February, 1987] © *1987 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, ***60***, *661—666 (1987)* 661

Optical Resolution by Preferential Crystallization of 1,1,3,3-Tetramethylbutylammonium Salt of *N*-Formyl-DL-*α*-phenylglycine

Tadashi SHIRAIWA,* Hideya MIYAZAKI, Tomoki IMAI, Michio SUNAMI, and Hidemoto KUROKAWA
Faculty of Engineering, Kansai University, Yamate-cho, Suita, Osaka 564

(Received July 29, 1986)

Infrared spectrum, solubility, and ternary phase diagram of solubility indicated that 1,1,3,3-tetramethylbutylammonium salt (DL-TMB salt) of *N*-formyl-DL-*α*-phenylglycine is a conglomerate. In order to resolve DL-TMB salt efficiently, crystallization of D- and L-TMB salts was examined by free energy of critical nucleation in supersaturated solutions. Successive preferential crystallization of DL-TMB salt was experimented at 10 °C for racemic solution in ethanol with the degree of supersaturation of 130%, and D- and L-*α*-phenylglycine with 100% optical purity were obtained from pure D- and L-TMB salts.

D-*α*-Phenylglycine (abbreviated as D-Phg) as a useful material of ampicillin has been chemically synthesyzed,[1] because Phg is not found in natural amino acids. Synthetic DL-Phg is then subjected to optical resolution by preferential crystallization[2–4] and diastereomeric procedures[5–7] to obtain D-Phg. For the optical resolution of DL-amino acids, their *N*-acyl derivatives have been purposely used. In the case of *N*-acetyl- or *N*-benzoyl-DL-*α*-phenylglycine, the Phg obtained is partially racemized during hydrolysis of the derivatives,[8] because Phg is apt to racemize on heating in an acid solution. It is expected that *N*-formyl-*α*-phenylglycine (FrPhg) is easier to be hydrolyzed under mild conditions than *N*-acetyl and *N*-benzoyl derivatives. It was attempted, therefore, to resolve organic ammonium salts of DL-FrPhg.

The organic ammonium salts of DL-FrPhg examined are propylammonium, isopropylammonium, butylammonium, *t*-butylammonium, 1,1,3,3-tetramethylbutylammonium (DL-TMB salt), cyclohexylammonium, benzylammonium, and dibenzylammonium salts. The racemic structure of organic ammonium salts of *N*-acyl-DL-amino acids has been determined from calculated values of free energy of formation by enthalpy of fusion and melting point described in our previous papers.[3,4,9] Since the salts of DL- and D-FrPhg decompose on heating, the racemic structure of these compounds was determined by comparing infrared spectra and solubility values of the DL-salts with those of the corresponding D-salts. The TMB salt of DL-FrPhg which seems to be in a conglomerate was subjected to resolution by a preferential crystallization. Conditions of hydrolysis of the D- and L-TMB salts resolved was examined to obtain D- and L-Phg without racemization. The optical resolution by preferential crystallization needs that unseeded enantiomer remains in the solution as a supersaturation state during crystallization. Although Hongo et al. reported on the stability of solution of DL-serine *m*-xylene-4-sulfonate dihydrate,[10] such problem has not been fully studied. Therefore, crystallization of D- and L-TMB salts was examined by free energy of critical nucleation to find appropriate conditions for optical resolution of DL-TMB salt.

Table 1. Preparation of Supersaturated Solutions of 1,1,3,3-Tetramethylbutylammonium Salt of *N*-Formyl-DL-*α*-phenylglycine[a]

Solvent	Degree of supersaturation[b]/%	Amount/g
Methanol	110	12.855
Methanol	120	14.024
Methanol	130	15.192
Ethanol	110	3.341
Ethanol	120	3.645
Ethanol	130	3.949

a) Solvent: 50 cm³. b) Temperature: 10 °C.

Experimental

Materials. DL- and D-Phg were purchased from Sigma Chemicals Co., and amines from Wako Pure Chemicals Ind.

***N*-Formylation.** DL- and D-FrPhg were prepared by formylating DL- and D-Phg, respectively, as usual as the preparation of ordinary amino acids.[11] DL-FrPhg: Mp 175 °C. D-FrPhg: Mp 178 °C; $[\alpha]_D^{20}$ −252° (*c* 1.00, methanol).

