmetric transformation of *N*-Ac-DL-Leu was established.

In the optical resolution process by preferential crystallization, the crystallization of the desired optical isomer (for example L-isomer) by seeding leads to an excess of the undesired opposite isomer (D-isomer) in the solution. This excess D-isomer also begins to crystallize spontaneously after a certain time, resulting in low enantiomeric purity of the product. Accordingly, the rate of racemization of the excess D-isomer in the optical resolution system is a very important factor in the asymmetric transformation by this type of optical resolution method. This is distinct from the common cases of a second order asymmetric transformation[2)] which use the difference in solubility between the diastereoisomeric salts with a chiral resolving agent. Therefore, the factors accelerating the rate of racemization of *N*-Ac-D-Leu were investigated.

Since the presence of acetic anhydride was essential for accelerating the racemization of *N*-Ac-D-Leu, the effect of amounts of acetic anhydride was investigated at 75 °C using acetic acid as a solvent. If the racemization of *N*-Ac-D-Leu is complete in several hours, the rate of racemization is sufficient for practical asymmetric transformation. Although the rate of racemization was decreased with the decrease of the molar ratio of acetic anhydride, as can be seen in Fig. 1, a racemization rate sufficient for the asymmetric transformation was obtained even at 0.1 molar ratio of acetic anhydride. The effect of temperature on racemization was investigated under various conditions. The rate of racemization was greatly influenced by temperature and varied with the composition of the reaction mixture, as shown in Fig. 2. A temperature higher than 40 °C was necessary for practical racemization. The stability of crystalline optically active *N*-Ac-Leu was also investigated under the above conditions for racemization in a liquid phase. As a result, crystals of optically active *N*-Ac-Leu were found to be not racemized even at 90 °C. Thus the most essential requirement for this kind of asymmetric transformation was fulfilled.

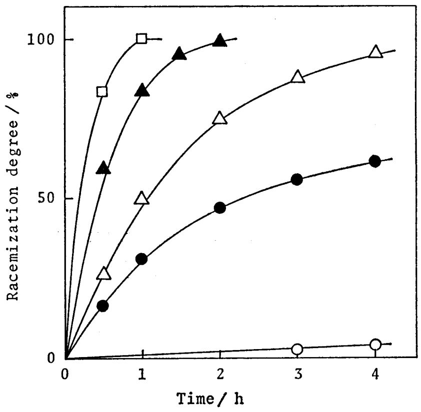

Fig. 1. Effect of acetic anhydride on racemization of *N*-Ac-D-Leu.
The solutions containing 6.0 g of *N*-Ac-D-Leu, 30 ml of AcOH and various amounts of Ac_2O were heated at 75 °C. Molar ratio of Ac_2O for *N*-Ac-D-Leu was varied from zero to 0.5: ○; zero, ●; 0.05, △; 0.1, ▲; 0.2, □; 0.5. Samples (0.5 ml) from the reaction mixture were diluted by methanol (10 ml) and optical rotations were measured. Racemization degree was calculated from the following equation:

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

Subsequently, optical resolution of *N*-Ac-DL-Leu by a preferential crystallization procedure was carried out at 80 °C under appropriate conditions to cause the rapid racemization of *N*-Ac-D-Leu in the liquid phase. After stirring for 1 h, all of the racemic modifications existing in excess as a supersaturation state, 12 g of *N*-Ac-DL-Leu per 100 ml of solvent, were converted to optically pure crystalline *N*-Ac-L-Leu and the filtrate was the saturated solution of *N*-Ac-DL-Leu. Figure 3 illustrates the above batch asymmetric transformation, compared with the ordinary optical resolution by a preferential crystallization procedure. In the asymmetric transformation, all of the racemic modifications existing in the supersaturation state are converted to crystalline optically active L-isomers and the supersaturation state is cancelled. On the other hand, in the ordinary optical resolution, some unseeded D-isomer remains in the liquid phase as an unstable supersaturation state. This is a marked difference between the two cases.

