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# Racemic Structures and Optical Resolutions by Preferential Crystallization of Organic Ammonium Salts of *N*-Formyl-DL-phenylalanine

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Racemic structures of the butylammonium salt, pentylammonium salt, benzylammonium salt, dicyclohexylammonium salt (DL-DCH salt), and 4-methylpiperidinium salt (DL-4-MP salt) of *N*-formyl-DL-phenylalanine were studied to explore the possibility of optical resolution by preferential crystallization. Free energies of formation of racemate ($\Delta G^F$) of the DL-salts and data at eutectic points in binary phase diagrams of melting points indicate that the DL-salts form racemic compounds in the vicinity of the melting points. However, according to $\Delta G^F$ of DL-DCH and 4-MP salts and infrared spectra and solubilities of the DL- and L-salts, these DL-salts transform into conglomerates around room temperature. It is possible to resolve DL-DCH and 4-MP salts by preferential crystallization at 10 °C. Successive preferential crystallization of DL-4-MP salt at 10 °C in 1-butanol gives the D- and L-salts with optical purities of 95—100%.

DL-Phenylalanine (abbreviated as DL-Phe) has been optically resolved by emzymatic, diastereomeric, and preferential crystallization procedures. Of these procedures, the preferential crystallization procedure is useful for industrial operation, since the optical resolution can easily be achieved by providing a small amount of one enantiomer as seed crystals in a supersaturated solution of racemic modifications. Optical resolutions by this procedure have been reported on the ammonium salt,[1] ethylammonium salt,[2] isopropylammonium salt,[3] butylammonium salt,[3] 1,1,3,3-tetramethylbutylammonium salt,[2] and cyclohexylammonium salt[4] of *N*-acetyl-DL-phenylalanine. It is expected that the hydrolysis of *N*-formylphenylalanine (FrPhe) is easier than that of *N*-acetylphenylalanine. However, no optical resolution of DL-FrPhe by preferential crystallization has been reported, though DL-FrPhe has been optically resolved through its diastereomeric salts with (−)-fenchylamine.[5] This paper describes an attempt to resolve organic ammonium salts of DL-FrPhe by preferential crystallization.

The racemic structures of the butylammonium salt (DL-BA salt), pentylammonium salt (DL-PTA salt), benzylammonium salt (DL-BZA salt), dicyclohexylammonium salt (DL-DCH salt), and 4-methylpiperidinium salt (DL-4-MP salt) of DL-FrPhe were determined on the basis of free energies of racemates ($\Delta G^F$)[2,6,7] and data at eutectic points in binary phase diagrams of melting points. The results indicated that the DL-salts form racemic compounds in the vicinity of the melting points. However, the $\Delta G^F$ values of DL-DCH and 4-MP salts suggested that the DL-salts are transformed into conglomerates at lower temperatures. This was supported by a comparison of solubilities and infrared spectra of the DL-salts with those of the corresponding L-salts. Optical resolutions of DL-DCH and 4-MP salts were attempted by preferential crystallization at 10 °C.

## Experimental

**Materials.** DL-Phe and L-Phe were purchased from Sigma Chemicals Co., and amines from Wako Pure Chemicals Ind.

***N*-Formylation.** DL- and L-FrPhe were obtained, respectively, by formylating DL- and L-Phe in the usual way.[8] DL-FrPhe: Mp 168 °C (lit,[5] 165—166 °C). L-FrPhe: Mp 168 °C (lit,[5] 167.0 °C); $[\alpha]_D^{20}$ +75.1° (*c* 1.00, ethanol) (specific rotation of D-FrPhe[5]: $[\alpha]_D^{20}$ −75.2° (ethanol)).

**Preparation of Organic Ammonium Salts.** DL- or L-FrPhe (0.01 mol) and 0.01 mol of an amine were dissolved in 30 cm$^3$ of methanol. To the solution was added 30 cm$^3$ of diethyl ether and the salt formed was collected by filtration.