Preparation of Organic Ammonium Salts. A solution of 0.01 mol of DL- or D-FrPhg and equimolar amine in 150 cm³ of acetone was allowed to stand overnight at 5 °C. The salt formed was filtered and recrystallized from ethanol.

D-TMB salt: C, 66.02; H, 9.08; N, 9.08% (Calcd for $C_{17}H_{28}N_2O_3$: C, 66.20; H, 9.15; N, 9.08%); mp 176—179 °C (decomp); $[\alpha]_D^{20}$ −124° (*c* 1.00, water) and $[\alpha]_D^{20}$ −109° (*c* 1.00, ethanol); solubility 14.964 g/(100 cm³ methanol), 3.571 g/(100 cm³ ethanol) at 10 °C. DL-TMB salt: C, 66.21; H, 9.07; N, 9.12%; mp 157—160 °C (decomp); solubility 23.373 g/(100 cm³ methanol), 6.075 g/(100 cm³ ethanol) at 10 °C.

L-TMB salt obtained from mother liquor in the optical resolution of DL-salt was recrystallized twice from ethanol to give L-salt with 100% optical purity; mp 177—179 °C (decomp); $[\alpha]_D^{20}$ +124° (*c* 1.00, water).

Optical Resolution. DL-TMB salt was dissolved in 50 cm³ of ethanol or methanol at approximately 40 °C to give racemic solutions with the degree of supersaturation of 110, 120, and 130% at 10 °C; results of preparation of these supersaturated solutions were summarized in Table 1. The solution was slowly cooled to 10 °C and seeded with 0.050 g of D-TMB salt. After stirring the solution at 30 rpm and 10 °C, the precipitated salt was collected by filtration, 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 a previous paper.[3]

Successive Preferential Crystallization. DL-TMB salt (3.949 g) was dissolved in 50 cm^3 of ethanol at 40 °C. After being cooled to 10 °C, the solution was seeded with 0.050 g of D-TMB salt and stirred for 15 min. The precipitated salt was collected by filtration, washed with a small amount of diethyl ether, and dried. DL-TMB salt (0.319 g) was dissolved in the filtrate at 40 °C. After being seeded with 0.050 g of the L-TMB salt at 10 °C, the mixture was treated similarly to the case of D-TMB salt. The degree of resolution of D- and L-TMB salts was calculated by

$$\text{Degree of resolution}/\% = [\text{YOPM}/\text{g}\times 100]/[(\text{Operation amount of D- or L-salt}/\text{g}) - 1.519/\text{g}]. \quad (1)$$

Preparation of Optically Active α-Phenylglycine. D- or L-TMB salt (8.00 g) with optical purity of approximately 95% was dissolved in 30 cm^3 of ethanol at an elevated temperature. The solution was allowed to stand overnight at 5 °C. The precipitated salt was collected by filtration to give the D- and L-TMB salts with 100% optical purity in 90% yield.

D- or L-TMB salt (3.08 g) was dissolved in a mixture of 8 cm^3 of 5 mol dm^{-3} hydrochloric acid, 22 cm^3 of water, and 20 cm^3 of ethanol. After being stirred for 2 h at 50 °C, the solution was allowed to stand overnight at room temperature, and dried under reduced pressure at 50 °C. The residue was dissolved in 40 cm^3 of methanol and pH was adjusted to 6 with concentrated aqueous ammonia. Precipitated Phg was filtered, washed with a small amount of each water and methanol, and dried. D-Phg: Yield 1.253 g; $[\alpha]_D^{20}$ −158° (*c* 1.00, 1 mol dm^{-3} HCl) (lit,[12] $[\alpha]_D$ −157.8°(dil HCl)). L-Phg: Yield 1.249 g; $[\alpha]_D$ +158° (*c* 1.00, 1 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} with a JASCO A-102 infrared spectrophotometer by the KBr disk method.

Saturated methanol or ethanol solutions of DL- and D-TMB salts were prepared at 10 °C and diluted appropriately. The absorbance was measured at 260 nm with a Shimadzu double-beam spectrophotometer UV-150-02 by using a quartz cell of 0.1 dm path length. Solubility values of the salts were determined from calibration curves.

