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Asymmetric Transformation of DL-*p*-Hydroxyphenylglycine by a Combination of Preferential Crystallization and Simultaneous Racemization of the *o*-Toluenesulfonate

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The asymmetric transformation of DL-*p*-hydroxyphenylglycine was achieved between two enantiomers by a combination of preferential crystallization of a desired enantiomer of *p*-hydroxyphenylglycine *o*-toluenesulfonate (HPG·*o*-TS) and the simultaneous racemization of the antipode. L-HPG·*o*-TS was easily racemized by heating at 100 °C in aqueous 95% (v/v) acetic acid in the presence of small amounts of salicylaldehyde and free DL-HPG. A supersaturated solution of DL-HPG·*o*-TS placed under such conditions for racemization was seeded with the crystals of D-HPG·*o*-TS, and added with DL-HPG and *o*-toluenesulfonic acid in order to provide continuously the supersaturated state of DL-HPG·*o*-TS as a driving force. As a result, 77.2% of DL-HPG added was transformed to D-isomer.

D-*p*-Hydroxyphenylglycine is useful as a side chain of semisynthetic penicillins or cephalosporins. Our previous paper[1] reported that DL-*p*-hydroxyphenylglycine was resolved by a preferential crystallization procedure in the form of salts with aromatic sulfonic acids such as benzenesulfonic acid, *o*-toluenesulfonic acid, and the like. The preferential crystallization procedure is considered to be a useful method for industrial purposes since it enables the desired optically active isomer to crystallize preferentially from a supersaturated solution of the racemic modification by the simple inoculation of the same isomer. In this procedure, however, there are several disadvantages[2-4] resulting from the fact that the crystallization of the desired isomer by inoculation leads to an excess of the undesired isomer in the solution and the resolution system is racemic as a whole.

The asymmetric transformation[3-5] by a combination of preferential crystallization of the desired isomer and the simultaneous racemization of the undesired isomer is very promising for industrial applications without the disadvantages described above. The asymmetric transformation of *N*-acyl amino acids has been successful[3,4] because *N*-acyl amino acids were easily racemized in the presence of a catalytic amount of acetic anhydride. Many DL-amino acids can be resolved in the form of salts with aromatic sulfonic acids or mineral acids.[1,6] In order to apply the asymmetric transformation to such amino acid salts, it is necessary to determine the conditions of racemization of amino acid salts.

We recently reported[7,8] that the salts of optically active amino acids with sulfonic acids or mineral acids could be racemized by heating moderately in a medium of acetic acid in the presence of small amounts of an aldehyde and free DL-amino acid, and the racemization method could be applied to asymmetric transformation of amino acids. Using the racemization method, we studied the asymmetric transformation by a combination of the optical resolution of DL-*p*-hydroxyphenylglycine *o*-toluenesulfonate (DL-HPG·*o*-TS) and the simultaneous racemization of the salt.

Since the solubility of DL-HPG·*o*-TS in acetic acid is very low (at 80 °C and 100 °C, 1.80 and 3.06 g/100 ml AcOH, respectively), it is preferable to use aqueous acetic acid to obtain a practical starting concentration for the asymmetric transformation. The effects of salicylaldehyde and DL-HPG on racemization of L-HPG·*o*-TS in aqueous acetic acid were examined. As shown in Table 1, the racemization of the salt was accelerated in the presence of salicylaldehyde, DL-HPG, or both. Although the rate of racemization, which is an important factor in this kind of asymmetric transformation, decreased with an increase in the water content of the acetic acid, a racemization rate sufficient for the asymmetric transformation was obtained by the presence of both salicylaldehyde and DL-HPG in aqueous 95% (v/v) acetic acid. Therefore, the asymmetric transformation was thereafter carried out in aqueous 95% (v/v) acetic acid. The rate of racemization was greatly influenced by temperature as shown in Fig. 1. A temperature higher than 90 °C was necessary for practical racemization.

When DL-HPG·*o*-TS was crystallized from a solution placed under the racemizing conditions as described above, the IR-spectrum and the melting point of the DL-HPG·*o*-TS obtained were identical with those of

TABLE 1. EFFECTS OF SALICYLALDEHYDE AND DL-HPG ON RACEMIZATION OF L-HPG·*o*-TS[a]

Additive[b]	Racemization degree/%		
	AcOH[c]	95% AcOH[d]	90% AcOH[d]
None	20	18	18
Salicylaldehyde	55	30	24
DL-HPG	76	66	49
Salicylaldehyde and DL-HPG	99	94	62

a) Reaction was carried out at 100 °C for 3 h. b) 0.2 Molar equivalent. c) Glacial acetic acid. d) Aqueous acetic acid.

