Calorimetric Investigation of *N*-Acyl Amino Acids

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The melting characteristics of *N*-acyl amino acids were examined using a differential scanning calorimeter (DSC). The melting-point temperature of *N*-dodecanoyl L-amino acid increased with the size of its amino acid residue, while the order was opposite for the DL-amino acids. The racemic derivatives formed racemic compounds in their solid states. Each *N*-dodecanoyl L-amino acid had a larger enthalpy and entropy-of-fusion than its racemic isomer. Differences in the thermodynamic quantities between the optically active and racemic isomers were larger for *N*-dodecanoylvaline and -leucine compared with those of *N*-dodecanoylalanine. A mixture of these molecules were also studied. Each racemic isomer of *N*-dodecanoylalanine and -leucine partly formed a racemic pair in the liquid state.

Attention is presently being given to the stereochemical effects not only of micelle-catalyzed reactions but also to condensed systems of long hydrocarbon chain compounds. Tachibana and his coworkers[1-4] have investigated chiral and racemic 12-hydroxyoctadecanoic acids in their crystal aggregate and monolayer states. Iwahashi, *et al.*[5] studied the thermodynamic and structual relationship between optically active α-monostearin and its racemic isomer in their crystalline, molten, and dissolved states. They suggested that (*R*)- and (*S*)-molecules in the (*RS*)-form interacted to form a racemic pair not only in the crystalline state, but also in the molten state. Miyagishi and Nishida[6] reported that the smaller critical micelle concentration for a sodium salt of the optically active *N*-acyl amino acid resulted from a difference in the micellization enthalpy. The object of this paper is to describe the chirality effect on the melting characteristics of *N*-acyl amino acids.

Experimental

N-Acyl amino acids were prepared according to a procedure described before[6,7] and are tabulated in Table 1. The recrystallization of *N*-acyl amino acids was repeated until the DSC curve and a peak temperature became constant. A differential scanning calorimeter (Daini Seikosha DSC, model SSC/560S) was used for the measurement of the heat-of-fusion and melting temperature. A sample (about 5 mg) was sealed in an aluminium vessel (50 mm^3). The scanning rate was 5 K min^{-1}. Standard samples of phenanthrene and indium were used for calibration. A mixed sample was prepared as follows. A mixture of a known composition was dissolved in methanol and the resulting solution was allowed to stand for several hours. After the methanol was evaporated, a part of the mixture was used for the DSC measurement.

In the mixed systems, two peaks appeared on the DSC curve and each was detected as a separated peak. When the peaks approached each other, they began to overlap and became one broad peak with two peaks superimposed on it. The temperature at each peak was measured.

Results and Discussion

Melting Temperature. The melting temperatures of *N*-acyl amino acids are shown in Table 1 along with the heats-of-fusions. The melting-point temperature increased with the size of the amino acid residue in an L-series, while the order was opposite in a DL-series. The branched isomers of *N*-dodecanoylvaline all had similar melting-point temperatures. The same tendency was observed among the branched isomers of *N*-dodecanoylleucine. *N*-Acylglycine and -alanine each showed a tendency for the melting-point temperature to increase with the chain length of an acyl group. *N*-Acyl-DL-norleucine, however, gave melting-point temperatures scattered between 354—360 K and had no regular tendency.

Latent Heat of Fusion. The latent heat ΔH_f and entropy-of-fusion ΔS_f for *N*-dodecanoyl amino acids are plotted against the carbon number of their amino

TABLE 1. PROPERTIES OF *N*-ACYL AMINO ACIDS

	Mp/K	ΔH_f/KJ mol^{-1}	[α]
N-Dodec-Gly	393.1	48.4	
N-Dodec-L-Ala	353.1	37.6	−18.7
N-Dec-DL-Ala	365.6	37.5	
N-Dodec-DL-Ala	379.1	44.7	
N-Tetradec-DL-Ala	381.3	54.0	
N-Hexadeca-DL-Ala	387.1	58.4	
N-Dodec-Abu[a]	389.1	41.4	
N-Dodec-L-Val	378.6	33.1	−2.6
N-Dodec-DL-Val	364.5	64.4	
N-Dodec-DL-*n*-Val[b]	362.1	59.5	
N-Dodec-L-Leu	381.1	33.5	−14.5
N-Dodec-L-Ile	380.1	30.6	+4.6
N-Dodec-DL-Leu	354.6	59.9	
N-Oct-DL-*n*-Leu[c]	359.1	33.9	
N-Dec-DL-*n*-Leu	355.3	29.9	
N-Dodec-DL-*n*-Leu	350.4	55.2	
N-Tetradec-DL-*n*-Leu	360.1	57.7	

a) *N*-dodecanoyl-2-aminobutanoic acid. b) *N*-dodecanoyl-DL-norvaline. c) *N*-Octanoyl-DL-norleucine.

acid part in Fig. 1. The values of ΔH_f and ΔS_f depended on the size and optical isomerism of the acyl amino acid (see also Fig. 2). We did not find a significant difference of thermodynamic quantity among the branched isomers.