Results and Discussion

Racemic Structure. Infrared spectra of DL-FrPhg ammonium salts were compared with those of the corresponding D-salts. DL-TMB salt shows infrared spectrum identical with that of D-salt, whereas the spectra of other DL-salts are different from those of D-salts. This result indicates that DL-TMB salt is either a conglomerate or a racemic solid solution but other DL-salts form racemic compounds.[13] Further, DL-TMB salt is more soluble than the D-salt as described in the experimental section, and the ternary phase diagram in Fig. 1 illustrates the patterns of a conglomerate.[14]

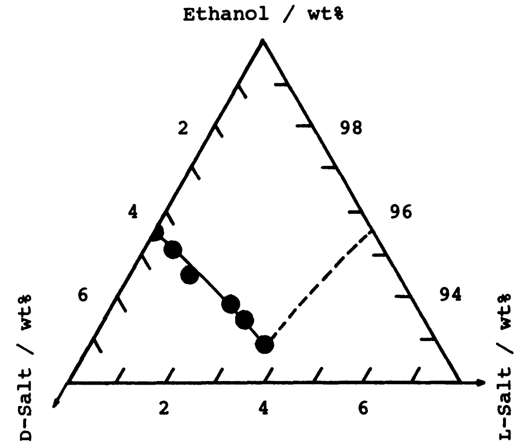

Fig. 1. Ternary phase diagram of solubility of 1,1,3,3-tetramethylbutylammonium salt of *N*-formyl-α-phenylglycine. Solvent: Ethanol. Temperature: 10°C.

The above results lead to the conclusion that only DL-TMB salt exists in a conglomerate at room temperature.

Preferential Crystallization and Free Energy of Critical Nucleation. Free Energy of Critical Nucleation: The crystallization of unseeded enantiomer from a supersaturated solution is relevant to the primary nucleation; therefore the crystallization is influenced by a free energy of critical nucleation (Δg_{max}/J nucleus^{-1}).[15-17] On the other hand, the crystallization of seeded enantiomer cannot be elucidated by the Δg_{max}, but is to be related to the secondary nucleation.[17] It is, however, estimated that Δg_{max} has some influence on the crystallization of seeded enantiomer, because the growth of critical nucleus is accelerated by seeding.[17] On the assumptions that no interaction exists between D- and L-TMB salts in a supersaturated solution and that the critical nucleus of TMB salt is spherical, the Δg_{max}[17] is calculated by

$$\Delta g_{max} = 4\pi r_{cr}^2\sigma_{cr} - (4\pi r_{cr}^3\kappa T/3V_0)\cdot\ln(C/C^*), \quad (2)$$

where r_{cr}/m is the radius of critical nucleus, σ_{cr}/J m^{-2} the interfacial energy, T/K the temperature, V_0[18]/m^3 the molecular volume of TMB salt, κ the Boltzmann constant, C^* the solubility of TMB salt at temperature T/K, and C the amount of TMB salt in supersaturated solution. The r_{cr} and σ_{cr} were calculated by Eqs. 3 and 4.[17,19]

$$r_{cr} = 2V_0A/\kappa T\cdot\ln(C/C^*) - 2r_0, \quad (3)$$

$$\sigma_{cr} = r_{cr}A/(r_{cr}+2r_0), \quad (4)$$

where r_0 is the molecular radius[18] and A is a constant. The constant A was calculated from a relationship between r and σ of nucleus;[19] $\sigma_{cr}(1+2r_0/r_{cr})$=constant $(A)=\sigma_{sa}(1+2r_0/r_{sa})$, where r_{sa} and σ_{sa} are the radius and interfacial energy of embryo in saturated solution, respectively.[20] The r_{cr}, σ_{cr}, and Δg_{max} in methanol and ethanol solutions with degrees of supersaturation of 110, 120, and 130% were calculated, and listed in Table 2. The optical resolution by preferential crystallization of DL-TMB salt was carried out in methanol and

Table 2. Properties of Critical Nucleus in Supersaturated Solutions of 1,1,3,3-Tetramethylbutylammonium Salt of *N*-Formyl-DL-α-phenylglycine[a)]

Solvent	Degree of supersaturation/%	r_{cr}[b)] ×10^{-8} m	σ_{cr}[c)] ×10^{-2} J m^{-2}	Δg_{max}[d)] ×10^{-17} J nucleus^{-1}
Methanol	110	3.64	1.49	8.28
Methanol	120	1.86	1.46	2.11
Methanol	130	1.26	1.43	0.95
Ethanol	110	4.77	1.96	18.7
Ethanol	120	2.45	1.92	4.83
Ethanol	130	1.67	1.89	2.21

a) Temperature: 10 °C. b) r_{cr}: Radius of critical nuleus. c) σ_{cr}: Interfacial energy of critical nucleus. d) Δg_{max}: Free energy of critical nucleation.