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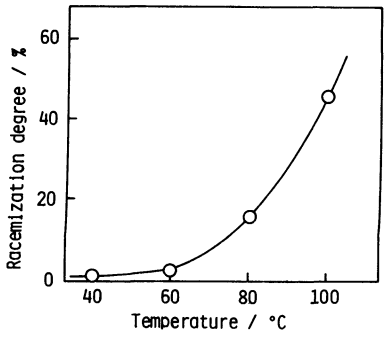

Fig. 1. Effect of temperature on racemization of L-HPG·*o*-TS.
The reactions were carried out for 1 h in aqueous 95% acetic acid in the presence of salicylaldehyde and DL-HPG.

racemic mixture of DL-HPG·*o*-TS obtained in our previous paper;[1] this indicates that DL-HPG·*o*-TS crystallizes as a racemic mixture suitable for the preferential crystallization procedure also under such racemizing conditions. The crystals of D-HPG·*o*-TS were confirmed to be not racemized under the above conditions for racemization in a liquid phase. Thus, the most essential requirement for this kind of asymmetric transformation was fulfilled.

Then, to find the possibility of the asymmetric transformation of DL-HPG, a batch-asymmetric transformation was carried out. The supersaturated solution of DL-HPG·*o*-TS placed under the racemizing conditions described above was seeded with the crystals of D-HPG·*o*-TS and stirred for 2 h. The crystallized crop and the second crop obtained from the mother liquor were analyzed. As a result, it was found that the preferential crystallization of seeded D-HPG·*o*-TS and the racemization of unseeded L-HPG·*o*-TS took place at the same time and 17% of DL-HPG·*o*-TS was transformed to D-HPG·*o*-TS.

Since the driving force for the preferential crystallization of a seeded isomer is provided only by the supersaturated state of the racemic modification, the yield of the enantiomer produced by this type of asymmetric transformation is dependent on the extent of the supersaturated state of the racemic modification. In a batch transformation, the yield is relatively low because the starting supersaturation degree is limited by the stability of the supersaturated state. To obtain a higher yield of the enantiomer, it is necessary to make up for the decrease of the supersaturation degree due to the preferential crystallization.

In the asymmetric transformation of DL-HPG·*o*-TS, the supersaturated state could not be continuously provided by cooling the solution because the racemization did not proceed at a low temperature (Fig. 1). In the present work, the supersaturated state was maintained continuously by suspending free DL-HPG in the reaction mixture and then by adding *o*-toluenesulfonic acid, thereto, to form the salt of DL-HPG·*o*-TS in the liquid phase. In this case, fortunately,

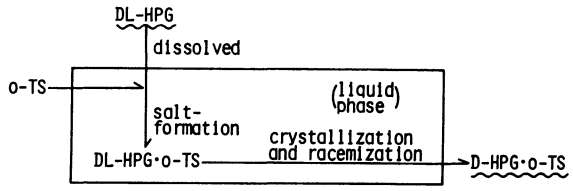

Fig. 2. Continuous asymmetric transformation of DL-HPG. ~~~, solid state.

the suspension of free DL-HPG is preferable since the presence of free DL-HPG in the liquid phase is essential for accelerating the racemization of L-HPG·*o*-TS. DL-HPG·*o*-TS resulting from free DL-HPG and the added *o*-toluenesulfonic acid can exist in a supersaturated state under an appropriate rate of the addition of *o*-toluenesulfonic acid, and can serve for the asymmetric transformation. The continuous asymmetric transformation presented here was carried out in such a suspension system in expectation of a repetition of the following process: dissolution of suspended DL-HPG, formation of DL-HPG·*o*-TS to result a supersaturated state, and preferential crystallization and simultaneous racemization toward the resulting DL-HPG·*o*-TS. The pathway is shown in Fig. 2.

