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Ph.D. Thesis

Physical Study on Crystal Growth and Polymorphic Transformations in *Cis*-Monounsaturated Fatty Acids

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Chapter 1

Introduction

1.1 Critical Importance of Unsaturated Fatty Acids in Biophysical Science and Food Science

Fatty acids are important both as components of phospholipids and glycolipids and fuel molecules. They are characteristic building-block components of most of lipids. Many different kinds of fatty acids have been isolated from the lipids of various species. They are primarily distinguished from each other in the presence of their double bonds; called "saturated" containing only single bonds, or "unsaturated" with one or more double bonds. In general, unsaturated fatty acids are twice as abundant as saturated fatty acids in both animal and plant lipids.[1] The double bonds of naturally occurring unsaturated fatty acids are in the cis geometrical configuration, which produces a rigid bend in the aliphatic chain.

The cis-monounsaturated fatty acids are synthesized from acyl-CoA in biological cells requiring NADH (reducted nicotinamide adenine dinucleotide), molecular oxygen, and cytochrome b_5.[2] Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain. Hence, mammals cannot synthesize linoleic acid (cis-ω-6,9-octadecenoic acid) and linolenic acid (cis-ω-3,6,9-octadecenoic acid).

Because they are necessary precursors for synthesis of other products such as arachidonic acid (*cis*-ω-6,9,12,15-eicosenoic acid), these fatty acids are required in the diet and are obtained from plant sources. They are therefore called essential fatty acids.3) Currently, there are immense scientific and medical interests in metabolism of arachidonic acid since this fatty acid is the precursor of prostaglandins. Postaglandins perform critical physiological functions and, rapidly becoming the newest "miracle drugs" in medicine.4)

Biological membranes are essential components of all living systems and, as monitors of the external environment of cells, are required to perform numerous crucial functions. The membranes consist of a complex assortment of lipids and proteins, and the view as a dynamic structure is embodied in the fluid mosaic model of membranes, proposed by Singer and Nicholson.5) The membrane lipids, mainly phospholipids and glycolipids, have a polar head group and two fatty acyl chains. In general, its *sn*-2-position is shared by a *cis*-unsaturated fatty acid, to promote fluidity and permeability of the membrane through conformational flexibility of the *cis*-unsaturated acyl chains.6,7) Specific interactions between the *cis*-unsaturated membrane lipids and membrane proteins has been known to modify the membrane activity.8) Therefore, the cis-unsaturation has attracted much attention in biophysical sciences.9)

The *cis*-unsaturated fatty acids are important also in food science as component of fats and oil content of foods such as margarine, shortening, lard etc. The major components of fats and oil are triacylglycerols. In a triacylglycerol, three fatty acids are bonded by ester linkages to a glycerol molecule. Hence, the physical chemical properties of each fatty acyl cain must determine a total of physical and chemical properties of fats and oil. Actually, the traditional chemical engineering techniques used in oil industry, e.g. mixing, hydrogenation, interesterification, fractionation etc., aim at modifying the fatty acid and glyceride compositions, and let the products serve their suitable properties.

The crystalline structure of fats decisively influences their physical properties, being determined by interactions between the fatty acyl chains.10) For example, physical properties of chocolate are determined by the polymorphs of cocoa butter crystals , in which the presence of oleoyl chains in triacylglycerols play important roles.11-14)

1.2 *Cis*-monounsaturated Fatty Acids

Figure 1-1 displays four principal *cis*-monounsaturated fatty acids which have been dealt with in the present work: oleic acid (*cis*-ω9-octadecenoic acid), erucic acid(*cis*-ω-9-docosenoic acid), petroselinic acid (*cis*-ω-12-octadecenoic acid), asclepic acid (*cis*-ω-7-octadecenoic acid). Oleic acid has been elucidated most intensely, since it is present in nature most abundantly, and thereby has attracted highest scientific as well as technological attention. Erucic acid is one of the important vegetable oil components. In our study, this substance has been chosen to comparatively examine effects of the difference in a total number of hydrocarbon and also in a relative position of the double bond on polymorphism in comparison to oleic acid: e.g., $C_{22:1}$,ω9, erucic acid has a double bond located closer to a CH_3-end group than oleic acid ($C_{18:1}$,ω9) in which the double bond is located at the central portion of the

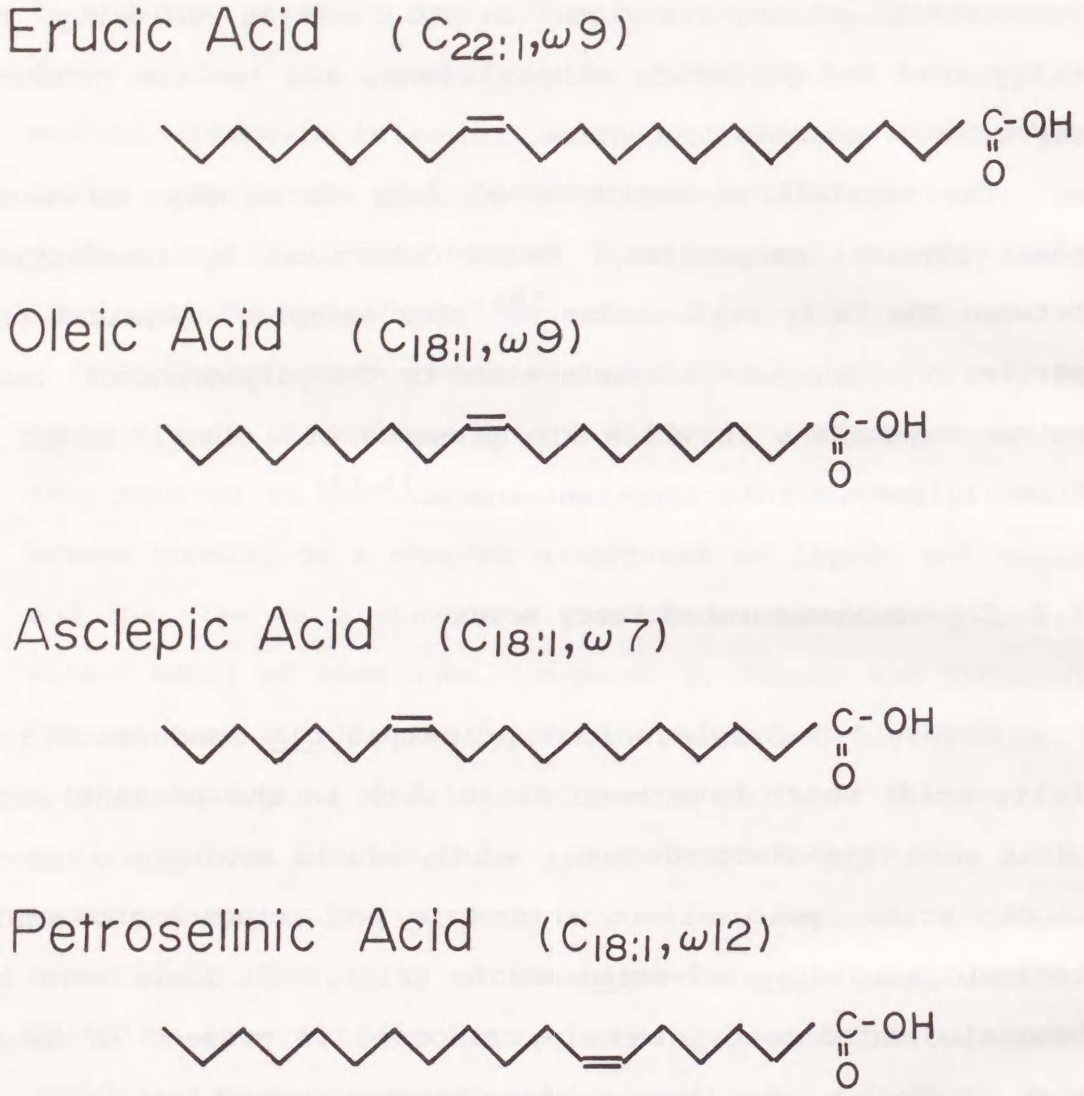


Figure 1-1 A schematic illustration of five *cis*-monounsaturated fatty acids.

aliphatic chain. Asclepic acid and petroselinic acid have been studied to examine the effects of the position of the double bond with the same aliphatic chain length as oleic acid. There have so far been little knowledge about this problem.

1.3 Polymorphism

To elucidate the functional roles of the cis-unsaturation of the aliphatic chains, **polymorphism** must be studied on a series of cis-unsaturated fatty acids in the crystalline phase. The polymorphism means occurrence and transformation of different crystal structures of a substance, under varying sets of thermodynamic and kinetic conditions (temperature, pressure, supercooling, supersaturation, etc.).10) A total behavior of polymorphism is directly related to the nature of molecular interactions in the crystal. Thus, comparative studies for the polymorphism of various cis-unsaturated fatty acids enable us to highlight the critical influences of cis-unsaturation on physical chemical properties of cis-unsaturated fats and lipids.

1.4 Review of Previous Studies

Despite of its critical importance, the polymorphism of cis-unsaturated fatty acids had been rather poorly understood: crystal structures,15-18) and Raman scattering study19) until a systematic research by Sato, Suzuki and Kobayashi initiated in 1985. The highest barrier, that has hindered molecular-level investigation of these compounds, may be difficulty in preparing highly pure samples which is necessary to obtain well-grown single crystals for X-ray diffractometry and vibrational spectroscopy. Furthermore, the polymorphism and polymorphic transformations are very complicated compared to those of saturated fatty acids, and are remarkably influenced by the contamination of small amounts of impurities.

