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Racemic Structure and Optical Resolution by Preferential Crystallization of Organic Ammonium Salts of *N*-Formyl-DL-tyrosine

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The Gibbs energy of formation of racemate, binary melting point diagram, and ternary solubility diagram indicated that dicyclohexylammonium salt of *N*-formyl-DL-tyrosine (DL-DCH salt) forms a racemic compound at melting point, but is a conglomerate at room temperature. The optical resolution of the DL-DCH salt was successfully performed by preferential crystallization. A successive preferential crystallization in methanol at 10 °C followed by purification gave D- and L-tyrosines with 100% optical purity.

L-Tyrosine (abbreviated as L-Tyr) as an important substance in vivo has been obtained by hydrolysis of proteins, because no industrially efficient synthesis has been established.[1] Optical resolution by preferential crystallization of DL-Tyr, therefore, has not been reported,[1,2] though DL-Tyr can be optically resolved by the diastereomeric procedure.[3,4] If DL-Tyr is synthesized in an industrial scale, the optical resolution is needed to obtain optically active tyrosines. It was attempted in this study to resolve organic ammonium salts of *N*-formyl-DL-tyrosine (DL-FrTyr) by preferential crystallization.

In the 16 ammonium salts, the salts showing respective melting points are pentylammonium (PTA), 1,1,3,3-tetramethylbutylammonium (TMB), dibutylammonium (DBA), dicyclohexylammonium (DCH), dibenzylammonium (DBZ), and 4-methylpiperidinium (4-MP) salts. The racemic structure of these DL-salts was determined by thermodynamic analysis.[5-7] As for other salts that decompose on heating, the racemic structure was examined by comparing infrared spectra of the DL-salt with those of the corresponding L-salt or ternary solubility diagram; these are propylammonium, isopropylammonium, butylammonium, isobutylammonium, *t*-butylammonium, cyclohexylammonium, benzylammonium, diisopropylammonium, diisobutylammonium, and *N*-methylcyclohexylammonium salts. The DL-DCH salt which seemed to be a conglomerate at room temperature was subjected to optical resolution by preferential crystallization.

Experimental

Materials. DL- and L-Tyrosine were purchased from Sigma Chemicals Co., and amines from Wako Pure Chemicals Ind. or Kanto Chemical Co., Ltd.

***N*-Formylation.** *N*-Formyl-DL- and -L-tyrosine were prepared by formylating each tyrosine similarly to the preparation of ordinary amino acids;[8] specific rotation of L-FrTyr $[\alpha]_D^{20}$ +84.4° (*c* 0.50, ethanol) (lit,[9] $[\alpha]_D$ +84.8° (ethanol)).

Preparation of Organic Ammonium Salts. A mixture of 0.01 mol of DL- or L-FrTyr and equimolar amine in 80 cm^3 of acetone was stirred for 30 min at room temperature. The formed salt was filtered and recrystallized from methanol.

L-DCH salt: Found C, 67.57; H, 8.76; N, 7.11% (calcd for $C_{22}H_{34}N_2O_4$: C, 67.66; H, 8.78; N, 7.17%); $[\alpha]_D^{20}$ +35.1° (*c* 0.50, water), $[\alpha]_D^{20}$ +43.1° (*c* 0.50, ethanol); solubility 3.660 g/(100 cm^3 methanol), 0.504 g/(100 cm^3 ethanol) at 10 °C. DL-DCH salt: Found C, 67.40; H, 8.78; N, 7.13%; solubility 7.181 g/(100 cm^3 methanol), 1.090 g/(100 cm^3 ethanol) at 10 °C.

D-DCH salt obtained from mother liquor in the optical resolution of DL-salt was recrystallized twice from ethanol to give D-salt with 100% optical purity; mp 232 °C; $[\alpha]_D^{20}$ −35.1° (*c* 0.50, water).

Optical Resolution. Preferential Crystallization: DL-DCH salt was dissolved in methanol or ethanol at 40 °C; 2.154 g in 25 cm^3 of methanol, 1.370 g in 100 cm^3 of ethanol. The solution was slowly cooled to 10 °C and seeded with L-salt; 0.050 g for methanol solution, 0.100 g for ethanol solution. After stirring the mixture at 10 °C, the precipitated salt was filtered, washed with a small amount of diethyl ether, and dried. The optical purity, yield of optically pure modification (YOPM), and degree of resolution of the salt were determined by the equations described in our previous paper.[10]