o-Toluenesulfonic acid has two moles of water of crystallization which can not be easily removed. The addition of the dihydrate into the reaction mixture results in an increase of the water content in the reaction system and consequently in the lowering of the racemization rate. Therefore, *o*-toluenesulfonic acid dihydrate was dissolved in acetic anhydride, which reacted with the water of crystallization at 100°C to form acetic acid. This was added to the reaction system in the form of an acetic anhydride solution of *o*-toluenesulfonic acid dihydrate. The pouring speed of the *o*-toluenesulfonic acid solution, *i. e.*, the speed of producing a supersaturated state, was controlled to meet the rate of crystallization of D-HPG·*o*-TS and the rate of racemization of L-HPG·*o*-TS. Namely, into a heterogeneous mixture consisting of 19.0 g of DL-HPG·*o*-TS, 1.25 ml of salicylaldehyde, 51.0 g of free DL-HPG, and 100 ml of aqueous 95% (v/v) acetic acid, 2.0 g of crystals of D-HPG·*o*-TS was seeded at 100°C. Then, the solution consisting of 62.5 g of *o*-toluenesulfonic acid dihydrate and 62.5 ml of acetic anhydride was poured at the rate of 5.0 ml/h into the mixture maintained at 100°C under stirring. The precipitated crystals were separated to give 82.8 g of almost optically pure D-HPG·*o*-TS. The material remaining in the filtrate was nearly racemic. The change in the composition of both enantiomers by the reaction is shown in Fig. 3. The result suggests that 77.2% of suspended DL-HPG was transformed to D-HPG *via* the pathway as shown in Fig. 2. The D-HPG·*o*-TS obtained above was easily decomposed to D-HPG without racemization by neutralization.

The asymmetric transformation between two dia-

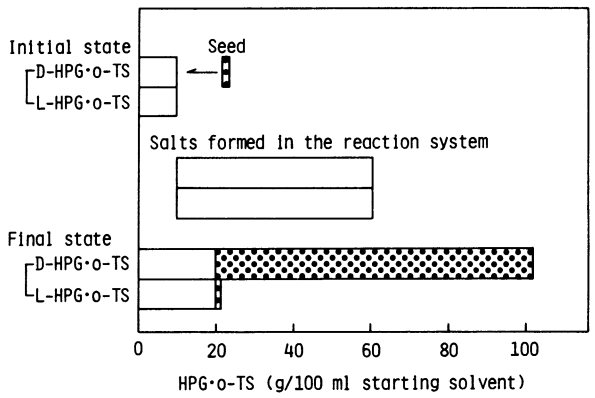

Fig. 3. The change in the composition of both enantiomers by continuous asymmetric transformation. ▩, crystals of HPG·*o*-TS; □, HPG·*o*-TS in solution.

stereoisomers by a combination of selective precipitation of the less soluble diastereoisomer and epimerization of the soluble diastereoisomer is well-known as an asymmetric transformation of the second order.[9] However, an asymmetric transformation between two enantiomers by a combination of preferential crystallization of a desired enantiomer and the simultaneous racemization of the antipode is unique and has not been reported except for a few examples.[3-5] It is necessary in this kind of asymmetric transformation to devise a way in which the supersaturated state is provided as a driving force. In the case of the asymmetric transformation of DL-HPG proposed here, the supersaturated state of DL-HPG·*o*-TS could be easily produced by adding DL-HPG and *o*-toluenesulfonic acid into the reaction system. If DL-HPG·*o*-TS in a supersaturated state is formed at a rate which meets the rates of preferential crystallization and racemization, the reaction proceeds in a high concentration of slurry state. The proposed method seems very promising for the industrial production of D-HPG because of its operational simplicity and high yield.

Experimental

Materials and Analyses. Optically active and racemic HPG manufactured by our company, Tanabe Seiyaku Co., Ltd., were used. *o*-Toluenesulfonic acid, salicylaldehyde, and other chemicals were obtained from Tokyo Kasei Kogyo Co., Ltd. Optically active and racemic HPG·*o*-TS were prepared in a manner described in a previous report.[1] All samples were dried overnight *in vacuo* at 40°C. Optical rotation was measured with a Perkin-Elmer 141 automatic polarimeter. The water content of samples was determined by the Karl-Fischer's method.

Racemization of L-*HPG·o-TS.* Unless otherwise noted, a mixture of L-HPG·*o*-TS (50 mg), DL-HPG (5 mg), salicylaldehyde (3 μl), and acetic acid containing 5% water (5 ml) was heated in a sealed tube at 100°C for 1 h or 3 h. After the reaction mixture was diluted with 1 M hydrochloric acid (5 ml (1 M=1 mol dm^{-3})), the optical rotation was measured. The racemization degree was calculated in the same way as described in a previous report.[7] The effect of DL-HPG or salicylaldehyde in acetic acid containing water and the effect of the reaction temperature are shown in Table 1 and Fig. 1, respectively.