Recently, Suzuki succeeded in preparing ultra pure samples of a series of cis-monounsaturated fatty acids. Then, Suzuki, Ogaki and Sato clarified for the first time a total of polymorphic behavior of oleic acid.20) They isolated three polymorphs of oleic acid, and examined the thermodynamic stabilities and polymorphic transformations by means of differential scanning calorimetry. In combination with this study, Kobayashi et al. clarified the molecular structures of three polymorphs using vibrational spectroscopy.21)

In these studies, a reversible order-disorder transformation of new type was discovered, occurring between gamma (order) and alpha (disorder) polymorphs in oleic acid. This transformation induced, in the disordered alpha form, conformational disordering at an aliphatic chain segment between a cis double bond and a CH_3 end group (omega-chain), keeping the conformation of the aliphatic chain segment between the double bond and the CH_3 end (delta-chain) ordered. They called this phenomenon, "interfacial melting", which assumes molecular fluidity at the omega-chain segment due to an introduction of the cis-double bond, as reflected on Raman scattering and entropy of fusion21): (a) After the transformation from gamma-form to alpha-form, a Raman C-C stretching band exhibiting the conformation of the omega-chain drastically decreased in intensity, whereas the corresponding Raman band of the delta-chain unchanged. (b) The entropy of fusion of alpha-form of oleic acid was smaller than that of beta-form, which assumes all trans conformation, by 25 %, due to the disordering in the omega-chain.20) The alpha-form, however, is not equivalent to a lamellar-type lyotropic liquid crystal of polar lipids, in which gauche conformation is extended over the all aliphatic chains.22) The major difference is that alpha-form contains a rigidly packed delta-chain and a "liquid-like" omega-chain which is located at the lamellar interface in the dimerized crystalline structure. Hence, the "melting" behavior of alpha-form has so far been observed only with spectroscopic and thermal methods (Figure 1-2). The vibrational spectroscopic study of Kobayashi's group has been extended to erucic acid, petroselinic acid and asclepic acid.[23] In parallel, to the spectroscopic study, crystal structure analysis using single crystals have also been carried out on the above cis-monounsaturated fatty acids.[23]

The effects of hydrostatic pressure on the gamma-alpha transformation in oleic and palmitoleic acids were examined by means of differential thermal analysis (DTA).24,25)

Quite recently, Iwahashi et al. found that oleic acid has three kinds of liquid structures depending on temperature: (1) in a range of temperature from melting point (13 C) to 30 C, the melt consists of clusters of quasi-smectic liquid-crystal: (2) in a temperature range between 30 and 55 C, the melt consists of clusters of a nematic-type liquid crystal: (3) An isotropic liquid occurs above 55 C.26) This anomaly in the melt structure is thought to be caused by a cis-double bond involved at the central position of an aliphatic chain of oleic acid.

Complexity of this kind in physical properties of oleic acid has never been observed in that of saturated fatty acids.27) The unique problem may thereby arise that the posi-

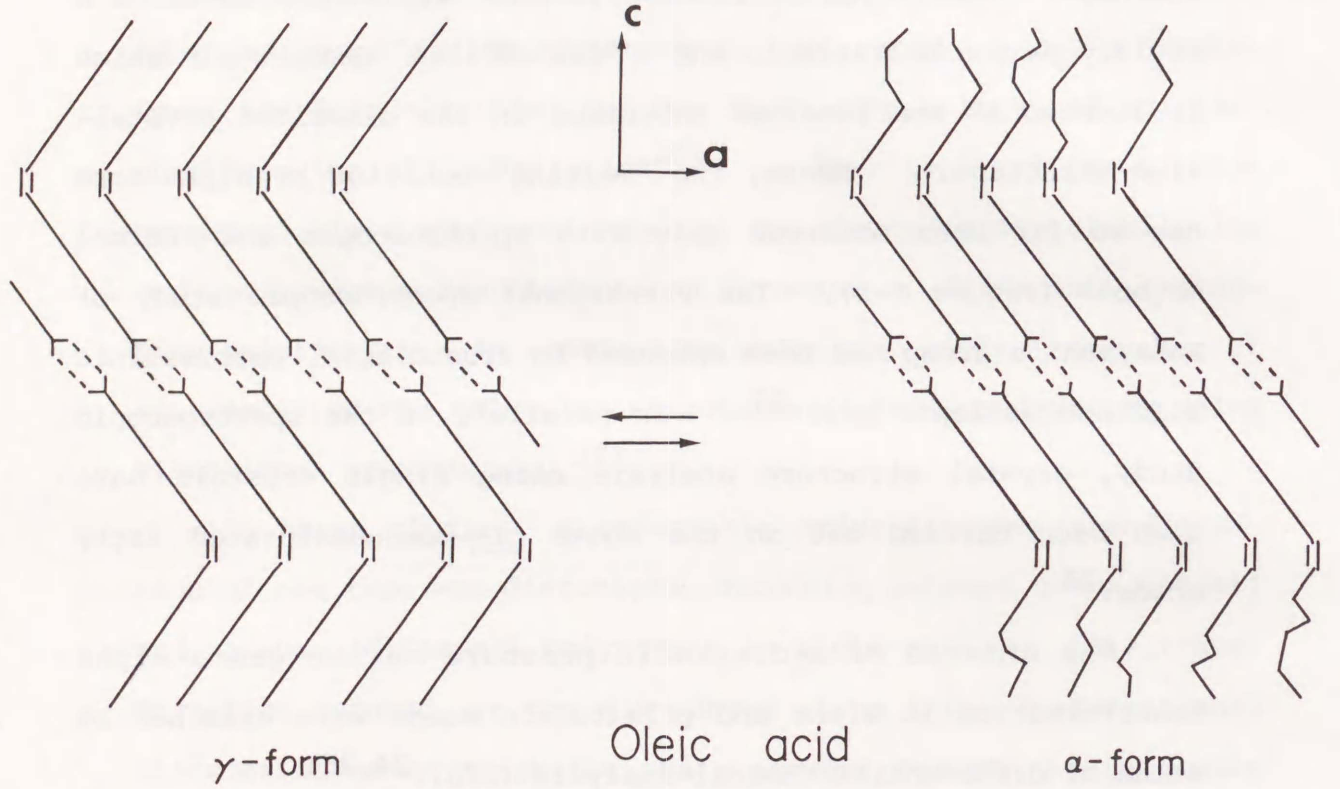


Figure 1-2 A schematic illustration of γ-α transformation in oleic acid.

tion of cis double bond in the aliphatic chain must be one of the key parameters to determine physical behavior in the polymorphism.[28)] In this regard one must remember the fact that the ability of a lipase from microorganisms to hydrolyze triacylglycerols is dependent on the position of the cis double bond.[29)]

To approach this we studied the polymorphic transformations of a series of cis-unsaturated fatty acids including erucic acid , petroselinic acid and asclepic acid by using X-ray diffractometry, polarized optical microscopy, differential scanning calorimetry and vibrational spectroscopy. Furthermore, to elucidate the positional effect of a cis double bond on the crystal growth kinetics, we measured crystal growth rates and nucleation rates of the polymorphs of oleic acid and erucic acid. The relationship between the melt structure and crystal growth mechanism was then examined in oleic acid. Finally, the interactions between cis-unsaturated fatty acids were discussed by observing phase behavior of binary mixtures of oleic acid and its positional isomers by thermal and structural measurements.

1.5 Methodology and Physical Backgrounds

a. Samples

All the fatty acid samples (>99.9% purity) we employed here were supplied by Nippon oil and Fats Co. The purity was determined by gas liquid chromatography (Shimadzu GC-9A or GC-14A; column SP-2340 or SP-2560, Supelco Inc.). Acetonitrile (99 % purity, Nakarai Chemicals) and n-decane (99 % purity, Tokyo Kasei Co.) were used as solvent.

b. Elements for Characterization of Polymorphism in Unsaturated Fatty Acids

To characterize the polymorphism of unsaturated fatty acids, we have taken into account of the following five elements. Two of them are regarding crystal structure; (1) subcell structure and (2) chain inclination, and the rest are related to molecular structures; (3) conformation of aliphatic chain, (4) conformation of an olefin group and (5) conformation of a COOH group.

b.1 Subcells

Most hydrocarbon chains in crystalline long-chain compounds are packed according to several possible ways. The best method to describe the chain packing is to employ a concept of subcell which was developed by Vand in connection with his X-ray diffraction studies of soaps.30) So far, theoretical analyses31-33) and reviews on the subcells have been described.9,34-36)

The subcell is defined by three axes: c_s for the translation between equivalent position within a chain and a_s and b_s for the lateral translations and three interaxial angles. Subcell with triclinic (T), orthorhombic (O), monoclinic (M), and hexagonal (H) symmetry were defined. It is recognized that the hydrocarbon chain packing defined by the subcell has the plane of the carbon-carbon-carbon zigzag of neighboring chains in either a mutually parallel (//) or perpendicular (⊥) orientation.9) The subcell structure can experimentally be speculated by means of powder X-ray diffraction short spacing, or polarized FT-IR, and determined exactly by means of X-ray single crystal analyses. In Figure 1-3, typical subcells are illustrated.

b.2 Chain Inclination

Due to the fact that the molecular interactions along the lateral direction in fatty acid molecule is stronger than that along the chain direction, the basic crystal structure of fatty acids is of lamella arrangement. It is recognized that the molecules are dimerized through hydrogen bond at their COOH groups. Hence, a plane between two lamella is constructed by methyl end groups. As shown in Figure 1-4, difference in crystal structure varies with the chain inclination. Therefore, the chain inclination can be an element which characterizes the polymorphism. The thickness of the lamella can be measured by X-ray long spacing diffraction.

b.3 Conformation of an Aliphatic Chain

In the most cases, saturated aliphatic chains assume the all-trans zigzag conformation in the most stable form of the crystalline phase. In the case of unsaturated fatty acids, however, the aliphatic chains do not always reveal the all-trans conformation in the crystalline state. A conformational disordering occurs in the aliphatic chain, as observed in alpha-form of oleic acid. Its conformation is understood that gauche conformers exist randomly at C-C bonds of the aliphatic chain.[21)] Therefore, the interfacial melting can be an additional element which characterizes the polymorphism.