Racemic compounds always had larger ΔH_f and ΔS_f values than the corresponding chiral isomers. For the optically active series, ΔH_f and ΔS_f decreased with the carbon number of the amino acid part, while for the racemic series, there were abrupt increases of ΔH_f and ΔS_f above a carbon number of 5. The difference of ΔH_f between the optically active and racemic isomers was 31.3 and 23.0 KJmol^{-1} for *N*-dodecanoylvaline and -leucine, and the difference of ΔS_f was 92 and 72 Jmol^{-1} K^{-1}, respectively. These values were very large compared to 7.1 KJmol^{-1} and 12 Jmol^{-1} K^{-1} for *N*-dodecanoylalanine.

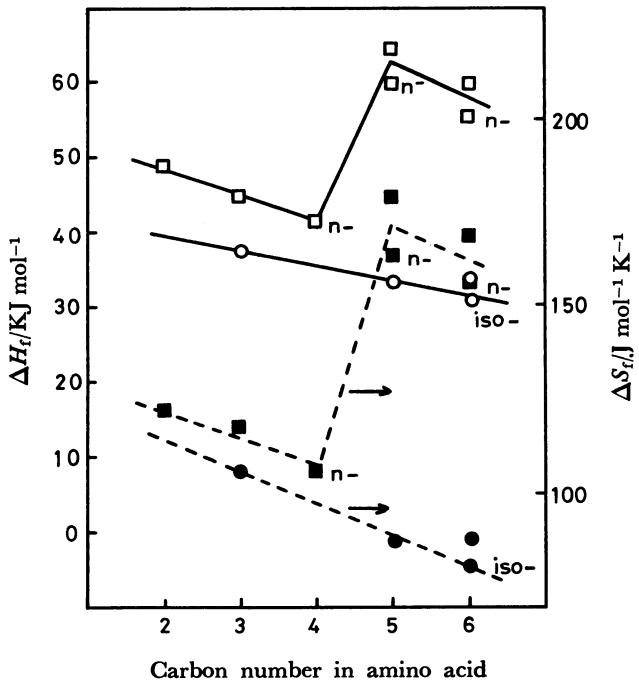


Fig. 1. Enthalpies and entropies of fusion of *N*-dodecanoyl amino acids. (○,●); L-form, (□,■); DL-form.

Figure 2 shows that the enthalpy and entropy-of-fusion depend on the size of an acyl group. For the alanine compounds, ΔH_f and ΔS_f increased linearly with the number of the methylene group in the acyl group. The enthalpy-of-fusion per methylene group was 4 KJmol^{-1}, which was identical with a figure found for normal alkane,[8] and monoglyceride.[9] The entropy-of-fusion increased by a factor 9 Jmol^{-1} K^{-1} for each additional methylene group added to the acyl chain. This value also agreed with that of normal alkane[8] and monoglyceride.[9] It can be concluded from the above results that the acyl group of the *N*-acyl DL-alanines behaves in the same manner as a normal alkane when it melts, and is hardly affected by the amino acid residue.

On the contrary, the theromodynamic quantities of *N*-acyl DL-norleucine varied, irregularly, with the size of the acyl group. It is suggested that the acyl group of the *N*-acyl DL-norleucines may interact somewhat with their amino acid residue, or at least perturbs the melting process.

Melting of Mixture. The temperatures cited in Figs. 3—6 are the peak temperatures on a DSC curve since it was difficult to determine the point at which the DSC curve started to deviate from the base line.

The melting-point temperatures of mixtures of *N*-

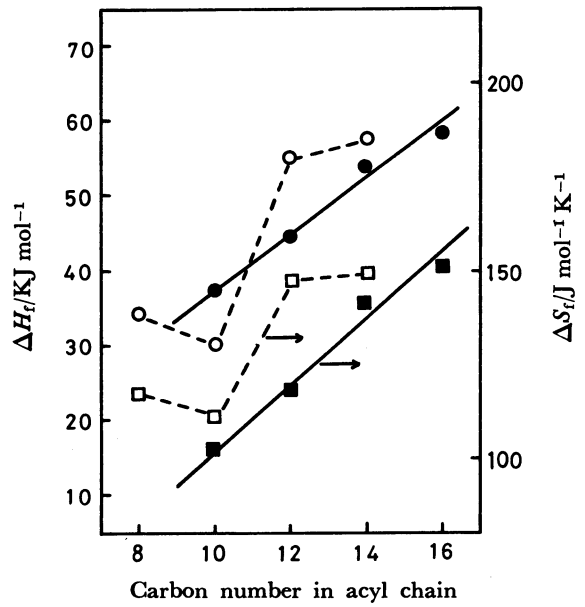


Fig. 2. Enthalpies and entropies of fusion of *N*-acyl DL-alanines and -DL-norleucines. (●,■); the alanines, (○,□); the leucines.

