

Crystallization and Transformation Mechanisms of α , β and γ -Polymorphs of Ultra-Pure Oleic Acid¹

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ABSTRACT

Crystallization and transformation mechanisms of ultrapure (99.999%) oleic acid were examined by Differential Scanning Calorimetry (DSC) and X-ray diffraction. X-ray diffraction spectra revealed three different polymorphs newly named α , β and γ , which differ from each other most significantly in the short spacing spectra. α and β were found to be equivalent to the previous data which Lutton named low and high melting polymorphs, whereas γ was newly identified in the present study. DSC studies have clarified the thermodynamic stability of the three polymorphs in a range of temperature from -20 to 16.2 C. β is always most stable, whereas α and γ are metastable, undergoing a reversible first-order solid-state transformation at -2.2 C (on heating). DSC also showed that the crystallization behaviors are strongly dependent on the polymorphs; α crystallizes at a much higher rate than β ; despite the fact that they have close melting points (α , 13.3 C; β , 16.2 C). It was demonstrated for the first time that the above peculiar polymorphic behaviors of oleic acid are quite different from those of stearic acid, a saturated fatty acid with the same carbon chain length.

INTRODUCTION

The use of unsaturated fatty acids and their derivatives recently has attracted significant attention in various fields of science and technology. To better understand the physico-chemical properties of the unsaturated fatty acids, fundamental research in a crystalline state is highly important. This is mainly because a single crystalline state can reveal characteristic features of the molecules in a well-defined molecular packing. Polymorphism is the main problem to which all long-chain fatty acid crystals are subjected. The polymorphism, revealing different molecular packing modes of similar lattice energies, also may have important implications for physiological characteristics of the unsaturated fatty acids in biological membranes (1).

However, few studies have been reported on the unsaturated fatty acids, even for the most typical, oleic acid. Lutton first measured powder X-ray diffraction patterns (2). Two polymorphs having lower and higher melting points were discovered. Abrahamsson et al. reported structural data (3) for the lower-melting polymorph, showing kinked structure at *cis*-double bond position. Koyama et al. recently studied (4) Raman spectroscopic analysis of various unsaturated fatty acids in a polycrystalline state. They found an increased overall fluidity of the molecules with an increasing number of the double bonds. Ernst studied the crystal structures (5) of some essential unsaturated fatty acids. However, detailed information is still needed on the occurrence of all possible polymorphs, thermodynamic and kinetic properties of crystallization and phase transformation of those polymorphs, the molecular conformation and dynamic behavior of the molecules and so on. As the first experiment of our systematic study on fatty acid crystals, this paper reports thermodynamic properties of the polymorphic modifications of ultrapure oleic acid.

MATERIALS AND METHODS

Samples

Ultrapure oleic acid was prepared by Nippon Oil and Fats Co. Ltd. The purity was guaranteed more than 99.999%.

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Figure 1 shows capillary gas-liquid-chromatographic data (Shimadzu GC-9A) with column: SP-2340, capillary: 50 m, carrier gas: He and temperature program of 0.2 C/min from 150 to 160 C and 2 C/min from 160 to 200 C. No positional and geometrical isomers and no different chain length fatty acids were detectable.

Differential Scanning Calorimetry (DSC)

Thermal analysis of the crystallization and polymorphic transformation of oleic acid were studied by the Seiko-Denshi DSC system (model SSC). The samples (about 10 mg) were sealed in aluminum pans. Before cooling, the sample was melted at 30 C for more than 30 min to dissociate molecular clusters which may remember the pre-existing crystal structures. The heating and cooling were performed by a computer program in DSC, which enabled us to lower the rates of temperature change to 0.01 C/min as well as to keep the temperature constant before or after rapid temperature changes. Thermodynamic data of transition, i.e., enthalpy (ΔH) and entropy (ΔS), were calculated by the computer from the equation: $\Delta S = \Delta H / T_{tr}$.

