Racemization of Optically Active Amino Acid Salts and an Approach to Asymmetric Transformation of DL-Amino Acids

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(Received June 13, 1983)

In connection with optical resolution of DL-amino acids, the racemization process of optically active amino acid salts was studied. A wide variety of salts of optically active amino acids with sulfonic acids or mineral acids could be racemized by heating in a medium of acetic acid at 80—100 °C for 1 h in the presence of 0.1 molar equivalents of an aldehyde and free DL-amino acid. From the reaction mixture, the racemized DL-amino acid salts were recovered in high yield (86—92%) and in high purity. An asymmetric transformation between two enantiomers of amino acid salt was attempted by the combination of preferential crystallization of the desired enantiomer and racemization of the antipode in the liquid phase. DL-Alanine *p*-chlorobenzenesulfonate was partially converted to L-isomer (yield 16%, optically purity 97%). Another approach was carried out by transformation between two diastereoisomeric salts. The combination of selective precipitation of less soluble diastereoisomeric salt and epimerization of soluble diastereoisomeric salt allowed DL-phenylglycine *d*-camphor-10-sulfonate to be partially converted to D-phenylglycine *d*-camphor-10-sulfonate (yield 68%, optical purity 96%).

Because of our interest in practical production of optically active amino acids, we have been studying the optical resolution of DL-amino acids and the racemization of the unwanted enantiomer obtained by the optical resolution process. A previous paper[1] proposed a new and facile method: Racemizing optically active amino acids in acetic acid solution in the presence of an aldehyde.

Now we extend this racemization method to the salts of optically active amino acids. The optical resolution is usually carried out in the form of salts of amino acids and the unwanted enantiomers are also obtained in the form of salts. For instance, chemical resolution is achieved by the formation of the diastereoisomeric salts, and preferential crystallization procedure can be applied to many amino acids by converting them to suitable salts such as DL-alanine *p*-chlorobenzenesulfonate,[2] DL-serine *m*-xylene-4-sulfonate,[2] DL-tryptophan *p*-phenolsulfonate,[3] DL-6-chlorotryptophan methanesulfonate,[3] and DL-*p*-hydroxyphenylglycine *o*-toluenesulfonate,[4] *etc.* If the racemization of the unwanted enantiomers can be easily accomplished in the form of the salts, the racemized DL-amino acid salts themselves can be advantageously reused as the starting material for the optical resolution. However, the racemization of the salts of amino acids is generally more difficult than that of free amino acids, although only a few salts[2,4] of optically active amino acids could be racemized by heating in water at a much higher temperature under high pressure. In this work, we could find a practical method for the racemization of various amino acid salts.

We attempted to apply this racemization method to an asymmetric transformation[5] of DL-amino acid salts by a simultaneous combination of the optical resolution and the racemization. The present paper describes the racemization of the salts of various amino acids and an approach to an asymmetric transformation of DL-phenylglycine and DL-alanine by using the present racemization method.

When the salts of L-amino acids were heated at 100 °C for 1 h in a medium of acetic acid in a manner similar to the racemization of free amino acids which is described in the previous report,[1] we found that the racemization of various amino acid salts was also accelerated by the addition of 0.1 molar equivalent of salicylaldehyde, but the rate of racemization was smaller than that in the case of free amino acids. When 0.1 molar equivalent

Table 1. Racemization of various amino acid salts[a]

Amino acid salt[b]	Racemization degree/%[c] in the presence of		
	None	SA[d]	SA[d] and DL-Amino acid
L-Alanine·HCl	3	55	100
L-Alanine·BS	0	13	88
L-Leucine·BS	0	7	64
L-Lysine·ABS[e]	0	59	59
L-Methionine·HCl	0	41	100
L-Phenylalanine·HCl[e]	14	59	100
L-Phenylalanine·pXS	0	19	100
L-Proline·BZ	15	28	32
L-Serine·mXS[f]	0	5	65
L-Valine·HCl	0	0	20

a) The reactions were carried out in acetic acid at 100 °C for 1 h. The amounts of salicylaldehyde and DL-amino acid used were 0.1 molar equivallents. b) BS, Benzenesulfonate; ABS, *p*-aminobenzenesulfonate; pXS, *p*-xylene-2-sulfonate; BZ, benzoate; mXS, *m*-xylene-4-sulfonate. c) $\frac{\text{Initial optical rotation} - \text{final optical rotation}}{\text{initial optical rotation}} \times 100$. d) SA, Salicylaldehyde. e) The reactions were carried out in a solid-liquid heterogeneous state. f) The reaction was carried out at 80 °C.