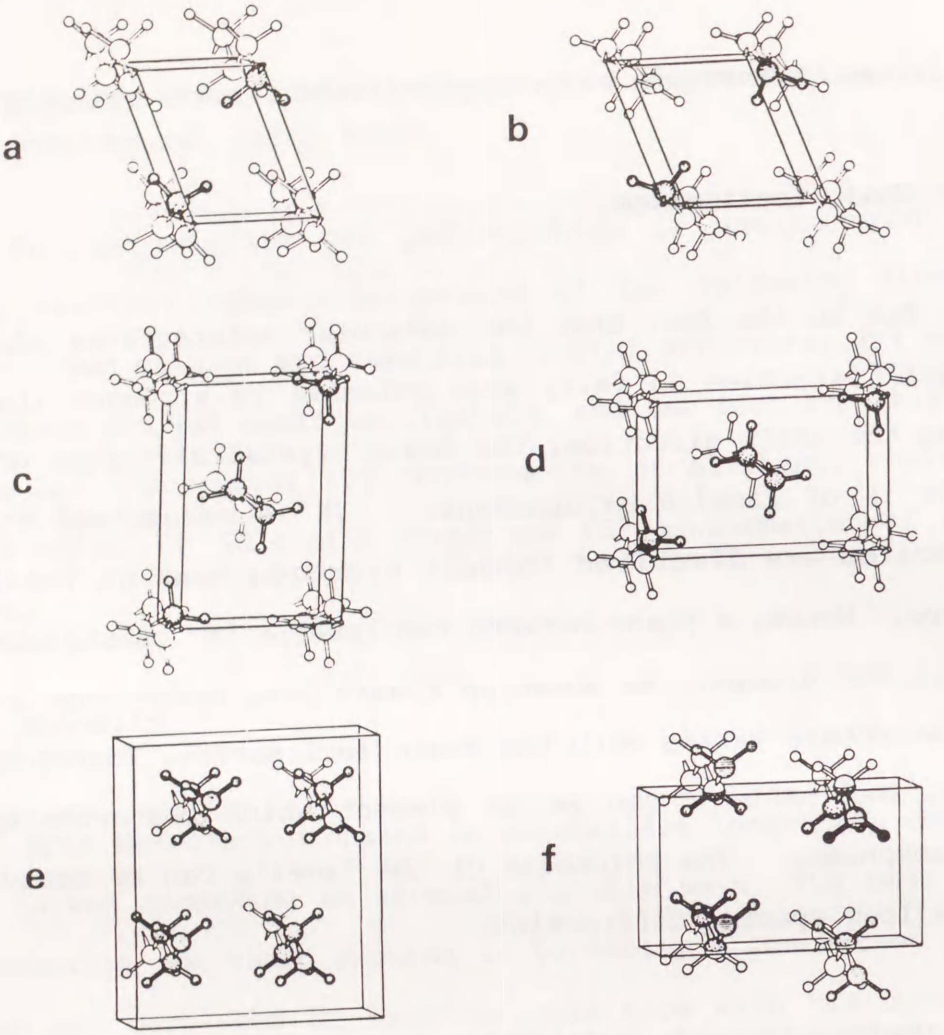


Figure 1-3 Simple hydrocarbon chain subcells. (a) $T_{//}$ type, (b) $M_{//}$ type, (c) $O_{\perp}$ type, (d) $O'_{\perp}$ type, (e) $O_{//}$ type, (f) $O'_{//}$ type. (From Ref. 9)

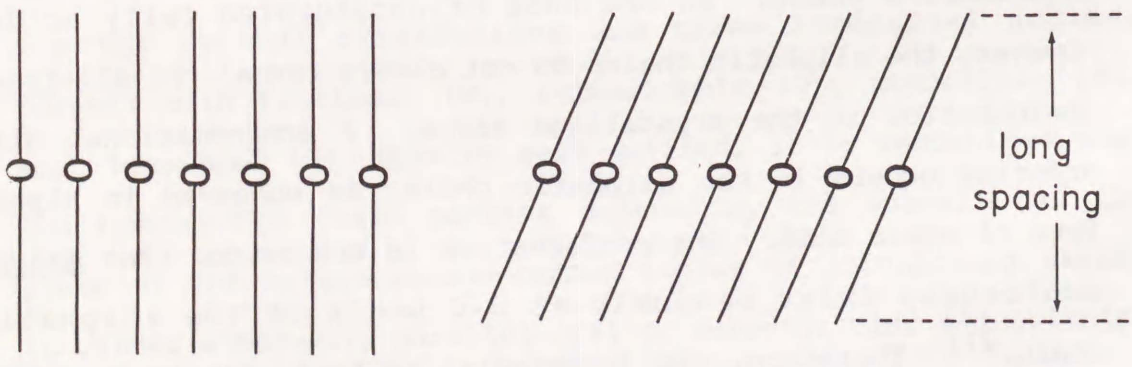


Figure 1-4 Chain inclination as an element for characterization of polymorphism of lipids.

b.4 Conformation of an Olefin Group

In the crystal structure of cis-unsaturated fatty acids, there exist structural variations in conformation of olefin group; the internal rotation of the C-C bonds linked to the C=C bond. In the case of oleic acid skew-cis-skew type (beta) and skew-cis-skew' type (gamma and alpha) were observed (Figure 1-5).

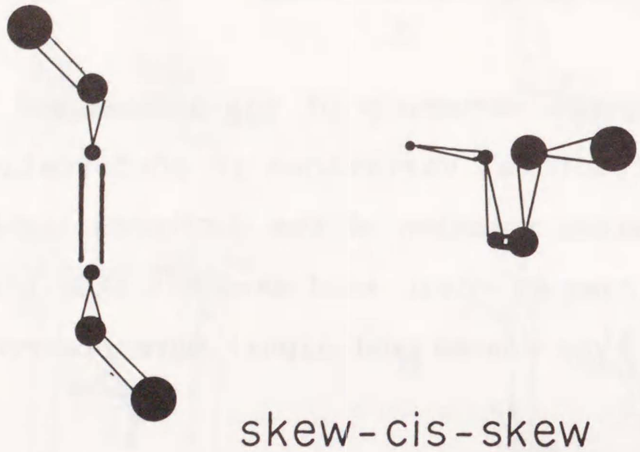
b.5 Conformation of a COOH Group

There also arises a structural variation with respect to the angle between the plane of the carboxyl dimmer and zigzag skeletal plane of the hydrocarbon chain. In the case of co-planar structure, there are two stable conformations, cis and trans (Figure 1-6).[23,37,38] Although both in the variations have been observed in the saturated fatty acids, the cis conformer has not been observed in the unsaturated fatty acids examined up to present.[39-40)

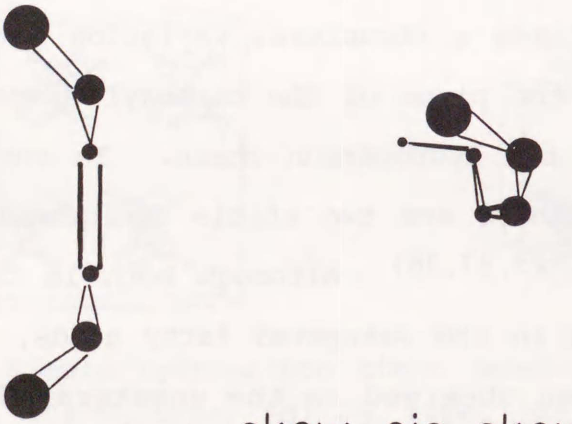
c. Methodology in Structural Analysis

c.1 X-ray Diffraction

To determine crystal structures, X-ray diffraction method using a single crystal is recognized to be the most powerful and direct one. However, there are various experimental difficulties in the direct application of X-ray three-dimensional structural analysis using single crystals to the lipid system. The polymeric feature of lipid compounds is very complicated and contamination with different modifications occurs very often,



skew-cis-skew



skew-cis-skew'

Figure 1-5 Two different conformation of an olefin group, skew-cis-skew type and slew-cis-skew' type.

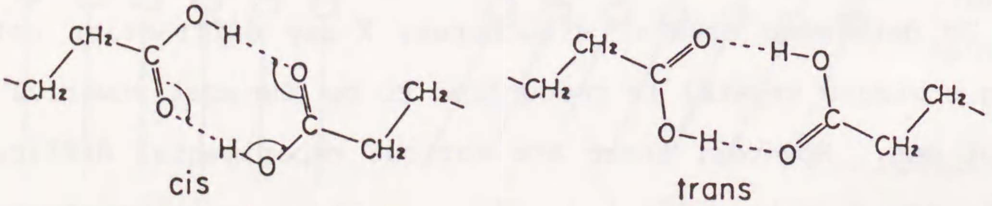


Figure 1-6 Two different conformation of dimerized carboxyl group, cis and trans. (From Ref. 41)

so that preparation of well-grown single crystals of particular modifications is rather difficult[36].

In the case of powder X-ray diffraction method, experimental difficulties are rather eliminated, although the number of detailed structural information is limited. It gives us some important informations such as long and short spacings. The lamella structure of long-chain compounds give rise to the long spacings, d_{hkl},001, observed in the spectra pattern of lower 2Θ range. These spacings are large in comparison to the other hkl diffractions and can be accurately measured and indexed. They have been used, in conjunction with other data, to calculate the carbon-carbon bond angles in the chain, the chain inclination with respect to the plane at the ends of the molecule, and the length the aliphatic chain.[9] The short spacing pattern is also obtained in the powder X-ray diffraction spectra pattern. These spacings correspond to the lateral packing of the aliphatic chain exhibited by the subcell. They also can be employed to identify the polymorphic modifications.

c.2 Vibrational Spectroscopy

Along with X-ray diffraction method, vibrational spectroscopy (infrared and Raman spectroscopies) is a powerful tool for the investigation of molecular-level structures in various physical states. With this method one is able to get valuable information of many kinds: molecular structures (functional groups, bond orders, conformations, hydrogen bonds), aggregation states of molecules (types of molecular packing, molecular motions), strength of the intra- and intermorecular forces, and so on. Furthermore, spectral data, particularly those in the low-frequency region, are related directly to the physical properties of bulk materials.[41)]

The following Raman-active modes are very sensitive to the conformational changes occurring in the hydrocarbon chain; antisymmetric CH_2 stretch $\nu_a(CH_2)$ (2885 cm^{-1}), CH_2 twisting $t(CH_2)$ (1298 cm^{-1}), symmetric CC stretch $\nu_s(CC)$ (1130 cm^{-1}), and antisymmetric CC stretch $\nu_a(CC)$(1060 cm^{-1}). They give rise to strong and sharp bands in highly crystalline samples, and smear into broad bands in molten states, where the molecules assume disordered conformation of a random mixture of the <u>trans</u> and <u>gauche</u> conformers. As to the subcell, the parallel (//) and perpendicular ($\perp$) alignments of carbon chains produce remarkably different patterns of band splitting due to interchain interactions. In the $O_\perp$-type subcell, the band associated with a molecular mode splits into a doublet of different polarizations. Furthermore, the conformation of an olefin group can be determined from frequency changes in CH stretch, C=C stretch, C=C-C bend, CH in-plane bend, and CH out-of plane bend modes.[41)]

c.3 Morphology

In the case of long-chain compounds, the shape of a single crystal generally exhibits a tabular shape with well-developed basal surface which is presumably normal to the long-chain axis. This is due to the fact that specific lamella-lamella interactions via CH_3-group planes are weaker than the lateral interactions. It is recognized that the equilibrium shape of the basal surface reflects the lateral packing, i.e. subcell structure.