Successive Preferential Crystallization: DL-DCH salt (2.154 g) was dissolved in 25 cm^3 of methanol at 40 °C. After being cooled to 10 °C, the solution was seeded with 0.050 g of L-salt and stirred for 130 min. The precipitated salt was collected by filtration, and DL-DCH salt (0.122 g) was dissolved in the filtrate at 40 °C. After being seeded with 0.050 g of D-salt at 10 °C, the mixture was treated similarly. The degree of resolution of D- and L-DCH salts was calculated by

$$\text{Degree of resolution}/\% = [\text{YOPM}/\text{g} \times 100]/[(\text{Operation amount of D- or L-salt}/\text{g}) - 0.898].$$

Preparation of Optically Active Tyrosine. D- or L-DCH salt (4.00 g) with optical purity of about 93% was dissolved in ethanol at an elevated temperature. After concentrating the solution to 100 cm^3 under reduced pressure at 40 °C, the resulting solution was allowed to stand for 2 d at 5 °C. The precipitated salt was collected by filtration to give about 3.5 g each of optically pure D- and L-salts.

D- or L-DCH salt (3.90 g) was dissolved in a mixture of 10 cm^3 of 1 mol dm^{-3} aqueous sodium hydroxide and 100 cm^3 of water. After removing the liberated dicyclohexylamine by extracting with diethyl ether, 10 cm^3 of 1 mol dm^{-3} hydrochloric acid was added to the aqueous solution. The solution was dried under reduced pressure at 50 °C. After being added 20 cm^3 of methanol to the residue, the mixture was filtered. To the filtrate was added 10 cm^3 of 1 mol dm^{-3} hydrochloric acid. After refluxing the solution for 2 h, the pH was adjusted to 6 with concentrated aqueous ammonia. Precipitated Tyr was filtered, washed with a small amount of cold water and methanol, and dried to give about 1.70 g each of D- and L-Tyr's. D-Tyr: $[\alpha]_D^{20}$ +11.8° (*c* 4.00, 5 mol dm^{-3} HCl).

Table 1. Thermodynamic Data for Organic Ammonium Salts of *N*-Formyltyrosine

Salt	Mp/K		ΔH^{f}[a]/kJ mol^{-1}		ΔG^{F}_{mp}[b]
	DL-Salt	L-Salt	DL-Salt	L-Salt	kJ mol^{-1}
PTA[c]	405	411	35.2	29.0	−1.91
TMB[d]	429	440	36.9	38.0	−1.52
DBA[e]	412	425	34.1	32.8	−1.37
DCH[f]	501	505	39.1	51.7	−2.48
DBZ[g]	437	442	45.4	46.7	−1.99
4-MP[h]	430	443	35.2	31.2	−1.56

a) ΔH^{f}: Enthalpy of fusion. b) ΔG^{F}_{mp}: Gibbs energy of formation of racemate at melting point. c) PTA: Pentylammonium salt. d) TMB: 1,1,3,3-Tetramethylbutylammonium salt. e) DBA: Dibutylammonium salt. f) DCH: Dicyclohexylammonium salt. g) DBZ: Dibenzylammonium salt. h) 4-MP: 4-Methylpiperidinium salt.

L-Tyr: $[\alpha]_D^{20}$ −11.8° (*c* 4.00, 5 mol dm^{-3} HCl) (lit,[11] $[\alpha]_D^{20}$ −11.8° (5 mol dm^{-3} HCl)).

Measurements. The specific rotation was measured by a Union Giken PM-101 digital polarimeter with a quartz cell of 0.5 dm path length. Infrared spectra were obtained in the range 4000—400 cm^{-1} by a JASCO A-102 infrared spectrophotometer by the KBr disk method.

Saturated methanol or ethanol solutions of DL- and L-DCH salts were prepared at 10 °C and diluted in appropriate concentrations when used. The absorbance was measured at 279 nm with a Shimadzu double-beam spectrophotometer UV-150-02 with a quartz cell of 0.1 dm path length. The solubility of the salts was determined from calibration curves.

Results and Discussion

Racemic Structure. Racemic Structure at Melting Point: The Gibbs energy of formation of racemate in the solid state at *T* K (ΔG^{F}_{T}) is calculated by the following equation:[5]

$$\Delta G^{F}_{T} = -\Delta\Delta H^{f} + T\Delta\Delta S^{f} - RT\ln 2 + \Delta C_p(\Delta T^{f} - T\ln T^{f}_{R}/T^{f}_{A}) + (\Delta C^{s}/2T^{f})(T^{f} - T)^2, \quad (1)$$