X-ray Diffraction

Powder X-ray diffraction was taken by Rigaku diffractometer ($\text{CuK}\alpha$: $\lambda = 1.542 \text{ \AA}$, Ni-filter) at temperatures ranging from -20 to 15 C. The crystals, precipitated either from melt or from solution, were mounted on the X-ray diffraction glass plate in a room at -25 C, and then sealed and placed in a temperature-controlled sample holder.

Solvent Crystallization

Oleic acid crystals were grown from organic solvents (acetonitrile and decane). A comparison was done for the crystals grown from melt and solvent as well. Furthermore, the stability of the polymorphs obtained also was evaluated in the solution phase. We observed the growth of the most stable polymorph at the expense of less stable ones in a

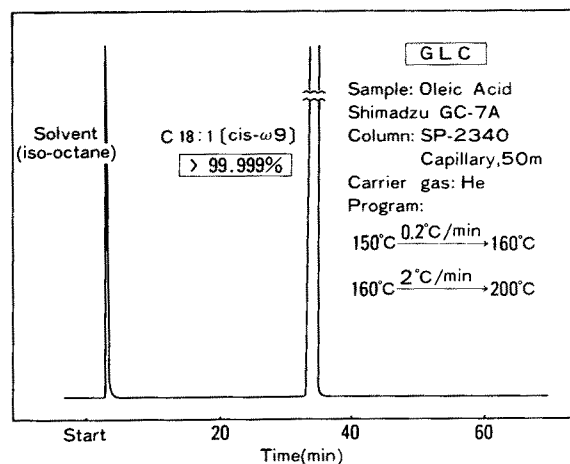


FIG. 1. A chart of gas-liquid-chromatographic (GLC) data of oleic acid.

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saturated solution. This method, which previously was applied to stearic acid polymorph (6), was employed as a complementary experiment to determine the polymorphic stability which was done mainly by DSC.

RESULTS

DSC Studies

DSC measurements have clarified that the crystallization and polymorphic transition were sensitively dependent on the rate of the temperature change.

The DSC data taken with the rate of 2 C/min for cooling and heating are shown in Figure 2. On cooling, two exothermic peaks appeared at 4.7 and -3.6 C, and two endothermic peaks appeared at -2.2 and 13.3 C on heating. Lutton reported (2) low melting (13.2 C) polymorph. So it is reasonable to conclude that the heating peak at 13.3 C is equivalent to this polymorph, and that the peak at 4.7 C on cooling corresponds to the crystallization of that polymorph. No previous data, however, is available for the peaks at -3.6 C (cooling) and -2.2 C (heating). So, these peaks were newly detected in the present experiment. As evidenced later on by X-ray diffraction, these peaks were found to correspond to a first order solid-state transformation between the low-melting polymorph (named by Lutton) and the newly-found polymorph.

Figure 3 shows the dependence of the crystallization behavior on the cooling rate in the DSC measurement. The sharp peak at 4.7 C is the same as that in Figure 2 with a rate of 2 C/min. When the rate was decreased to 0.05 C/min, a broad peak first appeared at 9.3 C and was followed rapidly by a sharp peak at 8.6 C. It is worthy to note that, when the sample exhibiting the double peaks at 0.05 C/min was heated at 2 C/min, two melting peaks at 13.3 and 16.2 C were obtained. A further decrease of the cooling rate, 0.02 C/min, yielded only one broad peak around 10.5 C. As the sharp peaks correspond to the crystallization of the low-melting polymorph, it is clear that the broad peaks reveal the crystallization of another polymorph which tends to occur at very low cooling rates from the melt phase. To verify this, the sample which first showed only one broad peak, as revealed in Figure 3 at 0.02 C/min, was cooled rapidly down to -50 C and heated again up to 30 C at 2 C/min (Fig. 4). Only one peak at 16.2 C was detectable. This was found to be equivalent to the second polymorph with high-melting point reported by Lutton (2).