Changes in the crystal morphology during the crystal growth process teach us the change in crystal growth mechanism.

d. Solubility

Solubility data are not only necessary to grow a single crystal, but also important to know valuable thermodynamic parameters describing the polymorphism of each substance. The relationship of Gibbs free energies between polymorphs can be determined by measuring the solubilities. Gibbs free energy of solute in nonideal solution at a given temperature T is expressed by

$$G = G^0 + RT \ln X + RT \ln \lambda \qquad [1.1]$$

where X is a molar fraction of the solute, λ is activity coefficients of the solute, G^0 is Gibbs free energy of the solute molecules in pure melt state, and R the gas constant. Here, molal Gibbs free energy of crystal, G^S and that of solute have the same value at equilibrium condition:

$$G^S = G = G^0 + RT \ln X + RT \ln \lambda \qquad [1.2]$$

Assuming that the state of solute molecules in the solution does not depend on the polymorphic form, the values of G^0, λ, R and T are the same for each polymorph. Hence, only the difference in molar fraction between the different polymorphs determine the difference in their Gibbs free energies; i.e., the polymorph having the lower solubility is thermodynamically more stable.

Furthermore, we may calculate the entropy (ΔS_d) and enthalpy (ΔH_d) of dissolution from the temperature dependence of solubility. Substituting the solubility for the vapor pressure, Clausius-Clapeyron equation is developed as

$$\Delta S_d = \frac{\Delta H_d}{T} = RT \left(\frac{\partial \ln a}{\partial \ln X}\right)_T \left(\frac{\partial \ln X}{\partial T}\right)_{\Delta G_d} \qquad [1.3]$$

where ΔG_d is free energy of dissolution and a is relative activity. Therefore, the entropy and enthalpy of dissolution may be calculated from the temperature dependence of solubility and the change in relative activity with concentration.42) The term involving the change in relative activity with concentration may be similar to the change of fugacity with vapor pressure, and has a value close to unity at low concentrations.43)

e. Crystal Growth

e.1 Nucleation and Crystal Growth

Crystallization involves two elementary processes: nucleation and crystal growth (Figure 1-7). Nucleation occurs when the solution or melt phases deviate from thermodynamic equilibria to greater extent. In more specified words, when supercooling of the melt or supersaturation of the solution, which are the driving force of crystallization, exceed certain critical values under a given set of external conditions, crystallization initiates. Three actual nucleation phenomena occur in a real system.44) They are homogeneous nucleation which occurs in a very pure system and at a higher driving force. Heterogeneous nucleation predominates either in an impure system or at a lower driving force. Secondary nucleation which becomes

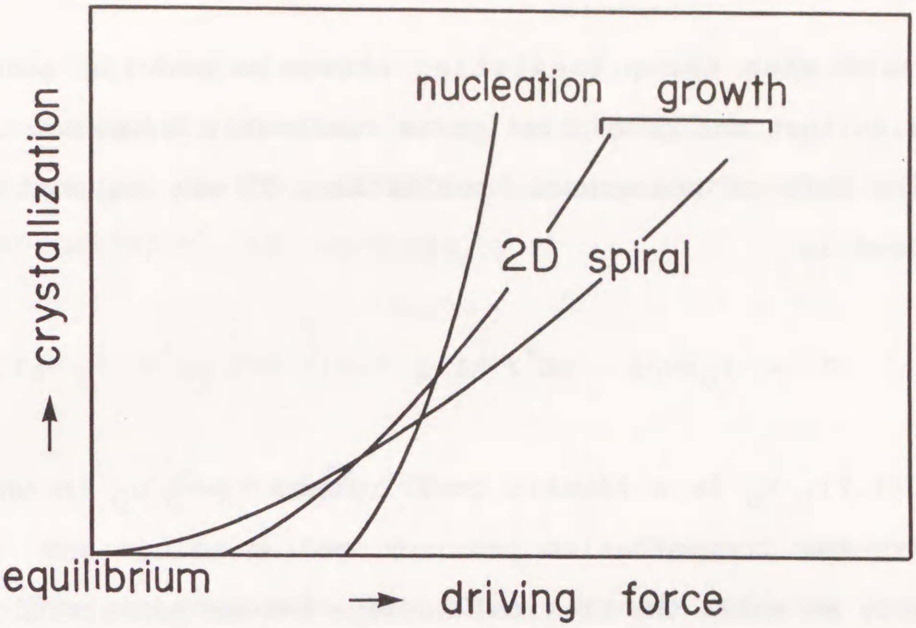


Figure 1-7 The rate of nucleation and crystal growth as a function of a driving force of crystallization. 2D is a two-dimensional nucleation growth, and spiral, is a spiral growth.

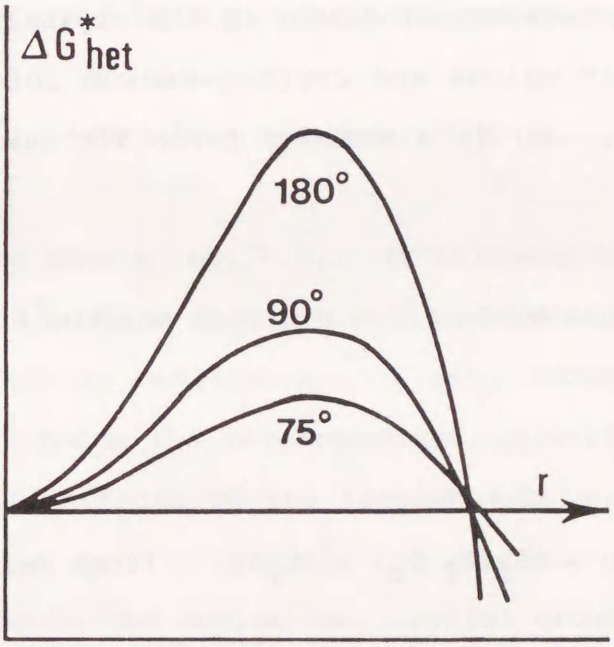


Figure 1-8 Activation free energy of heterogeneous nucleation, ΔG^*_h, as a function of a radius of a crystal nucleus. Decrease in ΔG^*_h with decreasing α value of eq.[1.9] is illustrated. (From Ref. 44)

important when the pre-existing crystals provide secondary nuclei so that the precipitation is remarkably enhanced.

The rate of homogeneous nucleation, J, may be most simply expressed as

$$J = K_0 \exp[- \Delta G^{*} / kT] \qquad [1.4]$$

In eq.[1.4], K_0 is a kinetic coefficient, $K_0 = N_0\ \nu_0$ in which N_0 is a monomer concentration per unit medium volume and ν_0 the frequency at which the critical nucleus become supercritical and transforms into the crystal. ΔG^{*} is an activation free energy for a formation of a critical crystal nucleus,

$$\Delta G^{*} = f\ \Omega^2\ \gamma^3 / (\Delta\mu)^2 \qquad [1.5]$$

where f is a geometrical factor of the crystal nucleus, Ω and γ are molecular volume and crystal-medium interfacial energy, respectively. $\Delta\mu$ is a driving force for nucleation which is expressed as

$$\Delta\mu = kT \ln \beta \qquad \text{(from solution)} \qquad [1.6]$$

and

$$\Delta\mu = \Delta S_f (T_f - T_c) = \Delta S_f \Delta T \qquad \text{(from melt)} \qquad [1.7]$$

where β is supersaturation ratio, ΔS_f entropy of fusion, T_f temperature of fusion, T_c crystallization temperature, ΔT supercooling. The factors to increase J for a given substance are both of thermodynamic and of kinetic nature; higher T_c, larger

$\Delta\mu$ and N_0, whereas smaller γ, all of which are thermodynamic parameters. The kinetic factor is involved in ν_0 which is determined by a reaction of monomer dimer cluster critical nucleus whose radius, r^*, is expressed by

$$r^* = 2\Omega\gamma / (\Delta\mu) \qquad [1.7]$$

$$\Delta G^* = (4\pi r^{*2}\gamma)/3 \qquad [1.8]$$

The heterogeneous nucleation depends on the interfacial relationship between crystal/medium, crystal/foreign substance and medium/foreign substance. The simplest consideration leads to a conclusion that an activation free energy of the heterogeneous nucleation, ΔG^*_h is expressed in terms of a contact angle α , which is made in a cusp-shaped crystal nucleus composed of the crystal/ foreign substance/ medium system,[44)]

$$\Delta G^*_h = \Delta G^*[\ 1/4(\cos^3\alpha) - 3/4(\cos\alpha) + 1/2\ ] \qquad [1.9]$$

where ΔG^* is the same as eq.[1.5]. It followed that, when α=180 degree, $\Delta G^*_h = \Delta G^*$, since the interaction between the solute and foreign substance is negligible. With decreasing α value, ΔG^*_h decreases, hence the heterogeneous nucleation is enhanced due to catalytic effects of the foreign substance as shown in Figure 1-8.

In contrast to the nucleation, crystal growth may occur at a relatively small driving force. A spiral growth occurs when the driving force is very small, since the surface steps are provided from screw dislocations protruding onto the growing surface. An alternate growth, two-dimensional (2D) growth, needs a certain amount of driving force, since newly-appearing surface nuclei must be formed onto the perfect crystal surface.[44)]

It follows that, in the actual crystallization processes, the different crystallization mechanisms in Figure 1-7 may occur simultaneously even in the same crystallization system, depending on varying driving forces.

e.2 Polymorphic Crystallization

Figure 1-9 exemplifies a typical crystallization behavior of two polymorphs, which are tentatively named A and B. A is more stable than B thermodynamically. The rates, however, both of nucleation and crystal growth are higher in B than A, as frequently observed. We attempt this general consideration, since many results of the unsaturated fatty acids examined in the present study can be interpreted according to the following idea.