where subscripts "A" and "R" represent the optically active and racemic modifications, respectively, *R* is the gas constant, ΔC_P is the difference in heat capacity between the liquid and solid states in the vicinity of melting point, and ΔC^{s} is that in solid state between racemic and optically active modifications; $\Delta\Delta H^{f} = \Delta H^{f}_{R} - \Delta H^{f}_{A}$ (ΔH^{f}, enthalpy of fusion), $\Delta\Delta S^{f} = \Delta S^{f}_{R} - \Delta S^{f}_{A}$ (ΔS^{f}, entropy of fusion), and $\Delta T^{f} = T^{f}_{R} - T^{f}_{A}$ (T^{f}, melting point); T^{f} represents T^{f}_{A} when $T^{f}_{R} > T^{f}_{A}$ and T^{f}_{R} when $T^{f}_{R} < T^{f}_{A}$. The term containing ΔC_p is ignored for the calculation of ΔG^{F}_{mp} at melting point, because the contribution is obviously smaller than that of the sum of the other terms in the right-hand side of Eq. 1.[5] The ΔG^{F}_{mp} of the salts of DL-FrTyr having melting points were calculated by Eq. 1 using ΔH^{f} and T^{f}.[5-7] Thermodynamic data of *N*-formyltyrosine salts are listed in Table 1. The values of ΔG^{F}_{mp} are negative, and further, these salts have eutectic points; the mole fractions of L-salt at eutectic point are 0.70—0.77 as listed in Table 2, and the binary melting point diagram of DCH salt is illustrated in Fig. 1. The above results indicate that these DL-salts form racemic compounds at melting point.

Table 2. Data at Eutectic Point for Organic Ammonium Salts of *N*-Formyltyrosine

Salt[a]	Mole fraction of L-salt[b]	Temperature/K	
		Found	Calcd[b]
PTA	0.77	398	399
TMB	0.70	424	425
DBA	0.70	411	410
DCH	0.75	494	493
DBZ	0.75	433	432
4-MP	0.71	425	426

a) See notes in Table 1. b) These values were calculated from Schröder-Van Laar and Prigogine-Defay equations.

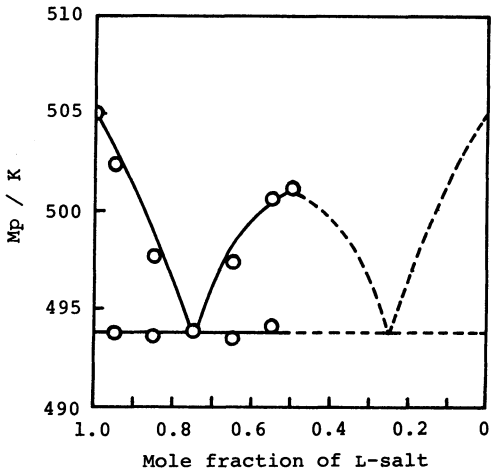

Fig. 1. Binary melting point diagram of dicyclohexylammonium salt of *N*-formyltyrosine.

Racemic Structure at Room Temperature: The ΔG^{F}_{283} at 283 K was also calculated by Eq. 1; in the calculation, ΔC_p was taken as 105 J mol^{-1} K^{-1} and ΔC^{s} as 31.4 J mol^{-1} K^{-1}.[5-7] Only the DL-DCH salt gives a positive ΔG^{F}_{283} value (+5.39 kJ mol^{-1}). This value suggests that the DL-salt does not form a racemic compound at 283 K. DL-DCH salt shows infrared spectrum identical with that of the L-salt and is more soluble than the L-salt as described in the experimental section.

Table 3. Preferential Crystallization of Dicyclohexylammonium Salts of *N*-Formyl-DL-tyrosine[a]

Solvent	Resolution time	Yield	Optical purity	YOPM[b]	Degree of resolution
	min	g	%	g	%
Methanol[c]	60	0.111	97.1	0.058	32.3
	90	0.141	95.7	0.085	47.3
	120	0.154	93.4	0.094	52.4
	175	0.185	87.9	0.113	63.0
	240	0.200	86.4	0.123	68.5
Ethanol[d]	45	0.141	96.0	0.035	25.0
	90	0.168	95.7	0.061	43.6
	120	0.179	97.7	0.075	53.6
	210	0.183	95.3	0.075	53.6
	300	0.188	91.7	0.072	51.4

a) Temperature: 10°C. b) YOPM: Yield of optically pure modification. c) Methanol: 25 cm³. Degree of supersaturation: 120%. Seed Crystals: 0.050 g of L-salt. d) Ethanol: 100 cm³. Degree of supersaturation: 126%. Seed crystals: 0.100 g of L-salt.