Consequently, a conclusion can be drawn that oleic acid possesses three different polymorphs; two of them reveal the reversible solid-state transformation, and two have different melting temperatures (13.3 and 16.2 C). We named them β (melting at 16.2 C), α (melting at 13.3 C) and γ (transforming to α at -2.2 C on heating). In order to display all of the three polymorphs of oleic acid, Figure 4 shows a DSC experiment performed by a programmed temperature control: the sample was first cooled from 30 C to -20 C and then heated up to 13.0 C at 2 C/min, then the heating rate was decreased to 0.01 C/min between 13.0 and 15.5 C. After that, the sample was heated at 2 C/min up to 30 C. A time scale was used so that an indirect transition from α to β was shown clearly in Figure 5. On heating at 2 C/min, the solid-state transition from γ to α revealed one sharp peak at -2.2 C. No sharp peak appeared at 13.3 C where α -melting was detectable in Figure 2. Instead, a zig-zag pattern was obtained between 13.2 and 14.3 C. At the first stage of the zig-zag peaks, the amplitude was small. On continuous heating, the amplitude increased until one sharp endothermic peak and a subsequent sharp exothermic peak appeared. This result was highly reproducible either when the heating rate was lower than about 0.2 C/min or when

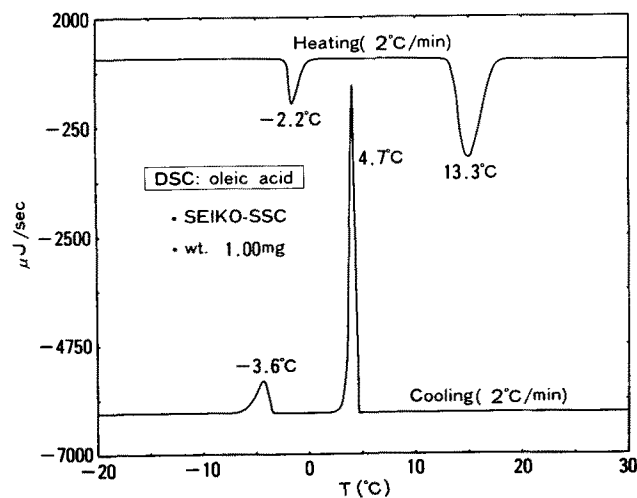


FIG. 2. DSC data of cooling and subsequent heating of oleic acid by the Seiko-SSC system. The rate of temperature change was 2 C/min. The weight of sample was calibrated to 1.00 mg. Before cooling the melt was kept at 30 C for more than 30 min.

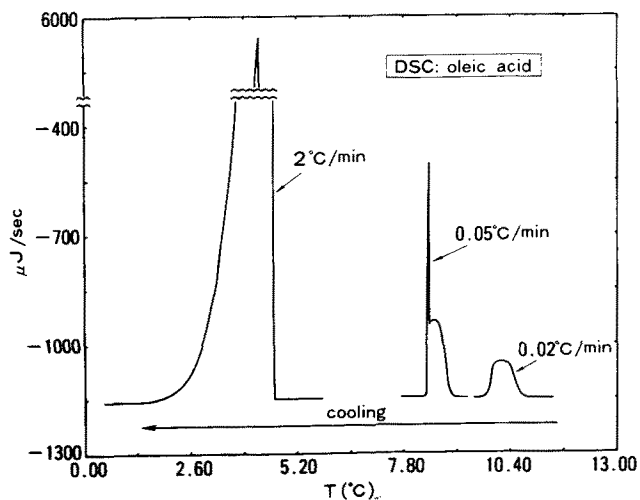


FIG. 3. DSC data of the crystallization behaviors of oleic acid with different cooling rates; 2, 0.05 and 0.02 C/min.