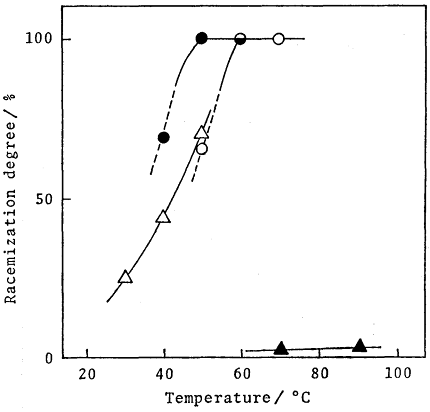

Fig. 2. Effect of temperature on racemization of *N*-Ac-D-Leu.
The mixture of the following composition was heated for 3 h. *N*-Ac-D-Leu/AcOH/Ac$_2$O (g/ml/ml): ○; 0.2/1.0/0.1, ●; 0.2/4.0/0.4, △; 0.2/8.0/0.8, ▲; 0.2/1.0/0.0. ----: The solid of *N*-Ac-D-Leu did not dissolve completely during the reaction. The whole reaction mixture was diluted with methanol (20 ml) and the optical rotation was measured. Racemization degree was calculated as shown in Fig. 1.

Since the driving force for the optical resolution by preferential crystallization procedure is provided only by the supersaturation degree of the racemic modification, the yield of the enantiomer produced by a simple batch experiment of this type of asymmetric transformation is dependent on the extent of the supersaturation state of the racemic modification dissolved in the reaction mixture. To obtain a higher yield of enantiomer, it is necessary to make up for the decrease of the supersaturation degree due to the preferential crystallization. This may be achieved by removal of solvent or addition of inert solvent effective for lowering the solubility of the racemic modification, as described in the previous report. In the present work, however, fresh racemic modification was added as a solid after completion of a batch asymmetric transformation and it was dissolved at an elevated temperature. In this case, fortunately, only *N*-Ac-DL-Leu in a solid mixture of newly added *N*-Ac-DL-Leu and previously crystallized *N*-Ac-L-Leu was dissolved and *N*-Ac-L-Leu was not dissolved because *N*-Ac-DL-Leu forms a true racemic mixture in the reaction system.[3] Thus a semicontinuous asymmetric transformation was able to be achieved by repeating the batch operation. The results are shown in Fig. 4, indicating that all of *N*-Ac-DL-Leu introduced successively into the reaction system was transformed to almost optically pure *N*-Ac-L-Leu. Using 2 g of seed crystals of *N*-Ac-L-Leu and 208 g of total *N*-Ac-DL-Leu on 100 ml scale, 130 g of *N*-Ac-L-Leu was obtained as 94% optically pure crystals. After the separation of *N*-Ac-L-Leu, 42 g of *N*-Ac-DL-Leu was recovered from the filtrate as the second crop.

From the practical view point, however, the above

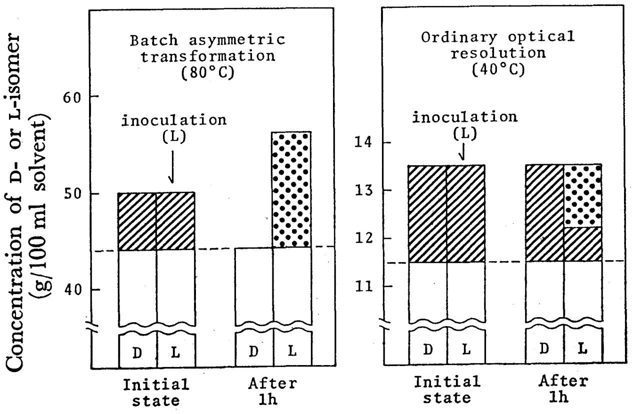

Fig. 3. Batch asymmetric transformation and ordinary optical resolution by preferential crystallization procedure of *N*-Ac-DL-Leu existing in excess as supersaturation state.
Batch asymmetric transformation was carried out under the conditions described in the text. Ordinary optical resolution was carried out at 40 °C using 0.1 g of seed crystals with the supersaturated solution of *N*-Ac-DL-Leu, 27 g/AcOH 100 ml. ----: Solubility of *N*-Ac-DL-Leu, 88 g/100 ml solvent at 80 °C and 23 g/100 ml AcOH at 40 °C, ▨: supersaturated part of *N*-Ac-Leu, ▒: crystals of *N*-Ac-L-Leu.