Table 4. Successive Preferential Crystallization of Dicyclohexylammonium Salt of *N*-Formyl-DL-tyrosine[a]

Run	Added amount of DL-salt	Operation amount[b]/g		Resolution time	Salt obtained			
					Yield	Optical purity	YOPM	Degree of resolution
	g	D-Salt	L-Salt	min	g	%	g	%
1	2.154	1.077	1.077	130	0.172	L 92.2	0.109	60.9
2	0.122	1.131	1.022	60	0.219	D 94.0	0.156	67.0
3	0.169	1.053	1.100	60	0.195	L 93.7	0.133	65.8
4	0.145	1.120	1.034	100	0.202	D 94.6	0.141	63.5

a) Solvent: 25 cm³ of methanol. Degree of supersaturation of initial solution: 120%. Seed crystals: 0.050 g of D- or L-salt. Temperature: 10°C. b) Operation amounts of D- and L-salts in solution were calculated from analyses of the salts obtained in Runs 1—3.

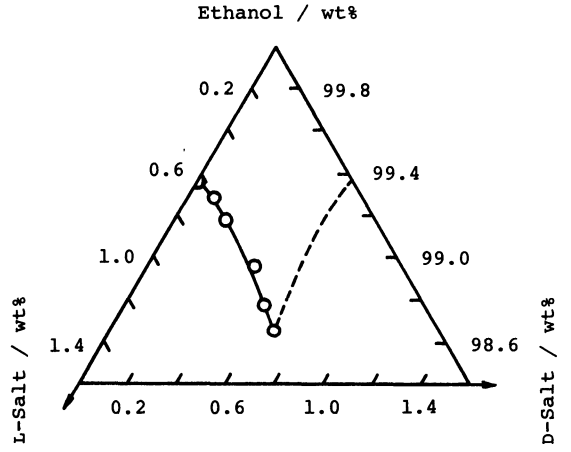

Fig. 2. Ternary solubility diagram of dicyclohexylammonium salt of *N*-formyltyrosine. Solvent: Ethanol. Temperature: 10°C.

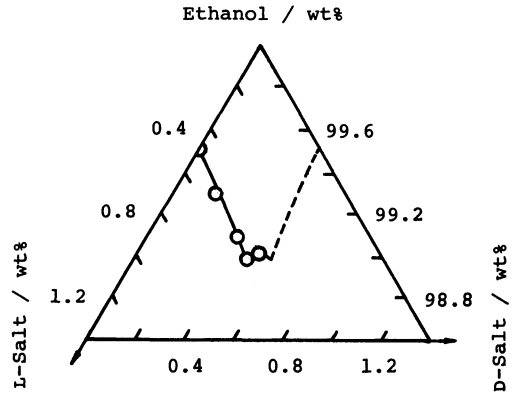

Fig. 3. Ternary solubility diagram of *t*-butylammonium salt of *N*-formyltyrosine. Solvent: Ethanol. Temperature: 10°C.

The ternary solubility diagram shows what is expected for a conglomerate as shown in Fig. 2.[2)]

For other DL-salts, the values of ΔG^{F}_{283} are negative, and the infrared spectra are different from those of the corresponding L-salts. These results indicate that DL-DCH salt exists in a conglomerate at room temperature, whereas other DL-salts form racemic compounds.

In the salts that decompose on heating, DL-*t*-butylammonium salt (DL-TBA salt) shows infrared spectrum excellently identical with that of L-salt, whereas the spectra of other DL-salts are different from those of the corresponding L-salts. DL-TBA salt is more soluble than the L-salt; DL-TBA salt 0.764 g/(100 cm³ ethanol), L-TBA salt 0.423 g/(100 cm³ ethanol) at 10°C. The ternary solubility diagram, however, suggests that DL-TBA salt is an unstable racemic com-

pound at room temperature as shown in Fig. 3,[2] because the mole fraction of L-salt at eutectic point is 0.55. This instability of DL-TBA salt may give infrared spectrum excellently identical with that of L-salt.

The above results lead to the conclusion that only the DL-DCH salt exists in a conglomerate at room temperature though the salt forms a racemic compound at melting point.

Optical Resolution of DL-Dicyclohexylammonium Salt. Preferential crystallization of DL-DCH salt is listed in Table 3; L-DCH salt was used as seed crystals.

It was possible to obtain L-DCH salt with optical purity of over 90%. Since the yield in methanol was nearly equal to that in ethanol, it is expected that the optical resolution from methanol solution in the same scale as that from ethanol solution gives L-DCH salt in about 4 times higher yield from ethanol. The optical resolution by a successive preferential crystallization for methanol solution was found to give D- and L-DCH salts with optical purity of 92—95% in degree of resolution of 60—67% as shown in Table 4. Optically pure D- and L-Tyr's were obtained from purified D- and L-DCH salts, respectively.

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