Crystallization of DL-*HPG·o-TS from Racemizing Solution.* A mixture of DL-HPG·*o*-TS (3.8 g), DL-HPG (0.2 g), and salicylaldehyde (0.25 ml) was dissolved in acetic acid (20 ml) containing 5% water under reflux and maintained at 100°C. The solution was seeded with finely pulverized crystals of DL-HPG·*o*-TS (10 mg) and stirred for 5 h at the same temperature. The precipitated crystals were quickly separated by filtration, washed with a small amount of AcOH, and dried to give DL-HPG·*o*-TS (0.92 g) crystallized from racemizing solution. The melting point (213—215°C, dec) and IR-spectrum of the DL-HPG·*o*-TS were identical with those of racemic mixture of DL-HPG·*o*-TS shown in previous report.[1]

Stability of Crystalline D-*HPG·o-TS under Conditions for Racemization.* A mixture of DL-HPG·*o*-TS (1.0 g), DL-HPG (40 mg), and salicylaldehyde (50 mg) was dissolved in AcOH containing 2% water under reflux to prepare a solution saturated with DL-HPG·*o*-TS at 100°C. To the saturated solution maintained at 100°C, crystals of D-HPG·*o*-TS (2.0 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 dried. The insoluble crystals proved to be optically pure D-HPG·*o*-TS (1.9 g), $[\alpha]_D^{25}$—66.6° (c=1, water).

Asymmetric Transformation. Batch Transformation: A mixture of DL-HPG·*o*-TS (3.8 g), DL-HPG (0.2 g), salicylaldehyde (0.25 ml) and AcOH (20 ml) containing 5% water was heated under reflux until a complete solution occurred and was maintained at 100°C. The supersaturated solution was seeded with finely pulverized crystals of D-HPG·*o*-TS (0.2 g) and stirred for 2 h at the same temperature. The precipitated crystals were quickly separated by filtration, washed with a small amount of AcOH, and dried to give D-HPG·*o*-TS (0.85 g), $[\alpha]_D^{25}$ −66.6° (c=1, water), optical purity 100%. Subtracting 0.2 g of seeded D-HPG·*o*-TS, 0.65 g of pure D-HPG·*o*-TS was obtained. After the separation of D-HPG·*o*-TS, the filtrate was stirred at 20°C for 2 h and the precipitated crystals were collected by filtration to give DL-HPG·*o*-TS (2.66 g), $[\alpha]_D^{25}$ +0.6° (c=1, water). The mother liquor did not show any optical rotation. Therefore, the whole reaction mixture became 17.1% enantiomeric excess.

Continuous Transformation: A mixture of DL-HPG·*o*-TS (19.0 g) and aqueous 95% (v/v) acetic acid (100 ml) in a three-necked flask fitted with a mechanical stirrer and a condenser was heated under reflux until complete solution occurred. Then the flask was placed in an oil bath controlled at 100°C. Salicylaldehyde (1.24 ml) was added to the solution and DL-HPG (51.0 g) was suspended therein. After 20 min, into the heterogeneous reaction mixture were added under stirring finely pulverized crystals of D-HPG·*o*-TS (2.0 g) as seed crystals. To this was poured a solution consisting of *o*-toluenesulfonic acid dihydrate (62.5 g) and acetic anhydride (62.5 ml) at the rate of 5.0 ml/h by a Micro Feeder JP-W (Furue Science Co., Ltd.). At 5 h and 20 h after the addition of the seed crystals, 1.3 ml and 0.7 ml of salicylaldehyde were added to the mixture, respectively. The mixture was stirred at the same temperature for total 30 h. The precipitated crystals were quickly collected by filtration, washed with a small amount of acetic acid, and dried to give D-HPG·*o*-TS (82.8 g), $[\alpha]_D^{25}$ −64.9° (c=1, water), optical purity 97.4%.

After the separation of D-HPG·*o*-TS, the filtrate was stirred for 2 h at room temperature and the precipitated crystals were collected by filtration to recover DL-HPG·*o*-TS (18.2 g), $[\alpha]_D^{25}$ 0.0° (c=1, water). The filtrate did not show any optical rotation. The change in the composition of both enantiomers by the reaction is shown in Fig. 3.

Preparation of D-*HPG.* The D-HPG·*o*-TS (82.0 g) obtained above was dissolved in water (230 ml) at an elevated temperature and was treated with charcoal. The solution was adjusted to pH 6 with 5 M sodium hydroxide and allowed to stand in a refrigerator overnight. The resulting precipitates were collected, washed with water, and dried to give D-HPG (34.0 g), $[\alpha]_D^{25}$ −158.2° (c=1, M-HCl).

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