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A New Method for the Determination of Optical Purity of Norleucine Based on Enthalpy of Transition in Solid-Solid Phase Transition

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Synopsis. A new method for determining the optical purity based on enthalpy of transition in solid–solid phase transition was introduced. The enthalpy of transition of norleucine recovered by an optical resolution procedure was measured by means of DSC. The magnitude of optical purity based on enthalpy of transition was in agreement with that based on specific rotation.

For the products obtained by optical resolution procedure or asymmetric synthesis, it is necessary to determine the magnitude of optical purity. The method for the determination of optical purity based on specific rotation has frequently been used.[1)] A large amount of sample (about 0.1 g) is required to measure its specific rotation with dissolution procedure, whereas only small amount of sample (a few mg) is required to measure its enthalpy of transition by DSC without dissolution procedure.

In the course of thermoanalytical and calorimetric studies on polymorphic behaviors of crystalline modification for aliphatic α-amino acids with normal alkyl chains, we have found that the α-form DL-norleucine (DL-NL) is transformed into the γ-form DL-NL at 390 K with the enthalpy of transition of 4.4 kJ mol^{-1} by DSC[3)] and that the α–γ transition of DL-NL is of the first order.[4)]

In this report, a new method for the determination of optical purity based on enthalpy of transition is introduced. The enthalpy of transition of norleucine recovered by optical resolution procedure was measured by means of DSC. The magnitude of optical purity based on enthalpy of transition was compared with that based on specific rotation.

Experimental

L-Phenylalanine (L-Phe) and L-tryptophan (L-Try) were purchased from Tokyo Kasei Co., and DL-norleucine (DL-NL) and D-norleucine (D-NL) from Sigma Chemical Co. All the samples used were the purest reagents commercially available: The purity of the compounds was guaranteed by each manufacturers to be more than 98%. These samples were further purified by recrystallization from aqueous solution. Active carbon, which was purchased from Wako Chemical Co., was G. R. grade. The procedure of optical resolution was based on the method described by Shiraiwa et al.[5)] The measurement of enthalpy of transition was carried out by means of DSC. The apparatus and procedure were tha same as described in the previous works.[3,4)] The specific rotation was determined by Japan Spectroscopic Co. Model J-20 for 5 mol dm^{-3} HCl solution by using a 0.1 dm path-length quartz cell.

DL-NL (0.02 mol) and L-Phe (0.01 mol) were dissolved in 35 cm^3 of 1 mol dm^{-3} sodium hydroxide solution and to the alkaline solution containing DL-NL was added 70 cm^3 of water. By adding 1 mol dm^{-3} HCl to the above solution, the pH was adjusted to 5—6 (isoelectric point). Precipitates obtained by stirring the solution for 10 min at 0 °C were collected by filtration. The similar procedure was performed in the case of DL-NL and L-Try. The precipitates were then dissolved in 50 cm^3 of water and active carbon (ten times of samples by weight) was added to the solution. The mixture was shaken at room temperature for 3 h and carbon powders were filtered off. The filtrate was evaporated to dryness at 40 °C under reduced pressure and then the residue was collected. The samples obtained by this procedure were dried in a desiccator and used (denoted as samples NL-A and NL-B) Other procedure for evaporating water from aqueous solution containing DL-NL was also performed. For example, a solution containing equimolar amounts (0.02 mol) of DL-NL and L-Phe in 350 cm^3 of water was concentrated to 180 cm^3 at 60 °C and the precipitates formed were collected by filtration (sample NL-C). A filtrate was again concentrated to 90 cm^3 at 60 °C and the resulting precipitates were collected by filtration (sample NL-D). Separation of free NL from the adduct was based on the adsorption difference between NL and Phe on active carbon.[6)]

Results and Discussion

The DSC method for determining optical purity, which is equal to enantiomeric purity is based on phase diagrams.[7)] Since α-amino acids, in general have no melting point and enthalpy of fusion, this method is not applicable to the amino acid samples.

In this study, we assume that crystalline amino acid that is racemic compound shows a solid–solid phase transition and that there is no interaction between DL- and D- or L-isomer in crystalline state. The magnitude of optical purity by thermal method, p_{th}, is defined as

$$p_{\mathrm{th}} = \frac{\Delta H_{\mathrm{t}}^{\mathrm{DL}} - \Delta H_{\mathrm{t}}^{\mathrm{obs}}}{\Delta H_{\mathrm{t}}^{\mathrm{DL}}}, \tag{1}$$

where $\Delta H_{\mathrm{t}}^{\mathrm{DL}}$ and $\Delta H_{\mathrm{t}}^{\mathrm{obs}}$ are the enthalpy of transition of racemic compound and the observed enthalpy of transition of mixture of DL-and D- or L-isomer in amino acids, respectively. The observed enthalpy of transition of amino acid recovered by optical resolution procedure, $\Delta H_{\mathrm{t}}^{\mathrm{obs}}$, is expressed as

$$\Delta H_{\mathrm{t}}^{\mathrm{obs}} = (1-y)\Delta H_{\mathrm{t}}^{\mathrm{DL}}, \tag{2}$$

where y is enantiomer excess.