Elemental analysis and specific rotation of L-DCH salt: C, 70.32; H, 9.15; N, 7.44%; $[\alpha]_D^{20}$ +36.1° (*c* 1.00, methanol). DL-DCH salt: C, 70.35; H, 9.15; N, 7.44% (calcd for $C_{22}H_{34}N_2O_3$: C, 70.55; H, 9.15; N, 7.48%). L-4-MP salt: C, 65.55; H, 8.27; N, 9.59%; $[\alpha]_D^{20}$ +45.7° (*c* 1.00, methanol). DL-4-MP salt: C, 65.46; H, 8.27; N, 9.60% (Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58%).

The D-4-MP salt (3.00 g) obtained from the mother liquor in the optical resolution of the DL-salt was dissolved in 15 cm$^3$ of 1-butanol at an elevated temperature. After standing overnight around 5 °C, the salt crystallized was collected by filtration, washed with a small amount of diethyl ether, and dried; yield, 2.57 g. The recrystallization was repeated twice: Mp 167 °C; $[\alpha]_D^{20}$ −45.7° (*c* 1.00, methanol).

**Optical Resolution. DL-Dicyclohexylammonium Salt:** DL-DCH salt (1.221 g) was dissolved in 100 cm$^3$ of ethanol around 40 °C to give a racemic solution with a degree of supersaturation of 161% at 10 °C. The solution was slowly cooled to 10 °C and seeded with 0.020 g of L-DCH salt. After stirring for 60—180 min at 10 °C, the salt precipitated was collected by filtration and washed with a small amount of diethyl ether. The optical purity, the yield of the optically pure modification (YOPM), and the degree of resolution of the salt obtained were determined by the equations in our previous paper.[9]

**DL-4-Methylpiperidinium Salt:** DL-4-MP salt (2.631 g) was dissolved in 50 cm$^3$ of 1-butanol around 40 °C to give a racemic solution with a degree of supersaturation of 160% at 10 °C. The optical resolution of DL-4-MP salt was carried out with this solution similarly to that of DL-DCH salt.

**Successive Preferential Crystallization:** DL-4-MP salt (10.560 g) was dissolved in 200 cm$^3$ of 1-butanol around 40 °C. The solution was slowly cooled to 10 °C and seeded with 0.050 g of L-4-MP salt. After stirring for 70 min at 10 °C, the salt precipitated was collected by filtration, and washed with a small amount of diethyl ether. DL-4-MP salt (1.268 g) was added to the filtrate, and dissolved around 40 °C. The solution was cooled to 10 °C and seeded with 0.050 g of D-4-MP salt. After stirring for 80 min, the salt precipitated was collected by filtration. The filtrate was treated similarly to above.

**Preparation of Optically Active Phenylalanine:** The D- or L-4-MP salt obtained by successive preferential crystallization (4.83 g) was added in 25 cm$^3$ of 1 mol dm$^{-3}$ hydrochloric acid without recrystallization, and the mixture was stirred for 40 min at 5 °C. The FrPhe was collected by filtration, washed with a small amount of water, and dried: yield of each of D- and L-FrPhe 3.09 g; for D-FrPhe $[\alpha]_{\rm D}^{20}$ −75.2° (*c* 1.00, ethanol); for L-FrPhe $[\alpha]_{\rm D}^{20}$ +74.5° (*c* 1.00, ethanol). The D- or L-FrPhe (2.50 g) was added in 20 cm$^3$ of 1 mol dm$^{-3}$ hydrochloric acid. After refluxing for 2 h, the solution was evaporated to dryness under reduced pressure around 50 °C. The residue was dissolved in 70 cm$^3$ of methanol, and the pH was adjusted to around 6 with ammonium hydroxide. The Phe crystallized was collected by filtration, washed with small amounts of water and methanol, and dried. D-Phe: yield 1.36 g; $[\alpha]_{\rm D}^{20}$ +34.4° (*c*. 1.00, water). L-Phe: Yield 1.32 g; $[\alpha]_{\rm D}^{20}$ −33.5° (*c* 1.00, water) (lit,[10] $[\alpha]_{\rm D}$ −34.5° (water)).