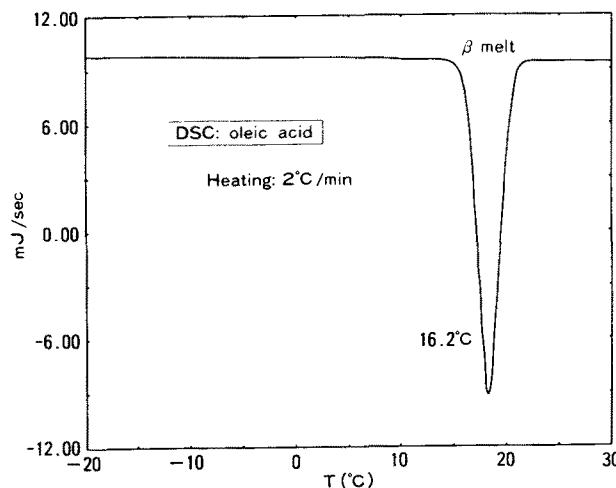


FIG. 4. DSC data of the heating of oleic acid which revealed only one broad peak on cooling at the rate of 0.02 C/min as shown in Fig. 3.

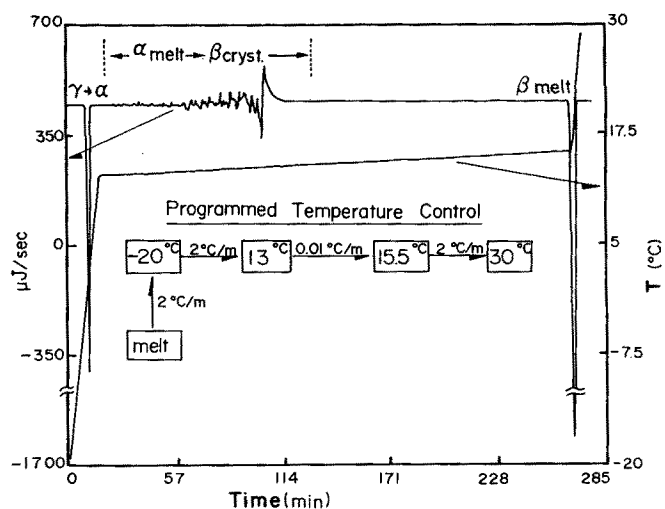


FIG. 5. DSC data on heating of oleic acid which was first cooled from 30 C to -20 C at the rate of 2 C/min. The heating rates were controlled in the way depicted within the figure. A zig-zag pattern, caused by the melting of α and successive crystallization of β , between 13.2 and 14.3 C, appeared only when the heating rate was extremely low. DSC curve (left) and temperature of the sample (right) are shown simultaneously. A time scale was used to better depict the transformations among α , β and γ polymorphs.

the temperature was kept at 13.3 C for a long period. This denies the possibility of nonlinearity in the heating rate. Instead, the zig-zag peaks between 13.2 and 14.3 C correspond to the repeated process of α -melting and subsequent β -crystallization. The indirect transition mentioned above is equivalent to this melting-recrystallization process. Thereafter, a further heating yielded one sharp endothermic peak of β -melting at 16.2 C.

X-ray Diffraction

To identify the polymorphic structures obtained by the melt crystallization and solvent crystallization, an X-ray diffraction study was carried out. Long and short spacings of the three polymorphs are shown in Figure 6. In the β form (10 C) a strong peak (3.68 A) and three weak peaks (4.63, 4.37 and 4.17 A) in the short spacing region and a long spacing of 41.2 A were obtained. In the α form (0 C), three strong peaks (4.32, 3.94 and 3.71 A) and a weak peak (4.12 A) revealed. The long spacing of α was 43.4 A. Finally, in γ (-10 C), three strong peaks (3.94, 3.72 and 3.42 A), each of which is followed by a shoulder peak, and another three weak peaks (4.71, 4.52 and 4.16 A) were the short spacing spectra; the long spacing was 41.9 A. All the data are given in Table I, together with those of the previous reports. Both the short and long spacing spectra of the three newly-found polymorphs are quite different from each other, implying different unit cell structures.