Equation 2 can be rewritten as

$$\Delta H_{\mathrm{t}}^{\mathrm{obs}} = 2x\,\Delta H_{\mathrm{t}}^{\mathrm{DL}} \quad \text{for } 0 \leqslant x \leqslant 0.5, \tag{3}$$

$$\Delta H_{\mathrm{t}}^{\mathrm{obs}} = 2(1-x)\Delta H_{\mathrm{t}}^{\mathrm{DL}} \quad \text{for } 0.5 \leqslant x \leqslant 1.0, \tag{4}$$

where x is mole fraction of D- or L-isomer of the amino acid. The correlation between $\Delta H_{\mathrm{t}}^{\mathrm{obs}}$ and x is schematically shown in Fig. 1.

In fact there is an example of racemic compound

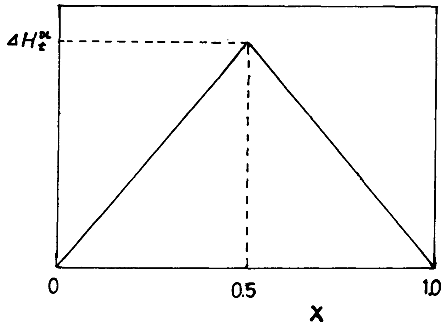

Fig. 1. The correlation between enthalpy of transition, ΔH_t^{obs} and mole fraction of D- or L-isomer, x.

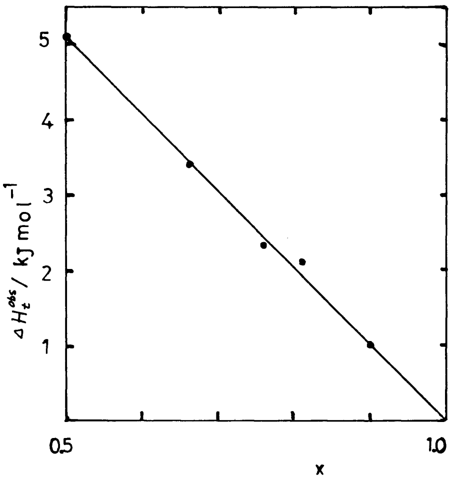

Fig. 2 A plot of enthalpy of transition, ΔH_t^{obs} against mole fraction of D-isomer.

Table 1. The Experimental Values of ΔH_t^{obs} at Enantiomer Excess of D-Norleucine by Arbitrary Mixing of DL- and D-Isomer

Enantiomer excess of D-isomer	Mole fraction of D-isomer	Peak temperature	Enthalpy of transition$^{a)}$
y	x	T/K	ΔH_t^{obs} /kJ mol^{-1}
0.32	0.66	392.5	3.4
0.52	0.76	392.4	2.3
0.61	0.81	392.4	2.1
0.80	0.90	392.5	1.0
0.00	0.50	392.2	5.1

a) The values of enthalpy of transition at heating rate, 5 K min^{-1}.

Table 2. The Comparison of Optical Purity Values Determined by DSC with Those by Specific Rotation

Sample	Enthalpy of transition ΔH_t^{obs} /kJ mol^{-1}	Optical purity by DSC %	Optical purity by specific rotation$^{a)}$ %	Configuration$^{b)}$
NL-A	4.7±0.0	7.8	8.2	L
NL-B	4.6±0.0	9.8	10	L
NL-C	1.6±0.0	69	84	D
NL-D	3.5±0.1	31	28	D

a) Ratio of $[\alpha]_D^{20}$ (c=0.5, 5 mol dm^{-3} HCl solution). b) Configuration obtained from specific rotation.

with a solid–solid phase transition.[2,3] The α-form DL-NL is transformed into the γ- form DL-NL at 390 K with the enthalpy of transition of 4.4 kJ mol^{-1} at null heating rate by DSC.[4] To confirm whether Eq. 4 holds in the case of NL, the enthalpy of transition by DSC was determined in the range of mole fraction of D-isomer between 0.5 and 1.0 at a heating rate of 5 K min^{-1}. The results obtained are summarized in Table 1. As is shown in Fig. 2, a plot of ΔH_t^{obs} against mole fraction of D-isomer was found to be linear. We can accordingly calculate the magnitude of optical purity of NL recovered by optical resolution from the enthalpy data obtained for mixtures of known mole fractions without dissolution procedure.

An attempt to resolve aliphatic amino acids by using L-phenylalanine as resolving agent has been introduced by Shiraiwa et al.[5] The results of optical resolution by their method are summarized in Table 2, in which the magnitudes of optical purity by DSC are compared with those by specific rotation. The samples of NL-A and NL-B in Table 2 were recovered by using L-Phe and L-Try as resolving agents, respectively. The samples of NL-C and NL-D were obtained by evaporating water from aqueous solution. The optical purity in Table 2 is the percent optical purity. As is shown in Table 2, the values of optical purity by DSC agree fairly well with those by specific rotation. Therefore, it is concluded that the method for determining the optical purity based on enthalpy of transition is applicable to the sample of norleucine.

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