**Measurements.** Specific rotations were measured with a Union Giken high sensitivity PM-101 digital polarimeter using a 0.5 dm path length quartz cell. Infrared spectra were obtained in the range 4000—400 cm$^{-1}$ with a JASCO A-102 infrared spectrophotometer by the KBr disk method. Solubilities of DL- and L-DCH salts in ethanol and those of DL- and L-4-MP salts in 1-butanol were measured at 10 °C in a similar manner as described in our previous paper.[2] These solubilities are given in Table 1. Enthalpies of fusion and melting points were determined by use of a differential scanning calorimeter (Rigaku Denki DSC-8230). Benzoic acid, potassium nitrate, and sodium nitrate were used as standard samples to obtain enthalpies of fusion.

Table 1. Solubilities of Dicyclohexylammonium and 4-Methylpiperidinium Salts of *N*-Formylphenylalanine

| Salt | Solvent | Solubility at 10 °C g/(100 cm$^3$ solvent) | |
|---|---|---|---|
| DCH[a] | Ethanol | DL : | 0.758 |
| | | L : | 0.561 |
| 4-MP[b] | 1-Butanol | DL : | 3.289 |
| | | L : | 1.628 |

a) DCH: Dicyclohexylammonium salt. b) 4-MP: 4-Methylpiperidinium salt.

## Results and Discussion

**Recemic Structures at Melting Piont.** Racemic structures of the organic ammonium salts of DL-FrPhe were examined, since the optical resolution by preferential crystallization requires that the racemic modification is a conglomerate. The free energies of formation of racemate at melting point ($\Delta G^{\rm F}_{\rm mp}$) of the DL-salts were calculated on the basis of the enthalpies of fusion ($\Delta H^{\rm f}$) and melting points of the DL- or L-salts.[2,6,7] These thermodynamic data are given in Table 2.

Since the $\Delta G^{\rm F}_{\rm mp}$ values of these DL-salts are negative (−2.8—−0.5 kJ mol$^{-1}$), it was suggested that the DL-salts would be racemic compounds or racemic solid solutions in the vicinity of the melting points and not conglomerates.[2,6]

If the DL-salts form racemic compounds, the eutectic points must be found in the mole fraction range of 0.5 to 1 in the binary phase diagrams of melting points and it is possible to calculate the mole fractions of the L-salts and temperatures for the eutectic points from the Schröder-Van Laar and Prigogine-Defay equations on the basis of the $\Delta H^{\rm f}$ and melting points of the DL- and L-salts.[11] The mixtures with the mole fractions of the L-salts calculated (0.58—0.85) were prepared, and the melting points were compared with the eutectic temperatures calculated. These results are given in Table 3.

The melting points obtained agreed very closely with the eutectic temperatures calculated, and the mixtures melted at the temperatures and not over a wide range. Although the mole fractions of L-DCH and 4-MP salts at the eutectic points (about 0.5) are relatively close to 0.5 and the melting points are equal to those of their respective DL-salts, the binary phase diagrams of melting points (Figs. 1 and 2) show that the eutectic mixtures consist of the D- and L-salts in the molar ratio of about 4:6. The above results indicate that BA, PTA, BZA, DCH, and 4-MP salts have the eutectic mixtures consisting of the D-

Table 2. Thermodynamic Data for Organic Ammonium Salts of *N*-Formylphenylalanine

| Salt | Mp/K | | $\Delta H^{\rm f\,a)}$/kJ mol$^{-1}$ | | $\Delta G^{\rm F}_{\rm mp}$ [b] |
|---|---|---|---|---|---|
| | DL-Salt | L-Salt | DL-Salt | L-Salt | kJ mol$^{-1}$ |
| BA[c] | 417 | 412 | 32.9 | 30.4 | −2.73 |
| PTA[d] | 398 | 392 | 36.9 | 32.8 | −2.78 |
| BZA[e] | 415 | 428 | 39.0 | 31.4 | −1.43 |
| DCH | 483 | 499 | 63.2 | 61.1 | −0.82 |
| 4-MP | 420 | 440 | 39.5 | 41.3 | −0.59 |

a) $\Delta H^{\rm f}$: Enthalpy of fusion. b) $\Delta G^{\rm F}_{\rm mp}$: Free energy of formation of recemate at melting point. c) BA: Butylammonium salt. d) PTA: Pentylammonium salt. e) BZA: Benzylammonium salt.

and L-salts in the molar ratios calculated, which leads to a clear conclusion that these DL-salts form racemic compounds in the vicinity of the melting points.