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Table 2. Effect of kind of aldehyde in racemization reaction[a)]

Aldehyde	Racemization degree/%			
	L-Alanine·BS		L-Methionine·HCl	
	With aldehyde	With aldehyde and DL-alanine	With aldehyde	With aldehyde and DL-methionine
None	0	0	0	5
Formaldehyde	14	46[b)]	20[c)]	42[b,c)]
Propionaldehyde	0	40[b)]	12	44[b)]
Butyraldehyde	0	45	12	49
Heptanal	0	79[b)]	27	73[b)]
Acrylaldehyde	0	48[b)]	16	38[b)]
Benzaldehyde	0	48	10	49
Salicylaldehyde	13	88	41	100
p-Hydroxybenzaldehyde	4	35	9	44
o-Nitrobenzaldehyde	0	39	17	54
5-Nitrosalicylaldehyde	14	76[c)]	42	86[c)]
Furfural	23	91[b)]	74	100[b,c)]

a) The reactions were carried out at 100 °C for 1 h in acetic acid. The amounts of aldehyde and DL-amino acid used were 0.1 molar equivalents. b) A 0.2 molar equivalent of DL-amino acid was used. c) A ninhydrin positive spot of degradation product was detected by TLC of the reaction mixture.

of free DL-amino acid corresponding to the L-amino acid moiety of the salt was added into the reaction system together with salicylaldehyde, the racemization of the amino acid salts was found to be greatly accelerated. These results are shown in Table 1. The content of amino acid in the reaction mixtures was confirmed to be almost 100% without significant decomposition. The racemization mechanism of amino acid salts seems to be the same as that of free amino acids. Namely, a part of amino acid moiety of optically active amino acid salt leads to the free state by adding free DL-amino acid and the free optically active amino acid is racemized by an imine formation from the amino acid and aldehyde, as described in our previous report. In the case of L-lysine *p*-aminobenzenesulfonate, the racemization was accelerated by only the addition of salicylaldehyde and the addition of free DL-amino acid was not effective. This seems to be because the *α*-amino group in lysine *p*-aminobenzenesulfonate exists already in the free state (uncharged form) predominantly without the addition of free amino acid. The effect of the kind of aldehyde on the racemization of L-alanine benzenesulfonate and L-methionine hydrochloride was examined at 100 °C for 1 h in glacial acetic acid. The results are shown in Table 2. Various aldehydes, such as aliphatic or aromatic aldehydes, accelerated the racemization of amino acid salts under the coexistence of free DL-amino acid. The effect of the amount of salicylaldehyde on the racemization of L-alanine benzenesulfonate and L-methionine hydrochloride was examined at 100 °C for 1 h under the coexistence of 0.2 molar equivalent of DL-alanine or DL-methionine in glacial acetic acid. The effect of the amount of free DL-leucine on the racemization of L-leucine benzenesulfonate was examined at 100 °C for 30 min or 3 h in the presence of 0.1 molar equivalent of salicylaldehyde in glacial acetic acid. The results are shown in Tables 3 and 4. Salicylaldehyde and free DL-leucine exerted their effects in the use of 0.001 molar equivalent and gave a sufficient racemization degree in the use of 0.05 molar equivalent.

Table 3. Effect of amount of salicylaldehyde[a)]

Molar equivalent of salicylaldehyde	Racemization degree/%	
	L-Alanine·BS	L-Methionine·HCl
0	0	9
0.001	13	15
0.005	48	53
0.01	70	71
0.05	100	100
0.1	100	100

a) The reactions were carried out in the presence of 0.2 molar equivalent of DL-amino acid at 100 °C for 1 h.

Table 4. Effect of amount of DL-leucine on the racemization of L-leucine benzenesulfonate[a)]

Molar equivalent of DL-leucine	Racemization degree/%	
	30 min	3 h
0	2	27
0.001	4	34
0.005	6	43
0.01	8	47
0.05	24	85
0.1	40	100
0.2	61	100

a) The reactions were carried out in the presence of 0.1 molar equivalent of salicylaldehyde at 100 °C.