DISCUSSION

First we discuss the stability and the transformation circuit of α , β and γ polymorphs. The different melting points, lower α and higher β , imply that β is more stable than α . The reversible transition from γ to α on heating in turn means that γ is more stable than α at lower temperatures than -2.2 C and vice versa. The next problem is the relative stability between γ and β , which did not reveal solid-state transformation. It was solved by examining the dissolution-nucleation experiments of β and γ at a fixed temperature in the solution. Many γ crystals were put in the decane solu-

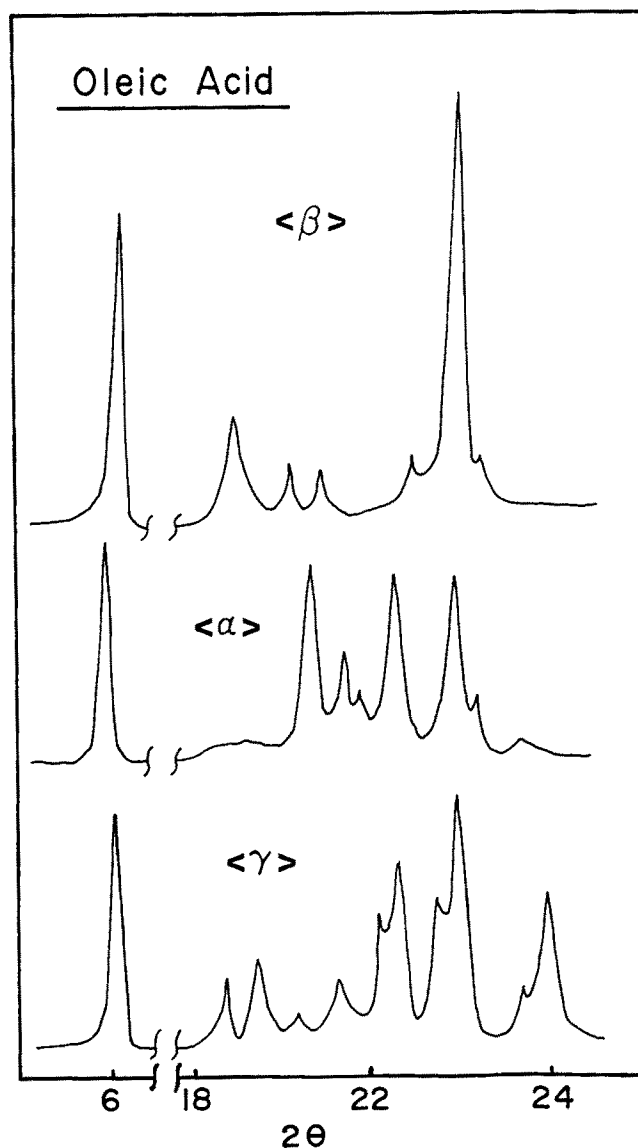


FIG. 6. X-ray diffraction patterns of α , β and γ polymorphs of oleic acid. The temperatures at which the data was taken were 10 C (β), 0 C (α) and -10 C (γ). CuK α ($\lambda=1.542$ A) with Ni-filter was employed.

TABLE I

X-ray Diffraction Spectra of Our Present Study and Lutton's (2)

	Present (Å)		Lutton ^b (Å)
β	41.2 (vs) ^a	high-melting	41.3 (w)
	4.63 (s), 4.37 (m), 4.17 (m)		4.66 (vs), 4.37 (m)
	3.87 (m), 3.68 (s), 3.38 (w)		3.67 (vs), 3.37 (w)
α	43.3 (vs)	low-melting	40.13 (vs)
	4.32 (s), 4.12 (w), 3.94 (s)		4.64 (m), 4.19 (s)
	3.71 (s), 3.49 (w)		3.80 (m), 3.59 (m)
γ	41.9 (vs)		
	4.71 (w), 4.52 (w), 4.16 (w)		
	3.94 (s), 3.72 (s), 3.42 (s)		

^aTo represent relative intensity of the spectrum, abbreviations are used, vs=Very strong, s=strong, m=medium and w=weak.

^bSome relatively strong peaks were presented selectively from the original paper.