However, the mole fractions of L-DCH and 4-MP salts at the eutectic points and the absolute values of $\Delta G^{\mathrm{F}}_{\mathrm{mp}}$ of the DL-salts are smaller than those of the other salts. Therefore, it is suggested that the DL-DCH and 4-MP salts are unstable racemic compounds in the vicinity of the melting points.[2,7] Such an unstable racemic compound has a possibility of transformation to a conglomerate at a lower temperature,[2,7] though stable ones in the vicinity of the melting points exist in racemic compounds even around room temperature.

Table 3. Mole Fractions and Temperatures at Eutectic Points for Organic Ammonium Salts of *N*-Formylphenylalanine

| Salt | Mole fraction of L-salt[a] | Temperature/K | |
|---|---|---|---|
| | | Found | Calcd[a] |
| BA | 0.84 | 403 | 404 |
| PTA | 0.85 | 387 | 386 |
| BZA | 0.70 | 412 | 412 |
| DCH | 0.60 | 483 | 483 |
| 4-MP | 0.58 | 420 | 420 |

a) These values were calculated from the Schröder–Van Laar and Prigogine–Defay equations.

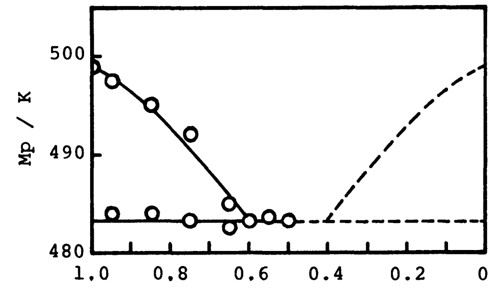

Fig. 1. Binary phase diagram of melting point of dicyclohexylammonium salt of *N*-formylphenylalanine.

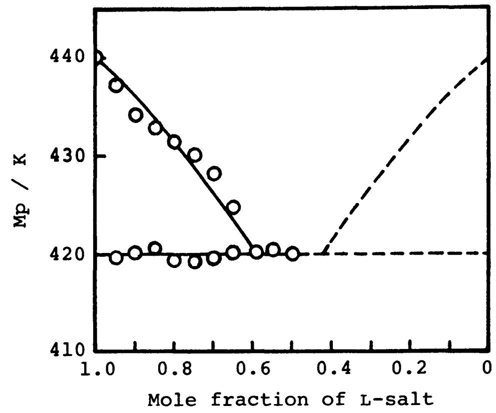

Fig. 2. Binary phase diagram of melting point of 4-methylpiperidinium salt of *N*-formylphenylalanine.

**Racemic Structures Around Room Temperature.** The above results suggest that DL-DCH and 4-MP salts have a possibility of existing in conglomerate around room temperature. Therefore, the transition temperatures ($T_0$) of DL-DCH and 4-MP salts were estimated according to previous papers;[2,7] the $\Delta G^{\mathrm{F}}$ is zero at $T_0$. The $T_0$ obtained was 314 K for DL-DCH salt and 324 K for DL-4-MP salt. The $T_0$ values indicate that DL-DCH and 4-MP salts do not form racemic compounds around room temperature, since the $\Delta G^{\mathrm{F}}$ values are positive at temperatures below $T_0$. The results are supported by the infrared spectra and solubilities of these DL- and L-salts; DL-DCH and 4-MP salts show infrared spectra identical with those of the corresponding L-salts, and are more soluble than the L-salts as shown in Table 1. The above results indicate that DL-DCH and 4-MP salts exist in conglomerate around room temperature, though the DL-salts form racemic compounds in the vicinity of the melting points.

Since the $T_0$ values estimated for DL-BA, PTA, and BZA salts are lower than 0 K and the infrared spectra of these DL-salts are different form those of the L-salts, it may be concluded that these DL-salts form also racemic compounds around room temperature.

**Optical Resolution by Preferential Crystallization.** Optical resolutions of DL-DCH and 4-MP salts have been attempted at 10 °C for racemic solutions with a degree of supersaturation of about 160%. The results are given in Tables 4 and 5.