Usually the crystallization prevails in the more stable polymorph at small driving forces. However, with raising driving force, the crystallization rate of the less stable polymorph, B, increases. Eventually the crystallization of B exceeds A both in the nucleation and crystal growth as shown in Figure 1-9(a). Therefore, it follows that the overall feature of crystallization is highly dependent on the magnitude of the driving force. For example, the concentration of the first-crystallized form at a fixed driving force changes from A to B with increasing driving force, as illustrated in Figure 1-9(b). Thus, the preferential crystallization of a particular polymorph is caused in a limited range of the driving force, where the

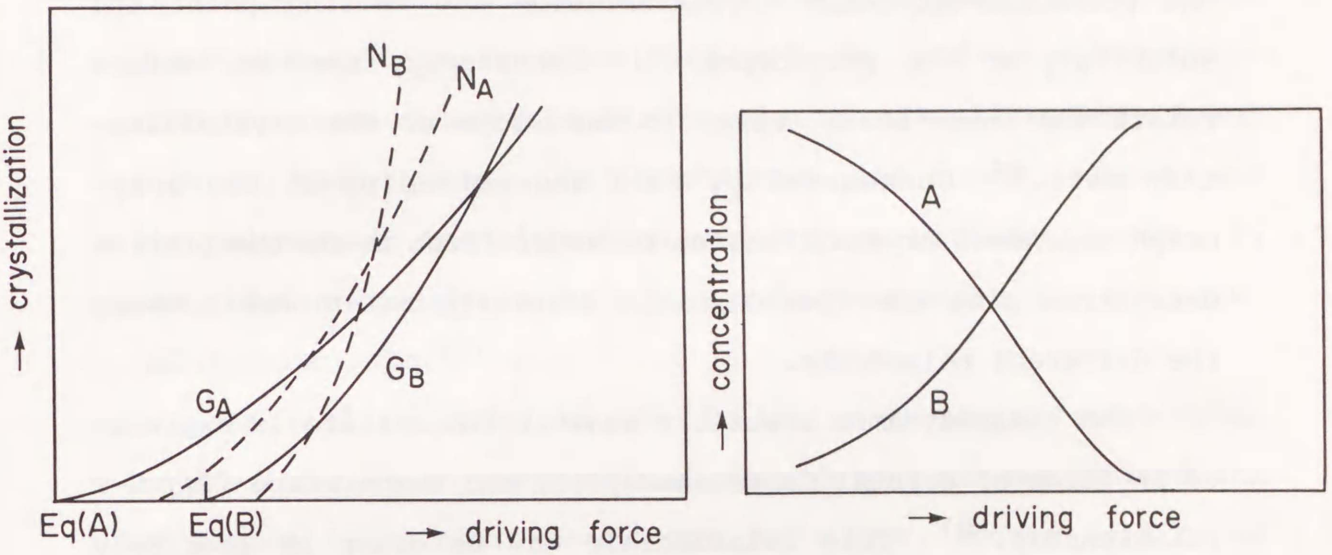


Figure 1-9 Rate of crystallization, (b) concentrations of crystallized materials of two different polymorphs, tentatively named A and B.

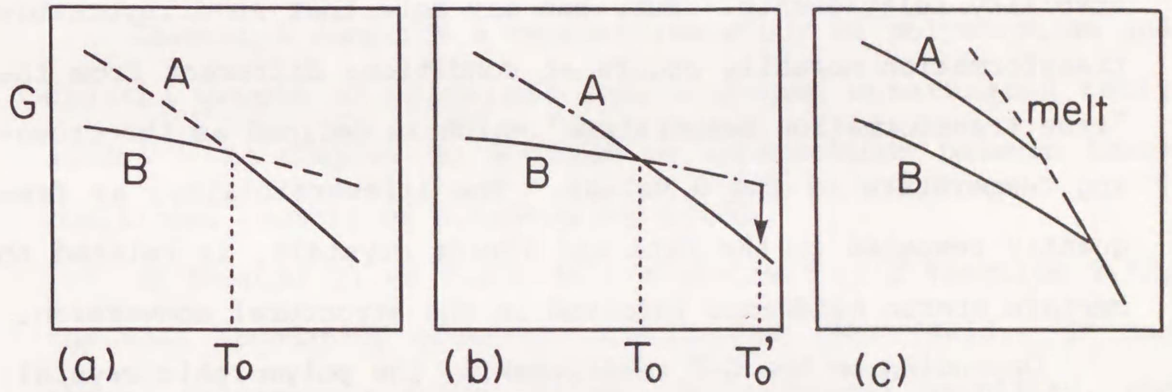


Figure 1-10 Classification of the relationship between crystal Gibbs energy (G) and temperature (T) for two polymorphs, A and B, and melt. T_0, crossing point of G(A) and G(B), T_0'an irreversible transformation temperature from B to A.

nucleation and crystal growth of that form are predominant.

The most decisive thermodynamic parameters in controlling the polymorph-dependent crystallization are melting point and solubility of the polymorphs. Conversely, kinetic factors relate the interfacial energy to the slope of the crystallization rate.[44] Consequently, full understanding of the polymorph-dependent crystallization behavior first needs the precise data concerning the thermodynamic stability relationship among the different polymorphs.

The thermodynamic stability against temperature is expressed in terms of crystal Gibbs energy (G) and temperature (T); G-T relationship.10) This relationship may be drawn by precisely measuring the melting point and solubility of each polymorph individually. The higher the melting point and, at the same time, the lower the solubility, the more the thermodynamical stabilitty. The transformation pathways among the different polymorphs in a crystalline state provide additional data on the stability relationship. But, one may note that an irreversible transformation normally occurs at conditions different from the "true transformation temperature" which is defined as the crossing temperature of the G values. The irreversibility, as frequently revealed in the fats and lipids crystals, is related to certain steric hindrance involved in the structural conversion.

Depending on the G-T relationship, the polymorphic crystallization are exemplified in three cases in Figure 1-10, using two polymorphs of A and B. In the first two cases, (a) and (b), the crossing temperature, T_o, of G(A) and G(B) is below the melting points of the two forms. In (a), the reversible transformation causes the crystallization of a single polymorph; A above T_o or B below T_o. In (b), the irreversible transformation occurs from B to A at T_o'. Hence two polymorphs can be crystallized simultaneously below T_o'. In the last case (c), A is always less stable than B, since the two forms have the same G value far above their melting points. If no solid-state transformation occurs, it is possible to crystallize both A and B at any temperature. On the contrary, the crystallization and the polymorphic transformation may compete with each other, in case that A transforms to B at a rate comparative to the crystallization rate.[45)]

As will be shown in the following chapters, polymorphic crystallization in oleic acid alpha and beta is categorized (c) of Figure 1-10, oleic acid gamma and alpha, erucic acid gamma and alpha (a), erucic acid alpha and alpha_1, petroselinic acid LM and HM (b).

1.6 Polymorphism of Principal Cis-monounsaturated Fatty Acids - Present Study -

Chapter 2 concerns a comparative study of polymorphism and crystal growth of principal four cis-mono unsaturated fatty acids. In Chapter 3, a study on interactions between three positional isomers of octadecenoic acids.

In Chapter 2, we first deal with oleic acid (section 2.1), the most abundantly occurring unsaturated fatty acid. It has three polymorphic forms, whose thermodynamic stability, and crystallization kinetics are discussed in terms of thermal property, molecular structure and polymorphic transformation.

Having greater concerns to the positional effects of the cis-double bond, we further describe erucic acid (section 2.2), petroselinic acid (section 2.3) and asclepic acid (section 2.4):

erucic acid has the same length of omega-chain as that of oleic acid, but the delta-chain is longer by 4 carbons. The later two acids, however, are positional isomers of oleic acid; omega-12 position of a double bond in petroselinic acid, and omega-7 position in asclepic acid. In these sections, it will be described that the position in the cis-double bond drastically influences major aspects of the polymorphic behavior.

Finally, we will deal with the behavior of melting and order-disorder transformations in the two binary systems of petroselinic acid/oleic acid and asclepic acid/oleic acid in Chapter 3.

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Chapter 2

Polymorphism of Principal Unsaturated Fatty Acids

2.1 Oleic Acid

a. Thermal and Molecular Properties of Oleic Acid Polymorphs

Three polymorphs of oleic acid, alpha, beta and gamma, were confirmed by means of differential scanning calorimetry,1) X-ray diffraction,1) IR and Raman spectroscopy2) and polarized optical microscopy.1) The transformation circuit of the three polymorphs is depicted in Figure 2.-1. Alpha-form is crystallized by chilling the melt, since it is thermodynamically metastable. Alpha- and gamma-forms undergo a reversible transformation in the solid-state at -2.2 C on heating. Gamma-form is the form whose structural determination was done in 1962.3) Beta-form, the most stable polymorph, crystallizes with very slow rates, both from solution and the melt phase. There is no solid-state transformation from alpha (or gamma) to beta-form in the melt-grown crystal due to a steric hindrance. Instead, the solution mediates the conversion. Lutton's "high-melting form"4) is equivalent to beta-form, as evidenced by melting point and X-ray diffraction spectra. Alpha-form, however, is contradictory to his X-ray data on "low-melting form", although they have the same melting point.

The single crystal shapes of the three forms are shown in

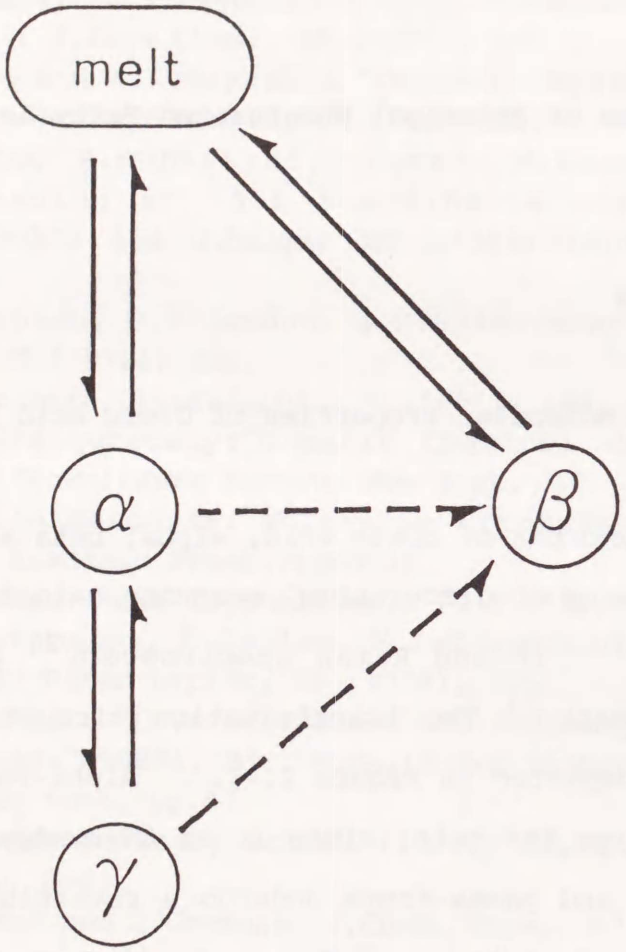


Figure 2-1 A transformation circuit of three polymorphs of oleic acid.