As a practical example, L-alanine benzenesulfonate, L-alanine *p*-toluenesulfonate, L-leucine benzenesulfonate, L-methionine *p*-chlorobenzenesulfonate, and L-*p*-hydroxyphenylglycine *o*-toluenesulfonate were racemized by the present method, and the respective racemic amino acid salts were directly separated from the reaction mixtures and analyzed. The results are shown in Table 5. The racemized DL-amino acid salts were

Table 5. Separation of racemized amino acid salts[a]

Amino acid salt[b]	Composition[c]			Separated salt[f]			
	AcOH (ml)	SA[d] (g)	DL-Amino[e] acid (g)	Yield /g	Yield /%[g]	$[\alpha]_D^{25}$/° (c=1, water)	Content of DL-form %
Alanine·BS	6	0.15	0.11	2.95	89.4	0	100
Alanine·pTS	6	0.14	0.10	3.05	92.4	0	100
Leucine·BS	6	0.13	0.14	2.85	86.4	0	100
Methionine·pCBS	6	0.11	0.13	3.00	90.9	0	100
p-Hydroxyphenylglycine·oTS	50[h]	0.22[i]	0.59[j]	3.66	87.9	0	100

a) The reactions were carried out at 100 °C for 2 h. b) BS, Benzenesulfonate; pTS, *p*-toluenesulfonate; pCBS, *p*-chlorobenzenesulfonate; oTS, *o*-toluenesulfonate. c) A 3 g amount of L-amino acid salt was used throughout. d) SA, Salicylaldehyde. e) Free DL-amino acid corresponding to L-amino acid moiety of the salt. f) No impurity was detected by TLC. g) Based on L-amino acid salt plus coexisting DL-amino acid. h) AcOH/water: 95/5(v/v). i) A 0.2 molar equivalent of SA was used. j) A 0.4 molar equivalent of DL-*p*-hydroxyphenylglycine was used.

obtained in high yield (86—92%) and in such high purity that no by-product was detected by thin-layer chromatography. The racemization method presented here seems to be a simple and practical way.

Ordinarily, the optical resolution of DL-amino acids is carried out under the conditions in which the amino acid is optically stable. On the contrary, we carried out the optical resolution of DL-amino acid under the racemization conditions described above in order to achieve an asymmetric transformation by a combination of optical resolution and simultaneous racemization (or epimerization). This type of asymmetric transformation is interesting because of its practical applications.[6-13]

An asymmetric transformation was carried out between two enantiomers by a combination of preferential crystallization and racemization. DL-Alanine can be resolved by preferential crystallization procedure in the form of *p*-chlorobenzenesulfonate.[2] This resolution method was combined with the present racemization method. Namely, a supersaturated solution consisting of DL-alanine *p*-chlorobenzenesulfonate (45.0 g), DL-alanine (2.0 g), salicylaldehyde (1.0 g), and glacial acetic acid (50 ml) was seeded with the crystals of L-alanine *p*-chlorobenzenesulfonate (0.5 g) and was gently stirred at 100 °C for 90 min. The precipitated crystals were quickly collected to give L-alanine *p*-chlorobenzenesulfonate (8.0 g), optical purity 97%, yield 16.7% (based on starting DL-salt). The mother liquor did not show any optical rotation. The results show that the preferential crystallization of L-alanine *p*-chlorobenzenesulfonate and the racemization of unseeded D-alanine *p*-chlorobenzenesulfonate took place at the same time, and that 7.3 g of L-alanine *p*-chlorobenzenesulfonate was transformed from DL-alanine *p*-chlorobenzenesulfonate existing as a supersaturation state.

Another approach was carried out by transformation between two diastereoisomeric salts. It is known that DL-phenylglycine can be chemically resolved by *d*-camphor-10-sulfonic acid as a resolving agent.[14] This resolution method was combined with the present racemization method. Namely, a mixture of DL-phenylglycine (15.1 g), *d*-camphor-10-sulfonic acid (22.1 g) and salicylaldehyde (0.24 g) was stirred in glacial acetic acid (70 ml) at 80 °C for 4 h. The precipitated crystals were collected to give D-phenylglycine *d*-camphor-10-sulfonate (26.1 g), optical purity 95.9%, yield 68.1% (based on DL-phenylglycine). The result shows that the selective precipitation of the less soluble D-phenylglycine *d*-camphor-10-sulfonate and the epimerization of the soluble L-phenylglycine *d*-camphor-10-sulfonate took place at the same time.