Table 3. Preferential Crystallization of 1,1,3,3-Tetramethylbutylammonium Salt of *N*-Formyl-DL-α-phenylglycine in Methanol[a)]

Degree of supersaturation %	Resolution time min	Yield g	Optical purity %	YOPM[b)] g	Degree of resolution %
110	20	0.120	83.3	0.050	8.6
	30	0.149	82.0	0.072	12.3
	40	0.173	84.0	0.095	16.3
	60	0.202	84.3	0.120	20.5
	70	0.263	76.4	0.151	25.9
	80	0.289	66.7	0.143	24.5
120	10	0.168	88.1	0.098	8.4
	15	0.239	89.1	0.163	14.0
	20	0.307	73.9	0.177	15.2
	30	0.463	60.7	0.231	19.8
	45	0.784	55.1	0.382	32.7
	60	1.029	46.2	0.425	36.4
	90	1.677	26.9	0.401	34.4
130	5	0.247	83.8	0.157	9.0
	10	0.573	71.4	0.359	20.5
	15	1.030	20.2	0.158	9.0

a) Solvent: 50 cm^3 of methanol. Seed crystals: 0.050 g of D-salt. Temperature: 10 °C. b) YOPM: Yield of optically pure modification.

Table 4. Preferential Crystallization of 1,1,3,3-Tetramethylbutylammonium Salt of *N*-Formyl-DL-α-phenylglycine in Ethanol[a)]

Degree of supersaturation %	Resolution time min	Yield g	Optical purity %	YOPM[b)] g	Degree of resolution %
110	30	0.071	93.7	0.017	11.2
	60	0.116	95.7	0.061	40.2
	90	0.158	90.1	0.092	60.6
	120	0.177	89.8	0.109	71.8
	130	0.200	75.0	0.100	65.8
	150	0.221	63.8	0.091	59.9
120	15	0.115	91.6	0.055	18.1
	30	0.155	94.8	0.097	31.9
	40	0.209	93.2	0.145	47.7
	60	0.285	95.1	0.221	72.8
	70	0.341	73.6	0.201	66.2
	90	0.519	30.6	0.109	35.9
130	7	0.193	97.4	0.138	30.3
	12	0.326	97.2	0.267	58.6
	17	0.415	96.4	0.350	76.8
	22	0.907	9.1	0.033	7.2

a) Solvent: 50 cm^3 of ethanol. Seed crystals: 0.050 g of D-salt. Temperature: 10 °C. b) YOPM: See note b) in Table 3.

Table 5. Retardation Time for Crystallization of L-Salt and Amount of Crystallization per Time for D-Salt[a)]

Solvent	Degree of supersaturation	Retardation time[b)]	Amount of crystallization per time[c)]
	%	min	$\times 10^{-3}\,\mathrm{g\,min^{-1}}$
Methanol	110	75	2.07
Methanol	120	18	11.5
Ethanol	110	125	1.12
Ethanol	120	65	3.82
Ethanol	130	20	22.8

a) Salt: 1,1,3,3-Tetramethylbutylammonium salt of *N*-formyl-α-phenylglycine. b) The retardation time for crystallization of L-salt was estimated from Figs. 2 and 3. c) The amount of crystallization per time for D-salt was calculated from linear plots in Figs. 2 and 3.

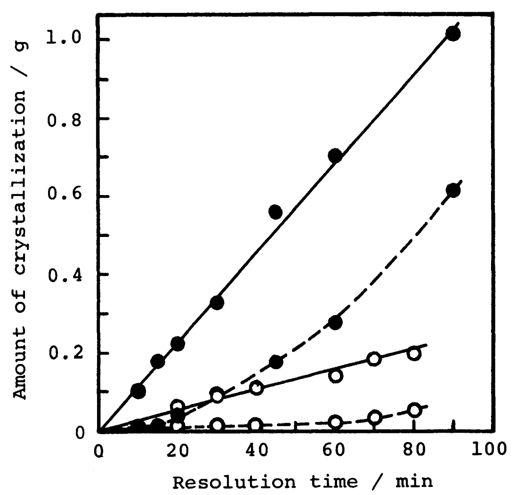

Fig. 2. Relationships between amounts of crystallization and resolution times in methanol. Temperature: 10°C. ——: D-Salt. ----: L-Salt. ○: Degree of supersaturation of 110%. ●: Degree of supersaturation of 120%.