Figure 2-2. All the crystals were of a tabular shape with a well-developed basal surface in a slightly supersaturated solution. Alpha-form reveals a slender hexagonal shape, while beta-form shows a truncated lozenge shape. The truncation occurs normal to a bisectrix of 55^0. Gamma-form reveals a rectangular shape which is consistent with the subcell structure of $O'_{//}$.3) The morphology of the three forms changes drastically to needle shape when the supersaturation of solution or supercooling is increased. Figure 2-3 and Table 2-1 show the X-ray diffraction patterns and the relative intensities of three polymorphs. The short spacing spectra of gamma-form corresponds to the subcell of $O'_{//}$. The other two forms reveal remarkably different patterns, implying different subcell structures. The long spacings are 4.34 nm (alpha), 4.12 nm (beta) and 4.19 nm (gamma).

Vibrational spectroscopic studies on alpha, beta and gamma forms of oleic acid may be summarized in the following:2)

(a) the gamma-alpha transformation is of an order(gamma)-disorder(alpha) type which is accompanied by a conformational disordering in the portion of aliphatic chain between the double bond and the terminal methyl group (omega-chain), keeping the conformation of the aliphatic chain portion between the double bond and the COOH end (delta-chain) ordered. The most conspicuous spectral change is seen in the low-frequency Raman spectrum (Figure 2-4b). All the sharp bands of gamma-form collapse to a broad band in alpha-form due to a loss of translational symmetry. This originates from a disordered structure. Additionally, a peculiarity, indicating the same conformational disordering, appeared in two strong bands due to a C-C

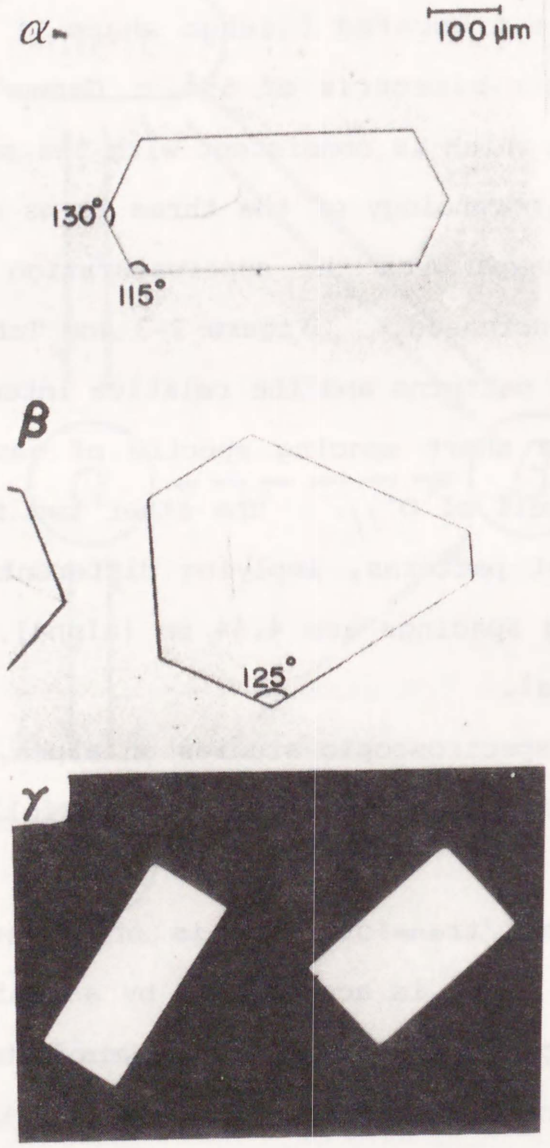


Figure 2-2 Crystal habits of alpha-form (4 C in acetonitrile), beta-form(7 C in acetonitrile) and gamma-form (-5 C in decane) of oleic acid.

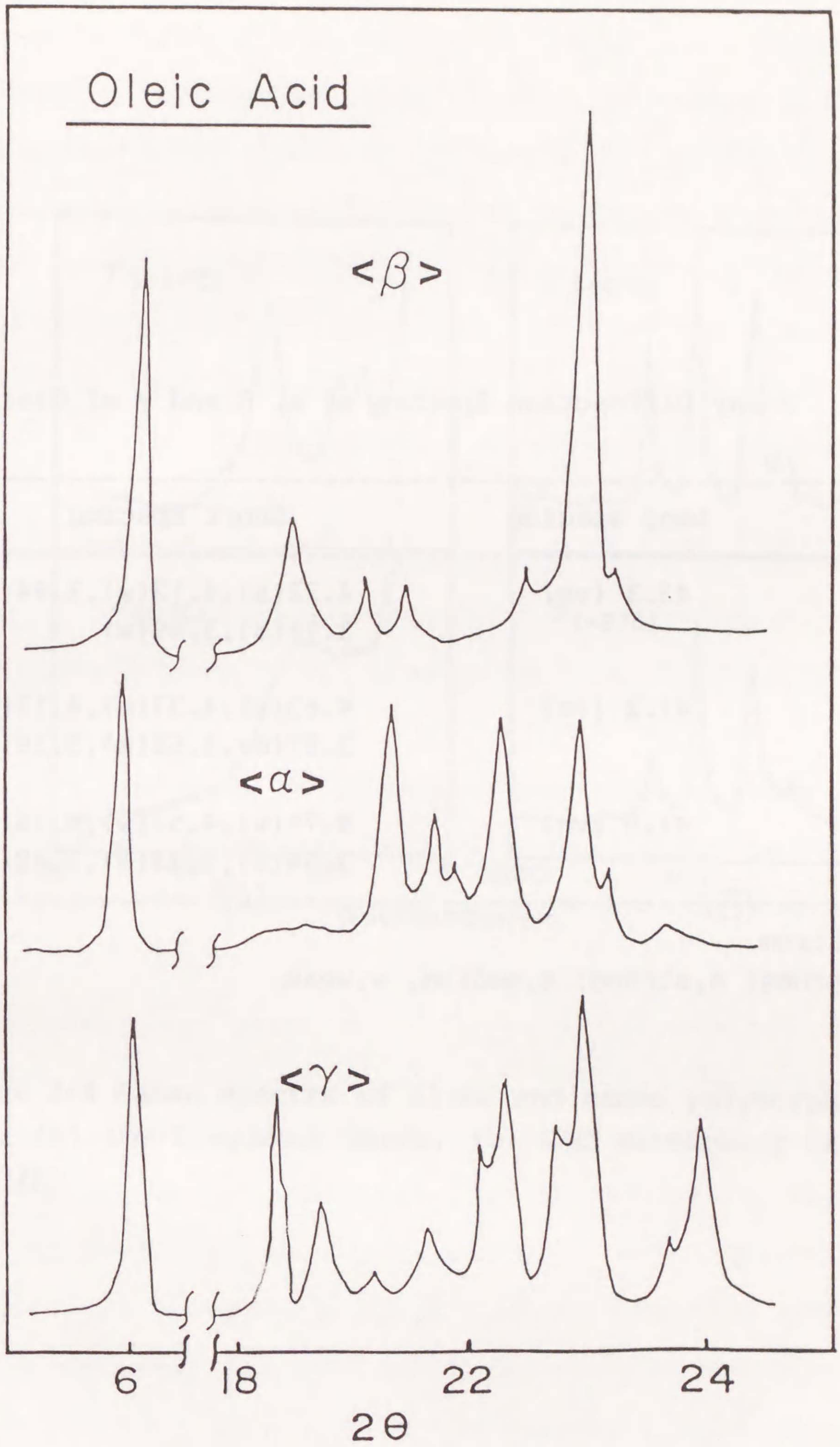


Figure 2-3 X-ray diffraction patterns of alpha, beta and gamma polymorphs of oleic acid.

Table 2-1 X-ray Diffraction Spectra of α, β and γ of Oleic Acid

	Long spacing	Short spacing
α	43.3 (vs)	4.32(s),4.12(w),3.94(s) 3.71(s),3.49(w)
β	41.2 (vs)	4.63(s),4.37(m),4.17(m) 3.87(m),3.68(s),3.38(w)
γ	41.9 (vs)	4.71(w),4.52(w),4.16(w) 3.94(s),3.72(s),3.42(s)

Unit, Angstrom.
va,very strong; s,strong; m,medium, w,weak.

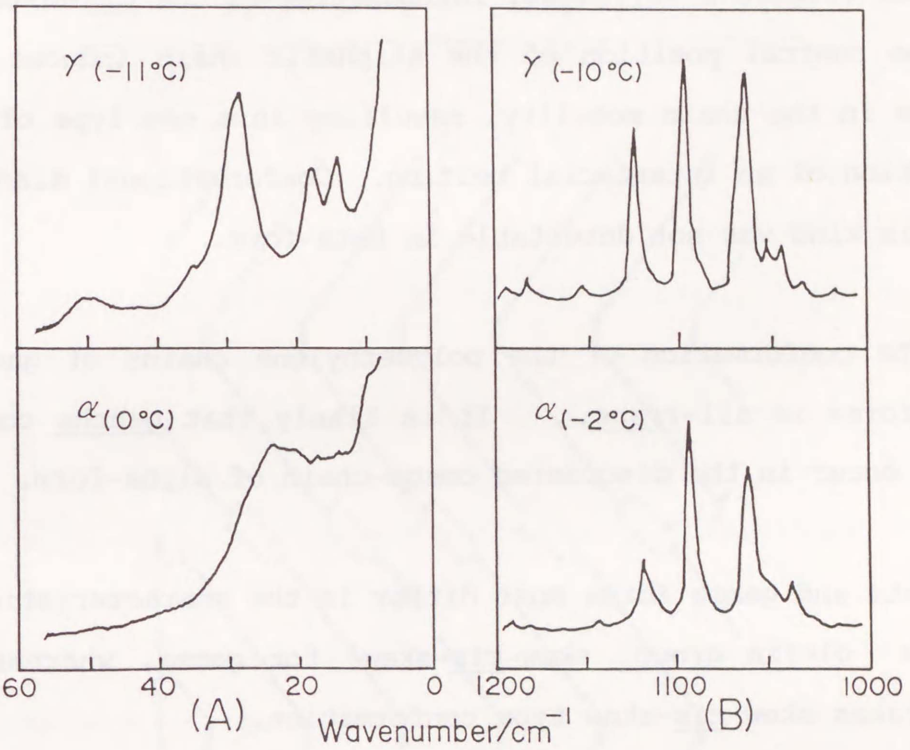


Figure 2-4 Raman spectra of alpha and gamma polymorphs of oleic acid, (a) low-frequency bands, (b) C-C stretching bands. (From Ref. 2)

streching mode. This mode is reflected in a single band of saturated acids. After the gamma-alpha transition, the 1125 cm^{-1} band of omega-chain drastically decreased in intensity, whereas the 1095 cm^{-1} band due to the delta-chain remained unchanged (figure 2-4b). Thus, introduction of one cis-double bond at the central position of the aliphatic chain induces an increase in the chain mobility, resulting in a new type of transformation of an interfacial melting. Conformational disordering of this kind was not detectable in beta-form.