The proposed racemization method and asymmetric transformation of amino acids seem to be very promising for industrial applications. The asymmetric transformation is under investigation.

Experimental

Materials and Analyses. Analytical standard grade amino acids manufactured by our company, Tanabe Seiyaku Co., Ltd., were used. All aldehydes and other chemicals were obtained from Tokyo Kasei Kogyo Co., Ltd. The salts of L-amino acids were prepared as described in previous reports.[2-4] Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter. Decomposition of amino acids was checked by thin-layer chromatography (solvent system: 1-butanol–acetic acid–water, 4: 1: 1, vol/vol/vol; plate: Merck's precoated Kieselgel $60F_{254}$). An accurate quantitative determination of amino acids was made by a Hitachi KLA-3B amino acid analyzer (resin, Hitachi Custom Resin; eluting buffer, citrate buffer [pH 3.25]; column temperature, 55 °C).

Procedure of Racemization. Unless otherwise noted, the racemization was carried out as follows. A mixture of L-amino acid salt (0.1 g), 0.1 molar equivalent of aldehyde (3—8 μl), 0.1 molar equivalent of DL-amino acid (3—8 mg) corresponding to the L-amino acid moiety of the salt, and glacial acetic acid (3.0 ml) was heated in a sealed tube at 100 °C for 1 h and was shaken occasionally. The conditions were varied according to each experimental design. After the reaction, the reaction mixture was diluted with 1 M hydrochloric acid (5 ml). Optical rotation of the solution was measured. The racemization degree was calculated as follows:

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

The reaction mixtures were checked by thin-layer chromatography or amino acid analyzer in order to determine whether or not the simultaneous decomposition of amino acid had occurred.

Preparation of DL-Amino Acid Salts by Racemization. A mixture of L-amino acid salt (3.0 g), 0.1 molar equivalent of salicylaldehyde (0.11 to 0.22 g, 0.2 molar equivalent for L-*p*-

hydroxyphenylglycine *o*-toluenesulfonate), 0.1 molar equivalent of free DL-amino acid (0.10 to 0.59 g, 0.4 molar equivalent for L-*p*-hydroxyphenylglycine *o*-toluenesulfonate) corresponding to the L-amino acid moiety of the salt, and acetic acid (6 ml, 50 ml for L-*p*-hydroxyphenylglycine *o*-toluenesulfonate) was stirred at 100 °C for 2 h. After the reaction, 0.1 molar equivalent of sulfonic acid or hydrochloric acid corresponding to the acid moiety of the salt was added in the reaction mixture in order to recover the free DL-amino acid existing in the reaction mixture as the corresponding amino acid salt. Then the reaction mixture was concentrated to dryness. The residual crystals were suspended in acetone (5—15 ml). The precipitated crystals were collected, washed with acetone, and air-dried at 55 °C. The L-amino acid salts used were L-alanine benzenesulfonate, L-alanine *p*-toluenesulfonate, L-leucine benzenesulfonate, L-methionine *p*-chlorobenzenesulfonate, and L-*p*-hydroxyphenylglycine *o*-toluenesulfonate. The yields and the purities of the separated amino acid salts are shown in Table 5.

Asymmetric Transformation of DL-*Alanine.* DL-Alanine *p*-chlorobenzenesulfonate (45.0 g), DL-alanine (2.0 g), and salicylaldehyde (1.0 g) were dissolved in glacial acetic acid (50 ml) at 120 °C. The solution was maintained at 100 °C, was seeded with finely pulverized crystals of L-alanine *p*-chlorobenzenesulfonate (0.5 g), and was gently stirred. During the reaction, the crystal growth of seeded L-isomer was observed. After 90 min, the precipitated crystals were quickly collected by filtration, washed with a small amount of glacial acetic acid, and dried to give L-alanine *p*-chlorobenzenesulfonate (8.0 g), $[\alpha]_D^{25}$ +3.5° (c=2, water), optical purity 97%, yield 16.7% (based on starting DL-salt). After the separation of the first crop, the second crop was crystallized by adding water (15 ml) and by stirring at room temperature and was collected by filtration to give DL-alanine *p*-chlorobenzenesulfonate (12.2 g), $[\alpha]_D^{25}$ 0.0° (c=2, water). The mother liquor did not show any optical rotation. Therefore, we subtract 0.5 g of seeded L-isomer from the net weight of L-isomer obtained above, and find that 7.3 g of L-alanine *p*-chlorobenzenesulfonate was transformed from DL-alanine *p*-chlorobenzenesulfonate existing as a supersaturation state.