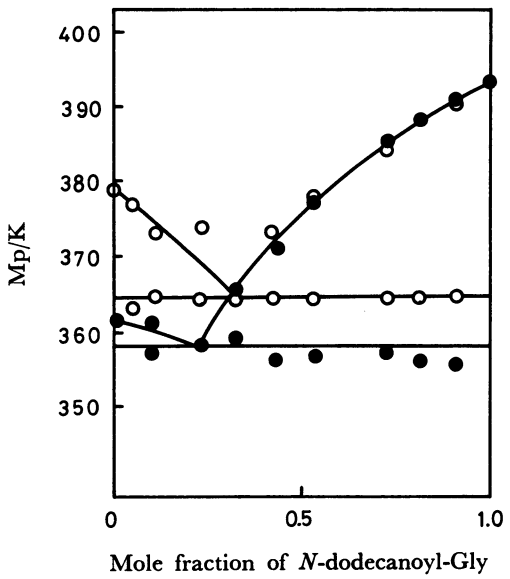


Fig. 3. Phase diagrams of mixtures of *N*-dodecanoylglycine and -valines. (○); L-form, (●); DL-form. The solid lines are the calculated values on the assumption of ideal mixing.

dodecanoylglycine and *N*-dodecanoyl-L-valine are shown in Fig. 3. This system has a single eutectic point and is a typical system with equilibrium between unmixed solid and mixed liquid states.

In a previous paper,[7] it was reported that a mixture of sodium salts of *N*-dodecanoylglycine and -L-valine could be treated as an ideal mixture in a micelle state. We attempted to apply the assumption of ideal mixing to the present system. The solid curves in Fig. 3 were calculated from Eq. 1[10] and are in good agreement with the experimental points.

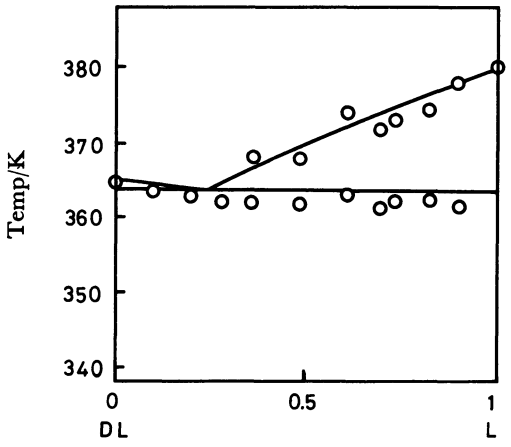
$$\ln x = (\Delta H_0/R)(1/T_0 - 1/T) \quad (1)$$

where x is mole fraction of the first component and ΔH_0 and T_0 are the latent heat-of-fusion and melting point.

The system, where racemic *N*-dodecanoylvaline was used instead of the optically active isomer, was also examined. We considered a system composed of A moles of *N*-dodecanoylvaline (1 mole is a mole of an optically active isomer) and B moles of *N*-dodecanoylglycine (mole fraction in Fig. 3 is equal to $B/(A+B)$). If the racemic *N*-dodecanoylvaline formed a racemic compound in the liquid state, the mole fraction of *N*-dodecanoylglycine would be $B/(A/2+B)$ in the liquid mixture (which differs from the mole fraction used in Fig. 3). Consequently, this phase diagram should be different in all regions from one containing the optically active isomer. The experimental results did not agree with such a prediction. When *N*-dodecanoyl-DL-valine is a racemic mixture in a liquid state, the mole fraction of the glycine derivative is given as $B/(A+B)$ in the liquid mixture. The results calculated from this mole fraction and Eq. 1 are given in Fig. 3. This figure indicates that the racemic *N*-dodecanoylvaline is a racemic mixture in a liquid state rather than a racemic compound, although it forms a racemic compound in the solid state (described later).

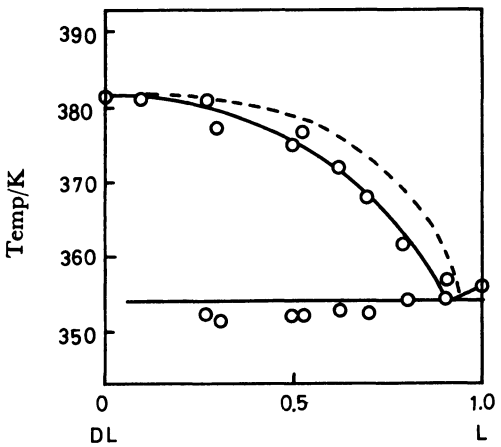
Mixture of Optically Active and Racemic Isomers. The systems shown in Figs. 4—6 were examined. In all of them, the formation of racemic compounds occured. These results reflect the fact shown in Fig. 1, that the ΔH_f and ΔS_f values of a racemic isomer are always larger than those of a corresponding optically active one. The difference may correspond to the formation energy of a racemic compound.