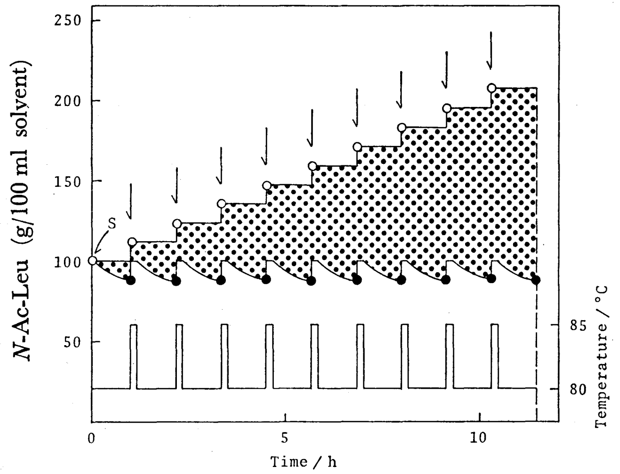

Fig. 4. Illustration of semicontinuous asymmetric transformation by repeating supply of DL-form in batch operation.
—○—: Total amount of *N*-Ac-Leu existing in the reaction mixture, —●—: concentration of *N*-Ac-Leu (almost DL-form) in the liquid phase, —: temperature, ▒: solid phase (almost L-form), ↓: addition of solid DL-form (12.0 g), S: seeding of *N*-Ac-L-Leu (2.0 g).

semicontinuous operation was considered to be complex and troublesome. The racemization experiments already demonstrated that the racemization proceeded at a rapid rate even at a relatively low temperature. Then, a continuous asymmetric transformation was carried out by a method in which the supersaturation state was continuously provided by cooling the solution from 100 °C to 40 °C. It is preferable that the rate of crystallization of the desired *N*-Ac-L-Leu is nearly equal to the rate of racemization of the undesired *N*-Ac-D-Leu in the solution. Therefore, the rate of cooling was controlled to meet those rates. When the temperature was decreased at a rate of 10 °C/h, which was selected as the optimum condition, the time course of the asymmetric transformation is shown in Fig. 5. In this experiment, acetic anhydride was supplemented after 2.5 h because its concentration had decreased at a relatively high rate, as can be seen in Fig. 5. Since the excess of the undesired *N*-Ac-D-Leu in solution was kept to a relatively low level (1±0.5 g/100 ml solvent) during the reaction, racemization seemed to proceed at a rapid rate to meet the preferential crystallization of *N*-Ac-L-Leu. The decrease in the concentration of acetic anhydride may be accounted for by the formation of a mixed anhydride or an azlactone of *N*-Ac-Leu, which are well known as racemizing intermediates.[4–6] After being cooled to 40 °C, the reaction mixture was allowed to stand for 30 min at the same temperature to complete racemization and crystallization. In this experiment, almost optically pure *N*-Ac-L-Leu was obtained in a high yield of 70%, based on the original weight of racemic modification (or 140% based on the enantiomer content of the racemic modification). The reproducibility of the reaction is demonstrated by the results of repeated runs given in Table 1.