Asymmetric Transformation of DL-*Phenylglycine.* DL-Phenylglycine (15.1 g, 100 mmol), *d*-camphor-10-sulfonic acid (22.1 g, 95 mmol), and salicylaldehyde (0.24 g, 2 mmol) were added into glacial acetic acid (70 ml). The mixture was stirred at 80 °C for 4 h. The reaction system was heterogeneous during the reaction. To the reaction mixture, *d*-camphor-10-sulfonic acid (1.1 g, 5 mmol) was added and the mixture was stirred at 20 °C for 2 h. The precipitated crystals were collected by filtration and dried to give D-phenylglycine *d*-camphor-10-sulfonate (26.1 g), $[\alpha]_D^{25}$ −47.1° (c=2, N-HCl), optical purity 95.9% (based on D-*d* salt $[\alpha]_D^{25}$ −49.7° (c=2, N-HCl) and DL-*d* salt $[\alpha]_D^{25}$ +13.6° (c=2, N-HCl)), yield 68.1% (136.2% based on D-form contained in DL-phenylglycine *d*-camphor-10-sulfonate).

D-Phenylglycine *d*-camphor-10-sulfonate (25.0 g) obtained above was recrystallized from water (120 ml) containing *d*-camphor-10-sulfonic acid (0.5 g) to give optically pure D-phenylglycine *d*-camphor-10-sulfonate (18.5 g), $[\alpha]_D^{25}$ −49.7° (c=2, N-HCl). The pure salt (18.0 g) was dissolved in water (70 ml) at an elevated temperature. The solution was adjusted to pH 6 with 10 M sodium hydroxide and allowed to stand in a refrigerator overnight. The precipitated crystals were collected, washed with water, and dried to give D-phenylglycine (6.6 g), $[\alpha]_D^{25}$ −168.5° (c=1, 5N-HCl).

References

1) S. Yamada, C. Hongo, R. Yoshioka, and I. Chibata, *J. Org. Chem.*, **48**, 843 (1983).
2) S. Yamada, M. Yamamoto, and I. Chibata, *J. Org. Chem.*, **38**, 4408 (1973).
3) S. Yamada, M. Yamamoto, C. Hongo, and I. Chibata, *J. Agric. Food Chem.*, **23**, 653 (1975).
4) S. Yamada, C. Hongo, and I. Chibata, *Agric. Biol. Chem.*, **42**, 1521 (1978).
5) M. M. Harris, "Progress in Stereochemistry," ed by W. Klyne and P. B. D. Mare, Butterworths Scientific Publications, London (1958), Vol. 2, p. 157.
6) J. C. Clark, G. H. Phillipps, M. R. Steer, L. Stephenson, and A. R. Cooksey, *J. Chem. Soc., Perkin Trans. 1*, **1976**, 471.
7) J. C. Clark, G. H. Phillipps, and M. R. Steer, *J. Chem. Soc., Perkin Trans. 1*, **1976**, 475.
8) W. J. Boyle, Jr., S. Sifniades, and J. F. Van Peppen, *J. Org. Chem.*, **44**, 4841 (1979).
9) S. Shibata, H. Matsushita, K. Kato, M. Noguchi, M. Saburi, and S. Yoshikawa, *Bull. Chem. Soc. Jpn.*, **52**, 2938 (1979).
10) K. Suzuki, S. Kiyooka, T. Miyagawa, and A. Kawai, *Nippon Kagaku Kaishi*, **1980**, 287.
11) S. Yamada, C. Hongo, and I. Chibata, *Chem. Ind. (London)*, **1980**, 539.
12) C. Hongo, S. Yamada, and I. Chibata, *Bull. Chem. Soc. Jpn.*, **54**, 3286 (1981).
13) C. Hongo, S. Yamada, and I. Chibata, *Bull. Chem. Soc. Jpn.*, **54**, 3291 (1981).
14) A. W. Ingesoll, *J. Am. Chem. Soc.*, **47**, 1168 (1925).