We could not assume ideal mixing in these systems, but could roughly estimate an interaction parameter between two corresponding chiral isomers in a liquid state. The excess thermodynamic potential of the first



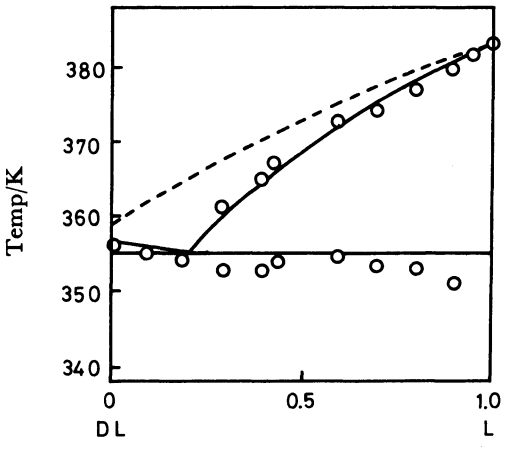
Mole fraction of dodec-L-Val

Fig. 5. Melting-point temperatures of mixture of *N*-dodecanoyl-L-valine and -DL-valine. The plotted points are experimental data, the solid and broken lines correspond the values calculated with β of 0.06 and 0, respectively, but two curves were almost overlapped.



Mole fraction of *N*-dodec-L-Ala

Fig. 4. Melting-point temperatures of mixture of *N*-dodecanoyl-L-alanine and -DL-alanine. The plotted points are experimental data, the solid and broken lines correspond the values calculated with β of -1.08 and 0, respectively.



Mole fraction of dodec-L-Leu

Fig. 6. Melting-point temperatures of mixture of *N*-dodecanoyl-L-leucine and -DL-leucine. The plotted points are experimental data, the solid and broken lines correspond the values calculated with β of -1.91 and 0, respectively.

component, $\mu^e(T,x)$ is in the most simple form[10]

$$\mu^e(T,\ x) = RT\ln f_1 = A(1-x_1)^2. \quad (2)$$

When we assume $A=RT\beta$, an activity coefficient f_1 is given by Eq. 3.

$$\ln f_1 = \beta(1-x_1)^2 \quad (3)$$

Substitution of Eqs. 2 and 3 into the following equation[11]

$$-\int_{T_1}^{T}\Delta S_f dT + RT\ln x_1 + \mu^e(T,\ x) = 0 \quad (4)$$

and a rearrangement gives:

$$T = \frac{\Delta H_1/R}{\Delta H_1/RT_1 - \ln x_1 - \beta(1-x_1)^2} \quad (5)$$
$$(0 \leqq x_1 \leqq x_{eu1},\ \text{and}\ x_{eu2} \leqq x_1 \leqq 1)$$

where x_1 and T_1 are the mole fraction and melting point of the first component. ΔS_f is its fusion entropy and is equal to $\Delta H_1/RT_1$. ΔH_1 is the latent heat-of-fusion. Our systems contain two components of L- and D-isomers, and the L-isomer corresponds to the first component. By using Eq. 3 and the method described by Oonk,[12,13] we obtain Eq. 6.

$$T = \frac{\Delta H_c}{\Delta H_c/T_c - R/2[\ln 4x_1(1-x_1) + \{x_1^2 + (1-x_1)^2 - 1/2\}\beta]} \quad (6)$$
$$(x_{eu1} < x_1 < x_{eu2})$$

Here, T_c is the melting temperature of the racemic isomer, ΔH_c is its latent heat-of-fusion and x_{eu1} and x_{eu2} are the compositions at the eutectic points ($x_{eu1} < x_{eu2}$). The melting-point temperature can be calculated from Eqs. 5 and 6.[15]

The interaction parameter (β) was estimated from the experimental values using Eqs. 2—6. The value of β was −1.08, 0, and −1.91 for *N*-dodecanoylalanine, -valine, and -leucine, respectively. These values suggest that the L- and D-isomers interact with each other to form at least a racemic pair in the systems of the alanine and leucine derivatives.

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13) Equation 6 can also be derivated from equation 23.10 in reference 14 on the assumption of Eq. 3.

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15) The values calculated in the region between x_1=0.5 and 1.0 are given Figs. 4—6 and the left side of the figures (mole fraction of DL- isomer is 1) corresponds to x_1=0.5 in Eqs. 5—6.