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tion which was saturated with respect to the β form at -10 C. After stirring the solution for one night at the same temperature, we confirmed by X-ray diffraction that the polymorphic modification of the suspended crystals in the solution became the β form. This is attributed to the fact that the β crystals could nucleate and grow at the expense of the γ crystals because of the lower solubility (lower crystal free energy) of the β form than of the γ form. To verify this, we directly observed by optical microscopy that new β crystals nucleated and subsequently grew in the decane solution in which the crystallization of γ lasted a long time at -10 C (K. Sato et al., unpublished data). The same result was obtained for the growth of β at the expense of α in the acetonitrile solution at 4 C.

Consequently, it can be concluded that α and γ are less stable than β at temperatures from -20 to 13.3 C.

Here we discuss the thermodynamic stability in terms of a crystal Gibbs free energy which can be evaluated from the melting, phase transformation and solubility experiments presented above. Figure 7 depicts the Gibbs free energy relationship among α , β , γ and melt as a function of temperature. As it is inferred that the crossing point between the crystal free energies of α and β would lie above their melting points, there is no solid-state transition between α and β . Depending on the crystal free energy relationship, the polymorphic transformations occur in two different modes. One is the direct transformation as revealed for α and γ in the solid-state. The thermal hysteresis of its transformation temperature gave evidence that the transformation is of the first order. Another is the indirect transformation as revealed for α - β and γ - β , which was achieved by

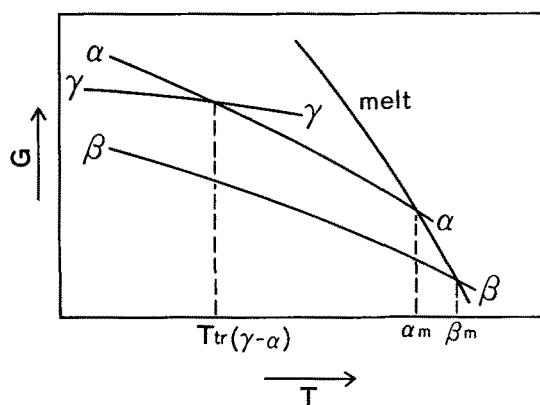


FIG. 7. Crystal free energy relationship of α , β and γ polymorphs of oleic acid. As a reference, the melt phase is also shown.

TABLE II

Thermodynamic Parameters of the Transformations of Oleic Acid, Stearic Acid, Tristearin and Triolein

	Oleic acid			Stearic acid ^a		Tristearin ^b	Triolein ^b
	$\gamma \rightarrow \alpha$	α (melt)	β (melt)	B \rightarrow C ^c	C (melt)	β (melt)	β (melt)
Temperature (C)	- 2.2	13.3	16.2	54	69.6	73	5
ΔH (kJ/mol)	8.76	39.6	51.9	4.3	61.3	191.4	107.8
ΔS (J/mol \cdot K)	32.3	138.4	179.3	13.1	293.4	553.1	387.6

^aDSC (13).

^bDSC (14).

^cA solid-state transition (13).

a mass transfer through appropriate medium (either melt or solution). We observed no solid-state $\alpha \rightarrow \beta$ transformation when α was kept at $0 \sim 10$ C over a few months, although Lutton reported that the lower-melting form transformed to the higher-melting form by holding the former one for six weeks at 10 C (2).

The overall polymorphic behavior of oleic acid is quite different from that of saturated fatty acids having similar numbers of carbon chains. For example, stearic acid has three typical polymorphs, A, B and C. The crystal free energies of these three polymorphs have crossing points around 30 C (7). On heating, the unstable polymorphs, A and B, at higher temperatures undergo the solid-state transformation below the melting point of the most stable form of C (69.6 C). Yet the overall polymorphic behaviors are similar to those of monoacid triglycerides. For example, tristearin has four polymorphs; α , β_1 , β_2 and β (8). β is the most stable, having the highest melting point. The other three are unstable. The crystal free energies of these four polymorphs have no crossing point at temperatures below the melting point of β . So, all the four polymorphs have their own melting points. Although the transformation from the unstable forms to the more stable ones occurs in the solid state of the triglycerides, the multiple melting phenomena of the oleic acid polymorphs has a closer similarity to the triglyceride polymorphs than to the saturated fatty acid polymorphs.