(b) The conformation of the polymethylene chains of gamma and beta forms is all-trans. It is likely that gauche conformations occur in the disordered omega-chain of alpha-form.

(c) Beta and gamma forms most differ in the characteristic bands of the olefin group; skew-cis-skew' for gamma, whereas beta-form takes skew-cis-skew type conformation.

(d) As for the subcell structure, gamma-form shows spectral bands characteristic of $T_{//}$. This supports the $O'_{//}$ subcell, because $O'_{//}$ may be included in the category of $T_{//}$. The beta-form assumes a specific subcell structure differing from $O'_{//}$ and $T_{//}$, according to C-C progressive bands reflected in IR spectra. The inferred subcell of beta suggests that the C-C zigzag planes of neighboring chains are not parallel to each other but, somewhat inclined.

Figure 2-5 and Table 2-2 show the crystal structure and the crystal data of gamma-for of oleic acid.[3)] The unit cell belongs to a monoclinic (or a pseudo-orthorhombic) system forming a layered structure. The conformation of the olefin

Table 2-2
Crystal Data of Oleic Acid Gamma-form
(From Ref. 3)

a (A)	9.51
b	4.74
c	40.6
β (degree)	90
V (A^3)	1830
Z	4
Space group	$P2_1/a$

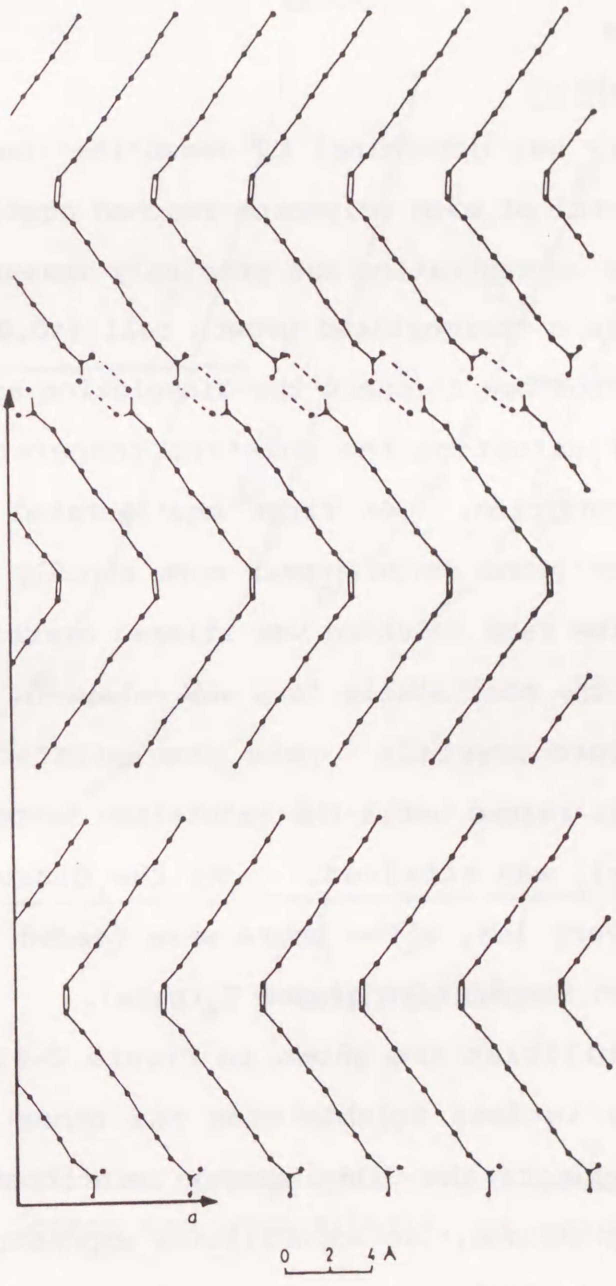


Figure 2-5 Crystal structure of gamma-form of oleic acid. (From Ref. 3)

group is described as $+133^0$ (nearly skew(S)), cis(C), and -133^0 (S'), or SCS'. With this particular conformation, the hydrocarbon chains at the both side of the olefin group become coplanar with each other. Such a coplanar molecule stacks above the other in the direction parallel to the b axis, constructing the $O'_{//}$ subcell arrangement.5)

b. Solubility

Solubility was determined by measuring the temperature at which the crystal of each polymorph reached equilibrium with the solution whose concentration was precisely measured. The solution was put in a thermostated growth cell (±0.05 C). We used an optical microscope to check the dissolution and growth of the crystals by fluctuating the solution temperature around the equilibrium condition. We first equilibrated the metastable forms, alpha or gamma which appear more rapidly than the beta-form. Then the same solution was stirred overnight so that the occurrence of the most stable form was enhanced. After all the excess beta-form crystals were precipitated, the solution temperature was raised until the saturation temperature of beta-form, T_S(beta), was attained. As the dissolution rate of beta-form is very low, a few hours were needed for the equilibration at each temperature around T_S(beta).

The solubilities are shown in Figure 2-6. It is clear that beta-form is less soluble than the other two Figure 2-6 forms. This reflects the Gibbs energy relationship among three polymorphs. In decane, two solubilities exponentially increased in an almost parallel way. The difference in T_S(gamma) and T_S(beta) at a fixed concentration was 4.9 ± 0.1 C. In

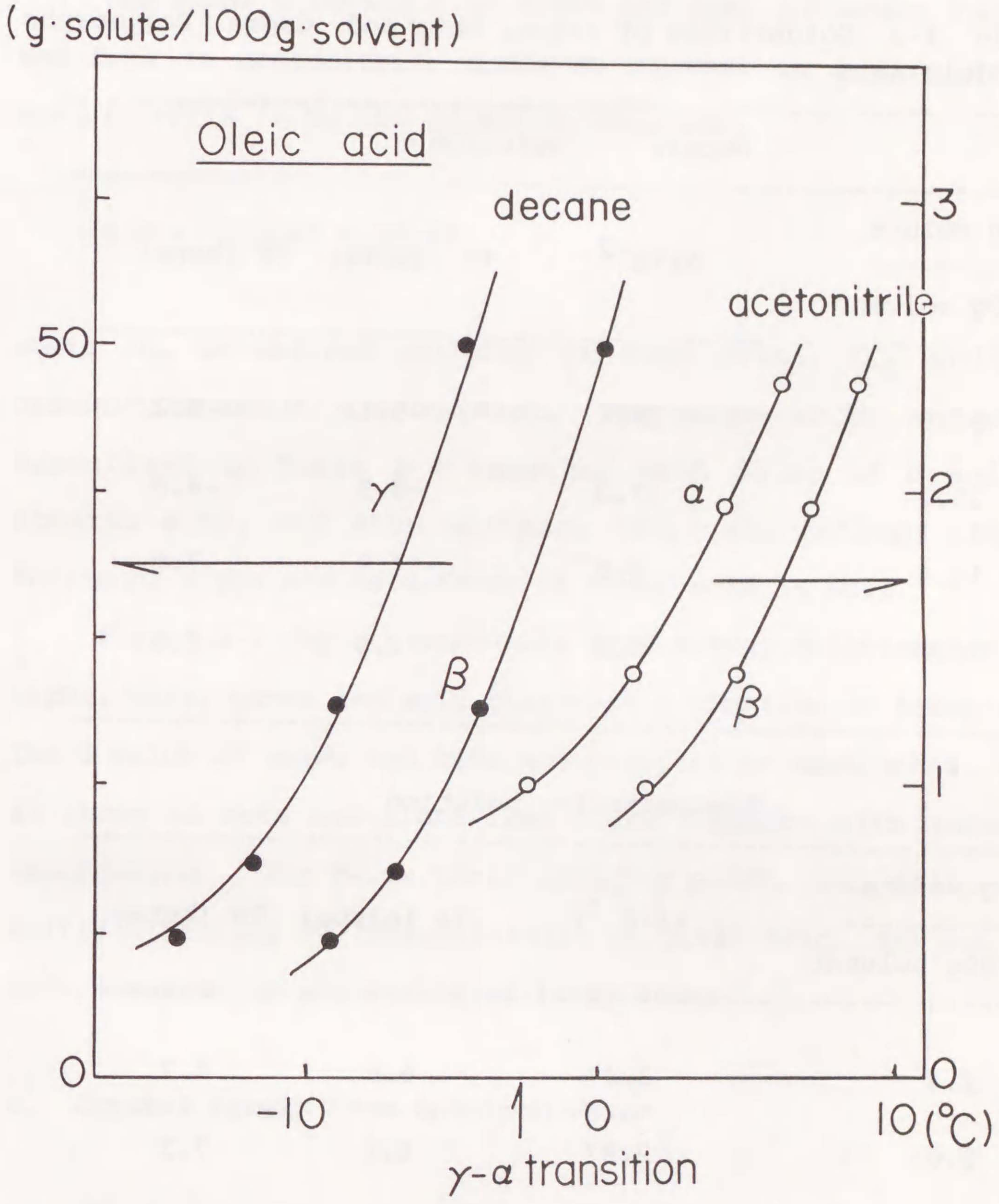


Figure 2-6 Solubilities of alpha, beta and gamma polymorphs of oleic acid in decane and acetonitrile, as expressed in molar fraction.

Table 2-3 Solubilites of Alpha, Beta and Gamma Polymorphs of Oleic Acid

Decane solution

g solute / 100g solvent	$X(10^{-2})$	Ts (gamma)	Ts (beta)
50.0	20.1	-5.1	-0.2
25.2	11.3	-8.9	-4.0
14.4	6.8	-11.9	-7.0
10.0	4.8	-13.5	-8.6

Acetonitrile solution

g solute / 100g solvent	$X(10^{-3})$	Ts (alpha)	Ts (beta)
2.4	3.47	6.0	8.7
2.0	2.87	4.1	7.3
1.4	2.10	1.1	4.5
1.0	1.46	-2.2	1.5

X, molar fraction, Ts, saturate temperture (^{0}C) acetonitrile, the difference in T_s(alpha) and T_s(beta) became a bit smaller with increasing concentration or temperature. The data are displayed in Table 2-3.