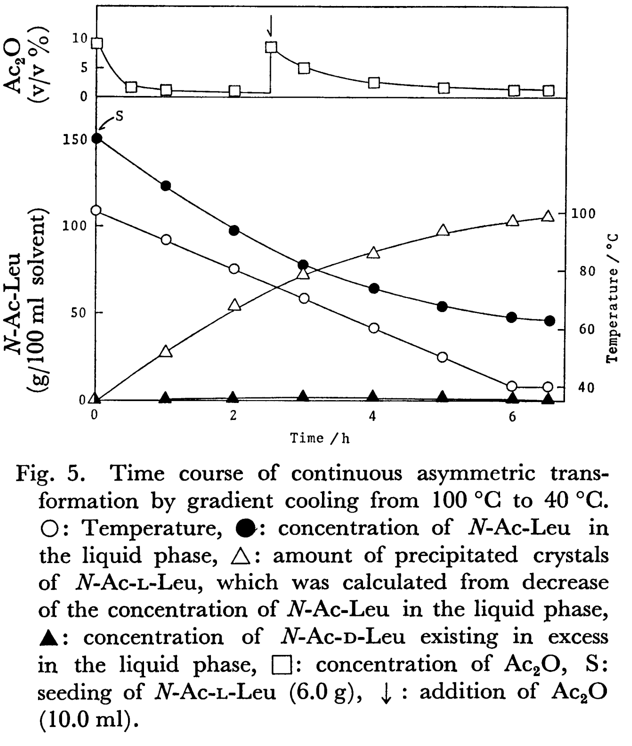

Fig. 5. Time course of continuous asymmetric transformation by gradient cooling from 100 °C to 40 °C. ○: Temperature, ●: concentration of *N*-Ac-Leu in the liquid phase, △: amount of precipitated crystals of *N*-Ac-L-Leu, which was calculated from decrease of the concentration of *N*-Ac-Leu in the liquid phase, ▲: concentration of *N*-Ac-D-Leu existing in excess in the liquid phase, □: concentration of Ac_2O, S: seeding of *N*-Ac-L-Leu (6.0 g), ↓: addition of Ac_2O (10.0 ml).

The main disadvantage of the ordinary optical resolution by preferential crystallization is that crystallization of the desired isomer by seeding leads to an excess of the undesired isomer in the solution and the resolution system is racemic as a whole (Fig. 3). The resolution is not finished until the crystals of the desired isomer are separated from the mother liquor containing the undesired isomer. Furthermore, the yield is limited by the stability of the supersaturated state of the undesired isomer and is usually low because the supersaturation is relatively unstable. On the contrary, the asymmetric transformation now presented causes a substantial change of the composition of D- and L-isomers in the reaction system and almost optically pure crystals of the desired isomer is obtained in a high yield up to 140%, based on the enantiomer content of the racemic modification. The simplicity of the operation is also one of the advantages of the present asymmetric transformation. Therefore, application of the present method for the industrial production of L-leucine, an essential amino acid, is considered to be very promising if combined with a proper synthetic method for *N*-Ac-DL-Leu. The further applications of the asymmetric transformation combining optical resolution process and racemization process to other amino acids or their derivatives are under investigation.

Experimental

Materials and Analyses. *N*-Ac-DL-Leu, *N*-Ac-L-Leu, *N*-Ac-D-Leu, and other chemicals were the same as described

TABLE 1. ASYMMETRIC TRANSFORMATION BY COOLING FROM 100 °C TO 40 °C[a)]

Exptl No.	First crop				Second crop[c)]	
	Yield g	$[\alpha]^{25}_{\mathrm{D}}$/° ($c$ 1, MeOH)	Optical purity %	Transformed[b)] amount g	Yield g	$[\alpha]^{25}_{\mathrm{D}}$/° ($c$ 1, MeOH)
1	112.6	−24.6	98.8	105.2	22.5	0.0
2	111.8	−24.4	98.0	103.6	25.4	0.0
3	113.5	−24.0	96.4	103.4	21.3	0.0
4	113.7	−24.3	97.6	105.0	18.5	0.0
5	110.2	−24.4	98.0	102.0	21.9	0.0

a) Reactions were carried out on a 100 ml-scale using 150 g of *N*-Ac-DL-Leu and 6.0 g of seed crystals of *N*-Ac-L-Leu. b) Amount of seed crystals was subtracted from the amount of net optically active form separated. c) The filtrate showed no optical rotation.

previously.[1] All samples for analyses were dried overnight *in vacuo* at 40 °C. Analytical and identification methods of *N*-acylamino acids were described in the previous paper.[1] The optical purity of separated crystals was determined by measuring the optical rotation with a Perkin-Elmer 141 automatic polarimeter. The total concentration of *N*-Ac-Leu in the reaction mixture was measured as total solute concentration with a Karl Zeiss immersion refractometer. The amount of *N*-Ac-D-Leu existing in excess in liquid phase was determined by refractometric and polarimetric measurements.[7] The amount of acetic anhydride remaining in a reaction mixture was determined by a Shimadzu gas chromatograph GC-3BT using a 3 m column of 20% Silicone DC-550 on Celite 545. Temperature control for reaction was carried out by using a Chino program setter NP-121-B and a Chino electronic recording controller ET-1561.

Racemization of N-*Ac*-D-*Leu*. *Effect of Amount of Acetic Anhydride*: *N*-Ac-D-Leu (6.0 g) was dissolved in AcOH (30 ml) at an elevated temperature and maintained at 75 °C. Various amounts (0.05, 0.1, 0.2, and 0.5 molar ratio) of acetic anhydride were added to the solution under stirring. The samples (0.5 ml) were taken out from the reaction mixture at intervals of 30 or 60 min, and were added into 10 ml of methanol. The optical rotation of the solution was measured and the racemization degree was evaluated on the basis of the initial optical rotation and its decrease. It was confirmed by thin-layer chromatography that significant degradation did not occur during the reaction. The data are shown in Fig. 1.