It was possible to obtain the L-salts with high optical purity (93—97%) by preferential crystallization. Although the L-DCH salt obtained at resolution times of 60 and 90 min had an optical purity of over 90%, the degrees of resolution were low (22—29%). On the other hand, in the optical resolution of DL-4-MP salt, L-salt with an optical purity of 96.8% was obtained in a relatively high

Table 4. Optical Resolution of Dicyclohexylammonium Salt of *N*-Formyl-DL-phenylalanine[a]

| Resolution time / min | Yield / g | Optical purity / % | YOPM[b] / g | Degree of resolution / % |
|---|---|---|---|---|
| 60 | 0.076 | 96.1 | 0.053 | 22.9 |
| 90 | 0.095 | 93.0 | 0.068 | 29.4 |
| 120 | 0.186 | 74.5 | 0.119 | 51.5 |
| 150 | 0.234 | 60.4 | 0.121 | 52.4 |
| 180 | 0.274 | 40.8 | 0.092 | 39.8 |

a) Solvent: 100 cm$^3$ of ethanol. Degree of supersaturation of racemic solution: 161%. Seed crystals: 0.020 g of L-salt. Temperature: 10 °C. b) YOPM: Yield of optically pure modification.

Table 5. Optical Resolution of 4-Methylpiperidinium Salt of *N*-Formyl-DL-phenylalanine[a)]

| Resolution time / min | Yield / g | Optical purity / % | YOPM[b)] / g | Degree of resolution / % |
|---|---|---|---|---|
| 30 | 0.280 | 95.1 | 0.246 | 49.9 |
| 60 | 0.332 | 96.8 | 0.301 | 61.0 |
| 90 | 0.339 | 87.2 | 0.276 | 56.0 |
| 120 | 0.551 | 25.8 | 0.122 | 24.7 |
| 180 | 0.602 | 15.8 | 0.075 | 15.2 |

a) Solvent: 50 cm$^3$ of 1-butanol. Degree of supersaturation of racemic solution: 160%. Seed crystals: 0.020 g of L-salt. Temperature: 10 °C. b) See note in Table 4.

Table 6. Successive Preferential Crystallization of 4-Methylpiperidinium Salt of *N*-Formyl-DL-phenylalanine[a)]

| Run | Added amount of DL-salt / g | Resolution time / min | Salt obtained: Configuration | Salt obtained: Yield / g | Salt obtained: Optical purity / % |
|---|---|---|---|---|---|
| 1 | 10.560 | 70 | L | 1.226 | 97.1 |
| 2 | 1.268 | 80 | D | 1.877 | 99.4 |
| 3 | 1.901 | 80 | L | 1.496 | 95.4 |
| 4 | 1.500 | 120 | D | 1.143 | 99.8 |

a) Solvent: 200 cm$^3$ of 1-butanol. Seed crystals: 0.050 g of D- or L-salt. Temperature: 10 °C.

degree of resolution (61.0%) at time 60 min. The L-4-MP salt partially resolved is easily purified to the L-salt with high optical purity by recrystallization. For example, the L-salt with an optical purity of 84.8% ($[\alpha]_D^{20}$ +38.8° (*c* 1.00, methanol)) was recrystallized once from 1-butanol to give L-salt with purity of 100% ($[\alpha]_D^{20}$ +45.7° (*c* 1.00, methanol)) similarly to the D-4-MP salt described in the experimental section. The L-salt obtained was treated with hydrochloric acid in the usual way to give L-Phe with an optical purity of 100% ($[\alpha]_D^{20}$ −34.5° (*c* 1.00, water)). These results indicate that the optical resolution of DL-4-MP salt is more favorable for obtaining the optically active Phe than that of DL-DCH salt. The optical resolution by successive preferential crystallization of DL-4-MP salt was found to give D- and L-Phe with high optical purity. The result is given in Table 6.

The D-4-MP salts obtained had optical purities of about 100%, and the L-salts those of over 95%. These D- and L-salts gave D-Phe with an optical purity of 99.8% and L-Phe with 97.0%, respectively.

It was found from the above results that DL-DCH and 4-MP salts are conglomerates around room temperature though the DL-salts form racemic compounds in the vicinity of the melting points, and that the optical resolution by successive preferential crystallization of DL-4-MP salt gives D- and L-Phe with optical purities of obout 100%.

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