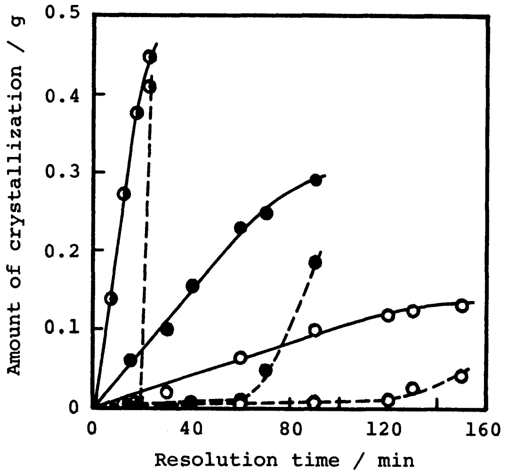

Fig. 3. Relationships between amounts of crystallization and resolution times in ethanol. Temperature: 10°C. ——: D-Salt. ----: L-Salt. ○: Degree of supersaturation of 110%. ●: Degree of supersaturation of 120%. ◑: Degree of supersaturation of 130%.

ethanol with D-salt as seed crystals; results are listed in Tables 3 and 4. From the results, amounts of crystallization[21)] of D- and L-TMB salts were calculated, and the relationships between amount and resolution time are shown in Figs. 2 and 3.

Since $\Delta g_{\max}$ is regarded as energy barrier for growth of the primary nucleus, it is predictable that the unseeded enantiomer tends to crystallize with a decrease in $\Delta g_{\max}$.[17)] This is supported by the retardation time for crystallization of L-TMB salt. The retardation time is defined as the resolution time until L-TMB salt begins to crystallize; the results estimated from Figs. 2 and 3 are listed in Table 5. Although Figs. 2 and 3 indicate the presence of L-TMB salt in the D-salt crystallized in short times, it will be due to the DL-salt adhered on filtration. Rapid crystallization of L-TMB salt requires longer retardation time with an increase in $\Delta g_{\max}$ values. The relationship between the retardation time (θ/min) and $\Delta g_{\max}$ was obtained by

$$\log \theta = -0.8913 \log(1/\Delta g_{\max}) + 16.18, \tag{5}$$

where the correlation coefficient is 0.972.

Figures 2 and 3 also suggest that seeded D-TMB salt tends to crystallize rapidly with a decrease in $\Delta g_{\max}$. The amount of crystallization of D-TMB salt per time (R_D/g min^{-1}) listed in Table 5 was calculated from linear plots in Figs. 2 and 3. Since the $\Delta g_{\max}$ values of D-TMB salt are equal to those of L-salt, the relationship between R_D and $\Delta g_{\max}$ was examined and is expressed by

$$\log R_D = 1.275 \log(1/\Delta g_{\max}) - 23.09, \tag{6}$$

where the correlation coefficient is 0.959. This result shows that the crystallization of seeded D-TMB salt is strongly influenced by $\Delta g_{\max}$.

Conditions of Optical Resolution: It is possible to estimate factors on crystallization of D- and L-TMB salts from Eqs. 5 and 6. In the optical resolution for methanol solution with a degree of supersaturation of 130%, the θ and R_D values calculated from Eqs. 5 and 6 are 10.2 min and 4.11×10^{-2} g min^{-1}, respectively. L-TMB salt begins to crystallize rapidly after 10 min, and

Table 6. Optical Resolution by Successive Preferential Crystallization of 1,1,3,3-Tetramethylbutylammonium Salt of *N*-Formyl-DL-α-phenylglycine[a]