Thermodynamic data of the transformations of $\gamma \rightarrow \alpha$, $\alpha \rightarrow$ melt and $\beta \rightarrow$ melt are summarized in Table II together with those of stearic acid, tristearin and triolein. It is interesting that the $\gamma \rightarrow \alpha$ transition of oleic acid has enthalpy and entropy values about twice as large as the B \rightarrow C transition of stearic acid. Meanwhile, the enthalpy and entropy values of fusion of α and β of oleic acid are smaller than those of stearic acid C. This contrast may be characteristic for the polymorphic behavior of oleic acid in comparison to stearic acid.

As for the X-ray diffraction data, there are a few contradictions between the present results and the previous ones. First for β , our data are consistent with those of Lutton for both long and short spacings (2). Abrahamsson et al. measured (3) X-ray analysis of the single crystal of the lower-melting polymorph grown from organic solutions, obtaining *cis* conformation at the double bond and pseudo-orthorhombic unit cell (space group: $P2_1/a$). Despite the fact that this structure was referred to as the lower-melting form, we have found that its diffraction spots are quite consistent with our polycrystalline data of the β form having higher melting point. Our examination was done after the determination of the whole polymorphic behavior of oleic acid by DSC. Thus, we conclude that the polymorph of the crystal structure analyzed by Abrahamsson et al. was not for α

but the most stable β form. In their literature another crystal structure was reported once, but not reproduced. This description may let us conceive an idea that some misleading result could happen due to rather complicated thermal behaviors of oleic acid crystals.

As for α and β , no previous data was consistent with the present ones. Lutton's lower-melting form has no common spectra with ours in either the short or long spacings (2). Furthermore, no equivalent short spacing spectra was found for triolein (9) or 2-oleyl-distearin triglyceride (10). So, the subcell structures of the α and γ forms of oleic acid may be quite unique. Infrared studies on the oriented crystals of α and γ (11) suggest that the methylene chains are packed in a parallel way. This parallel packing is the same as that of β . The more detailed structures of α and γ need to be clarified by using single crystals.

Lastly, the crystallization rates were found to be dependent on the polymorphs. The β form nucleates and grows quite slowly in comparison to the α form. A direct in situ measurement on the growth rate from melt at ca. 10 C indicates (12) that α grows faster than β by a factor of 1000. This difference in the growth rates is enormous. No such an extraordinary feature in the polymorph-dependent crystallization behavior was obtained in the saturated fatty acids. Because the melting points of α and β differ by only 3 C, there is no appreciable difference in the values of supercooling. Some remarkable differences in the molecular structures of α and β may cause the results. Further studies on the details of the crystal structures, molecular structures and those dynamic aspects will be necessary. Some are in progress by our group.

ACKNOWLEDGMENTS

We are indebted to M. Okada of Hiroshima University for his encouragement. M. Kobayashi of Osaka University provided valuable discussion and unpublished data. K. Sato thanks the staffs of the Institute of Low Temperature Science, Hokkaido University, for providing him the facility to work on the crystallization experiments at very low room temperatures.

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ERRATUM

Two mistakes occurred in "Hydrazine Reduction in the Gas Liquid Chromatographic Analysis of the Methyl Esters of Cyclopropenoic Fatty Acids," which appeared on pages 1340 through 1343 of the September issue of *JAOCs*.

The 15th and 16th lines from the bottom of the left-hand column on page 1343 appeared erroneously. The sentences which include those lines should read: "Thus, the difference in the peak areas of 19:CA before and after

hydrogenation gives the true normalized peak area of 19:CE. The percentage of original 19:CE on the Supelco-wax-10 column was calculated to be 10%, which is in good agreement with the decomposition studies of authentic methyl sterculate described earlier."

In the following paragraph, the first sentence should read, "In addition to the hydrogenation of the cyclopropene ring, slight hydrogenation of 18:2n6 to form 18:1n6 and 18:1n9 occurred."

The paper was written by R. G. Ackman, J. Conway and W.M.N. Ratnayake.