Effect of Temperature: Since the solubility of *N*-Ac-D-Leu varied largely with temperature, the reaction was carried out in three levels of the concentration of *N*-Ac-D-Leu corresponding to the reaction temperatures. A mixture of *N*-Ac-D-Leu (0.2 g) and various amounts of AcOH–$\mathrm{Ac_2O}$ (10:1,v/v) solution was heated at various temperatures in a sealed tube and shaken occasionally. After 3 h, the mixture was added into 20 ml of methanol and the optical rotation was measured. The racemization degree evaluated from the decrease of optical rotation is shown in Fig. 2.

Stability of Crystalline N-*Ac*-L-*Leu under Conditions of Racemization*. *N*-Ac-DL-Leu (12.5 g) was dissolved in 10 ml of AcOH–$\mathrm{Ac_2O}$ (10:1, v/v) solution at an elevated temperature to prepare the solution saturated with *N*-Ac-DL-Leu at 90 °C. To the saturated solution maintained at 90 °C, the crystals of *N*-Ac-L-Leu (10.0 g) were added. The heterogeneous reaction mixture was stirred for 5 h at the same temperature. The insoluble crystals were quickly separated by filtration, washed with a small amount of AcOH and then toluene, and dried. The insoluble crystals were proved to be optically pure *N*-Ac-L-Leu (8.5 g), $[\alpha]^{25}_{\mathrm{D}}$ −24.9° (*c* 1, MeOH).

Asymmetric Transformation. *Batch Transformation*: *N*-Ac-DL-Leu (100.0 g) was dissolved in 100 ml of AcOH–$\mathrm{Ac_2O}$ (10:1, v/v) solution at 90 °C and maintained at 80 °C. In this case, 12.0 g of *N*-Ac-DL-Leu existed as a supersaturation state, because the solubility of *N*-Ac-DL-Leu at 80 °C was 88.0 g/100 ml solvent. The supersaturated solution was seeded with finely pulverized crystals of *N*-Ac-L-Leu (2.0 g) and stirred for one hour to achieve preferential crystallization of *N*-Ac-L-Leu and simultaneous racemization of *N*-Ac-D-Leu. The precipitated crystals were quickly collected by filtration, washed with a small amount of AcOH, and dried to give *N*-Ac-L-Leu (14.5 g), $[\alpha]^{25}_{\mathrm{D}}$ −24.1° (*c* 1, MeOH), optical purity 96.8% (14.0 g of *N*-Ac-L-Leu and 0.5 g of *N*-Ac-DL-Leu). Subtracting 2.0 g of seeded *N*-Ac-L-Leu, 12.0 g of pure *N*-Ac-L-Leu was obtained. After the separation of *N*-Ac-L-Leu, the filtrate was stirred at 20 °C for 2 h and the precipitated crystals were collected by filtration to give *N*-Ac-DL-Leu (57.6 g), $[\alpha]^{25}_{\mathrm{D}}$ 0.0° (*c* 1, MeOH). The filtrate showed no optical rotation. Consequently, all of *N*-Ac-DL-Leu (12.0 g) existing as supersaturation was completely transformed to *N*-Ac-L-Leu. The schematic comparison of the transformation with the ordinary optical resolution is shown in Fig. 3.