Run	Added amount of DL-salt	Operation amount[b]/g		Salt obtained			
	g	D-Salt	L-Salt	Yield g	Optical purity %	YOPM[c] g	Degree of resolution %
1	3.949	1.975	1.975	0.369	D 95.5	0.302	66.2
2	0.319	1.823	2.125	0.577	L 95.1	0.499	82.3
3	0.527	2.173	1.876	0.584	D 93.3	0.495	75.7
4	0.534	1.915	2.123	0.495	L 96.7	0.429	71.0

a) Solvent: 50 cm^3 of ethanol. Degree of supersaturation of initial racemic solution: 130%. Seed crystals: 0.050 g of D- or L-salt. Temperature: 10 °C. b) The operation amounts of D- and L-salts in solutions were calculated on the basis of analyses of the salts obtained in runs 1—3. c) YOPM: See note b) in Table 3.

the amount of D-salt crystallized at a resolution time of 10.2 min is 0.419 g; this amount corresponds to 23.5% of theoretical value. This agrees well with the results shown in Table 3; D-salt with optical purity of 71.4% was obtained in degree of resolution of 20.5% at resolution time of 10 min, the amount of crystallization of D-salt was 0.441 g, and further, the optical resolution at 15 min gave D-salt with low optical purity (20.2%). However, the above result suggests that the optical resolution for methanol solution with degree of supersaturation of 130% is not favorable for obtaining D-TMB salt with high optical purity in high degree of resolution.

The optical resolution for ethanol solution with the degree of supersaturation of 130% gave D-TMB salt with optical purity of 96.4% and degree of resolution of 76.8% at the resolution time of 17 min. The resolution for 15 min seems to be more favorable for stable optical resolution than 17 min, because it is estimated by θ values that L-TMB salt begins to crystallize rapidly after 20 min. We can estimate from R_D that 0.342 g of D-TMB salt crystallizes at resolution time of 15 min and the amount corresponds to 75.0% of theoretical value. This optical resolution gives D-TMB salt with high optical purity in relatively high degree of resolution.

Optical Resolution of Successive Preferential Crystallization. Successive Preferential Crystallization: According to the above suggestion, the successive preferential crystallization of DL-TMB salt was carried out at 10 °C at resolution time of 15 min for the ethanol solution with degree of supersaturation of 130%, as shown in Table 6. The D-TMB salt with 95.5% optical purity was obtained in degree of resolution of 66.2% from the initial solution. Since the amount of crystallized D-salt was 0.310 g, this result supports the preceding suggestion.

The successive preferential crystallization gave D- and L-TMB salts with optical purity of approximately 95% in degree of resolution of 66—82%.

Purification of Salt Partially Resolved: The D- and L-TMB slats obtained by successive preferential crystallization were easily purified by recrystallization from ethanol as described in the experimental section. TMB salt with low optical purity was purified to optically pure salt in high yield with the optimum amount of ethanol calculated from the solubility of DL-TMB salt in ethanol.[22,23] For example, D-TMB salt with optical purity of 40.9% (1.477 g) was recrystallized from 15 cm^3 of ethanol; the optimum amount calculated is 14.37 cm^3. Solution of D-TMB salt dissolved at 40 °C was stirred for 30 min at 10 °C to crystallize 0.548 g of the D-salt with 100% optical purity; the theoretical yield of the optically pure D-salt is 0.604 g.

Hydrolysis of *N*-Formyl-α-phenylglycine: The D- or L-FrPhg with 100% optical purity was hydrolyzed by refluxing in 1 mol dm^{-3} hydrochloric acid for 2 h to give D- and L-Phg with optical purity of approximately 95%. This result indicates that these Phg were partially racemized by refluxing in hydrochloric acid. The hydrolysis of D- and L-salts with 100% optical purities was carried out by the procedure described in the experimental section. The D- and L-Phg were not racemized under these conditions, and obtained in 83% yields. Although the hydrolysis of *N*-acetyl-D-α-phenylglycine (D-AcPhg) was also attempted under similar conditions, D-Phg did not crystallize from a methanol solution at pH 6 adjusted with concentrated aqueous ammonia. AcPhg is not hydrolyzed under such conditions. This result suggests that DL-FrPhg is more favorable for the optical resolution than DL-AcPhg.

The above results indicate suitable conditions for the optical resolution of DL-TMB salt on the basis of Δg_{max} in supersaturated solutions and procedures to give optically pure D- and L-Phg by successive preferential crystallization of DL-TMB salt.

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