Semicontinuous Transformation by Repeating a Batch Run: *N*-Ac-DL-Leu (100.0 g) was dissolved in 100 ml of AcOH–$\mathrm{Ac_2O}$ (10:1, v/v) solution at 90 °C and cooled to 80 °C. The solution was seeded with the crystals of *N*-Ac-L-Leu (2.0 g, 65—100 meshes) and stirred for one hour at the same temperature for preferential crystallization of *N*-Ac-L-Leu and simultaneous racemization of *N*-Ac-D-Leu. Then, finely pulverized crystals of *N*-Ac-DL-Leu (12.0 g) were added into the heterogeneous system consisting of crystals of *N*-Ac-L-Leu and the solution saturated with *N*-Ac-DL-Leu. To dissolve the added *N*-Ac-DL-Leu, the mixture was heated at 85 °C for 10 min under stirring. All of the added *N*-Ac-DL-Leu was dissolved and most of the crystallized *N*-Ac-L-Leu remained in a solid state. The mixture was cooled again to 80 °C and stirred for one hour at the same temperature for the subsequent transformation. This operation was repeated nine times under heterogeneous conditions. After the last run, the precipitated crystals were quickly collected by filtration, washed with a small amount of toluene, and dried to give *N*-Ac-L-Leu (130.0 g), $[\alpha]^{25}_{\mathrm{D}}$ −23.3° (*c* 1, MeOH), optical purity 93.6% (121.7 g of *N*-Ac-L-Leu and 8.3 g of *N*-Ac-DL-Leu). If we subtract 2.0 g of seeded *N*-Ac-L-Leu, we see that 119.7 g of *N*-Ac-L-Leu was obtained. After the separation of *N*-Ac-L-Leu, the filtrate was stirred for 2 h at 20 °C and the precipitated crystals were collected by filtration to give *N*-Ac-DL-Leu (41.8 g), $[\alpha]^{25}_{\mathrm{D}}$ 0.0° (*c* 1, MeOH). The filtrate showed no optical rotation. Consequently, 119.7 g of the total *N*-Ac-DL-Leu (12.0 g × 10) used to make supersaturation state was transformed to *N*-Ac-L-Leu. The process of this experiment is illustrated in Fig. 4.

Continuous Transformation by Gradient Cooling of the Solution: *N*-Ac-DL-Leu (150.0 g) was dissolved in 100 ml of AcOH–$\mathrm{Ac_2O}$ (10:1, v/v) solution under reflux. The solution was cooled to 100 °C, seeded with finely pulverized crystals of *N*-Ac-L-Leu (6.0 g), and then the cooling was continued at the constant speed of 10 °C/h under stirring. After 2.5 h, $\mathrm{Ac_2O}$ (10.0 ml) was added into the reaction mixture at 75 °C. When the temperature reached 40 °C, the cooling was stopped and the reaction mixture was stirred for 30 min at the same temperature to complete racemization and crystallization. The precipitated crystals were quickly collected by filtration, washed with a small amount of AcOH, and dried to give *N*-Ac-L-Leu (112.6 g), $[\alpha]^{25}_{\mathrm{D}}$ −24.6° (*c* 1, MeOH), optical purity 98.8% (111.2 g of *N*-Ac-L-Leu and 1.4 g of *N*-Ac-DL-Leu). If we subtract 6.0 g of seeded *N*-Ac-L-Leu, we see that 105.2 g of *N*-Ac-L-Leu was obtained. After the separation of *N*-Ac-L-Leu, the filtrate was concentrated *in vacuo* to about half the initial volume, and allowed to crystallize. The precipitated crystals were collected by filtration to give *N*-Ac-DL-Leu (22.5 g), $[\alpha]^{25}_{\mathrm{D}}$ 0.0° (*c* 1, MeOH). The filtrate showed no optical rotation. Consequently, about 70% of the initially used *N*-Ac-DL-Leu was transformed to *N*-Ac-L-Leu. A typical time course of the transformation is shown in Fig. 5. The results of the five experiments carried out by the same procedure as described above are shown in Table 1.

References

1) C. Hongo, S. Yamada, and I. Chibata, *Bull. Chem. Soc. Jpn.*, **54**, 3286 (1981).

2) M. M. Harris, "Progress in Stereochemistry," ed by W. Klyne and P. B. D. Mare, Butterworths Scientific Publications, London (1958), Vol. 2, p. 157.

3) C. Hongo, M. Shibazaki, S. Yamada, and I. Chibata, *J. Agric. Food Chem.*, **24**, 903 (1976).

4) H. E. Carter and C. M. Stevens, *J. Biol. Chem.*, **133**, 117 (1940).

5) H. E. Carter and C. M. Stevens, *J. Biol. Chem.*, **138**, 627 (1941).

6) A. Neuberger, *Adv. Protein Chem.*, **4**, 297 (1948).

7) C. Hongo, S. Yamada, and I. Chibata, *Bull. Chem. Soc. Jpn.*, **54**, 1905 (1981).