The molar fraction X of gamma and beta in decane and alpha and beta in acetonitrile could be plotted in a straight line against $1/T(K^{-1})$ by the following equation:

$$\ln X = -\Delta H_d/RT + \Delta S_d/R \qquad [2\text{-}1]$$

where ΔH_d is defined enthalpy of dissolution, ΔS_d entropy of dissolution and R gas constant. The values of ΔH_d and ΔS_d are summarized in Table 2-4 together with those of B and C of stearic acid, and with enthalpy (ΔH_f) and entropy (ΔS_f) of fusion of alpha and beta-forms of oleic acid as well.

Figure 2-7 depicts the Gibbs free energy relationship among alpha, beta, gamma and melt phases as a function of temperature. The G value of gamma and beta are parallel to each other, whereas those of beta and alpha come close together with increasing temperature. Far below their crossing point, they melt. This multiple melting is characteristic of oleic acid, and has never been observed in the saturated fatty acids.

c. Crystal Growth from Solution Phase

Crystal growth rates from solution phase were measured for alpha and beta forms independently as a function of supersaturation. After a single crystal was put in a supersaturated solution, the growth rate of the lateral face was measured by using polarized optical microscope equipped with a video system. The supersaturation was controlled by varying the temperature of the

Table 2-4 Entharpy (ΔH) and Entropy (ΔS) for Dissolution for Alpha, Beta and Gamma of Oleic Acid and for B and C of Stearic Acid, Fusion of Alpha and Beta of Oleic Acid and for a Solid State Transformation From Gamma to Alpha

	Oleic acid							Stearic Acid	
	Dissolution							Dissolution	
	Decane		Acetonitrile		Fusion		Trans-formation	Decane	
	γ	β	α	β	α	β	$\gamma \rightarrow \alpha$	B	C
ΔH(kJ/mol)	100	100	59.4	76.0	39.6	51.9	8.8	138	128
ΔS(J/mol K)	360	352	222.9	279.7	138.4	179.3	32.3	410	379

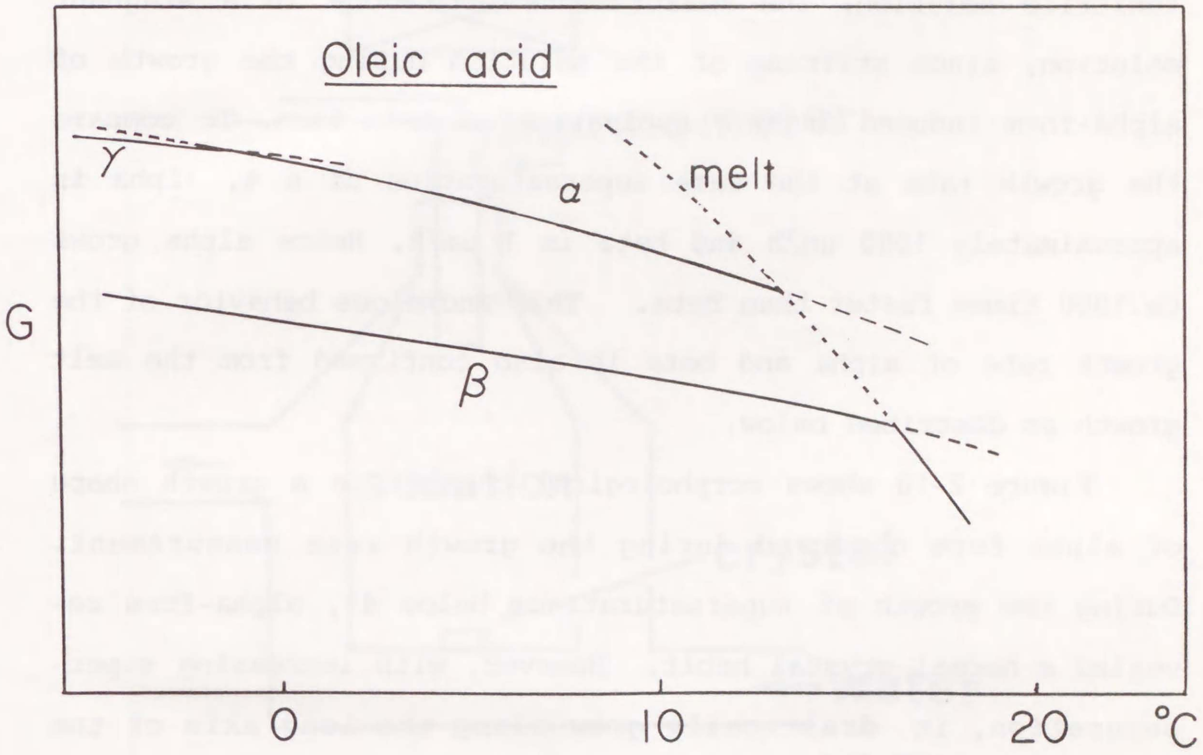


Figure 2-7 Crystal free energy relationship of alpha, beta and gamma polymorphs of oleic acid. As reference, the melt phase is also shown.

solution whose initial solute concentration was precisely measured. Figure 2-8 shows a schematic illustration of an experimental equipment. The solution was sealed in a grass-made cell whose temperature was controlled by thermostated water (± 0.02 C). Figure 2-9 and Table 2-5 show the growth rate of lateral face of single crystals of alpha and beta forms in acetonitrile solution. The measurements were done in a stagnant solution, since stirring of the solution during the growth of alpha-form induced drastic nucleation of beta-form. To compare the growth rate at the same supersaturation of 6 %, alpha is approximately 1000 μm/h and beta is 1 μm/h. Hence alpha grows ca.1000 times faster than beta. This anomalous behavior of the growth rate of alpha and beta is also confirmed from the melt growth as described below.

Figure 2-10 shows morphological changes in a growth shape of alpha-form observed during the growth rate measurement. During the growth at supersaturations below 4%, alpha-form revealed a normal crystal habit. However, with increasing supersaturation, it drastically grew along the long axis of the crystal, and the interface became rough, as shown in Figure 2-9 by two arrows at the supersaturation values for the two polymorphs. It seems that the crystal growth mechanism may be changed at these supersaturation values where the growth rate also drastically increased.

As to the beta-form, similar morphological change was observed. Figure 2-11 shows the morphological change of a single crystal of beta-form observed during the measurement of growth rates from solution phase. At low supersaturations, a single crystal of beta-form grew keeping the truncated lozenge shape. However, with increasing supersaturation, the growth

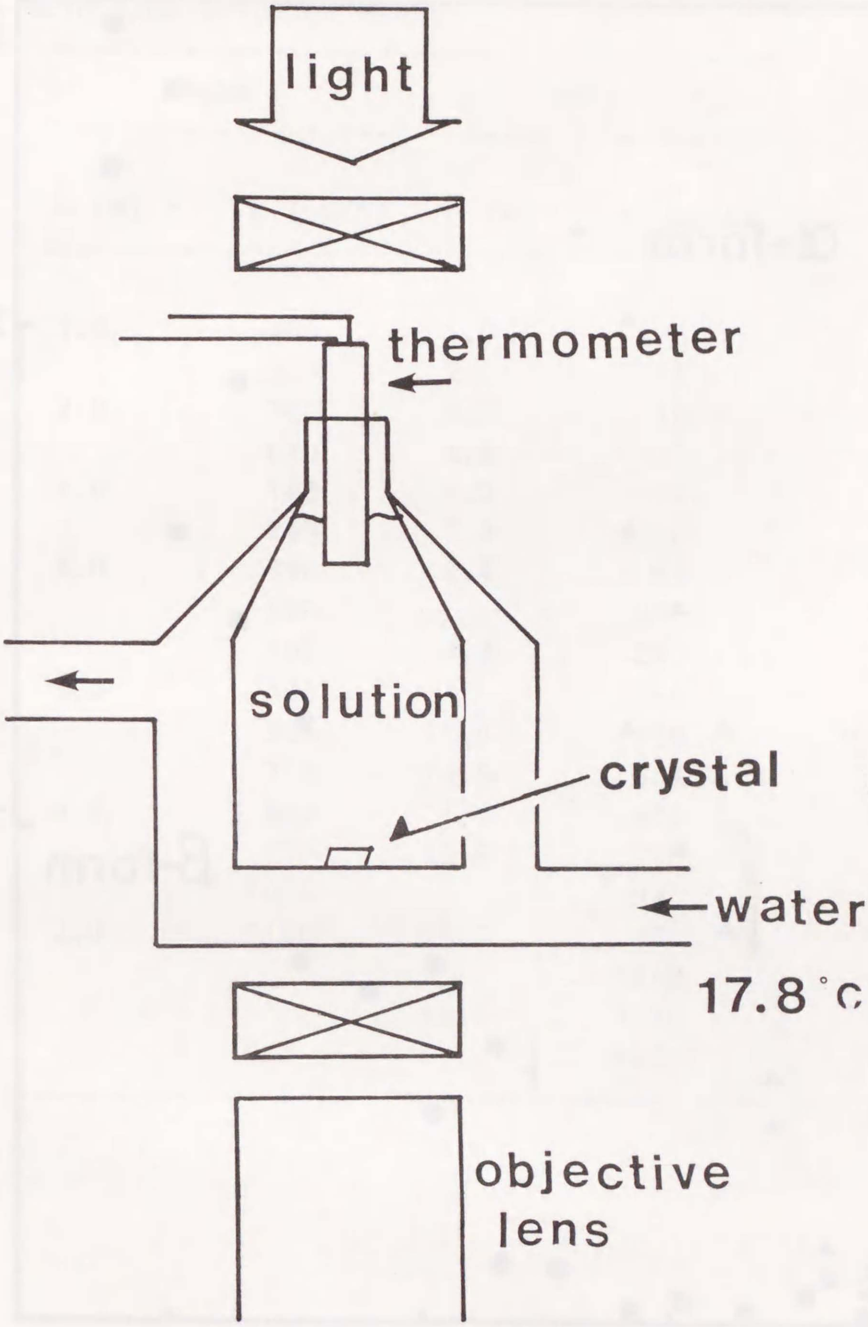


Figure 2-8 A measurement system for growth rate of a single crystal from solution phase.

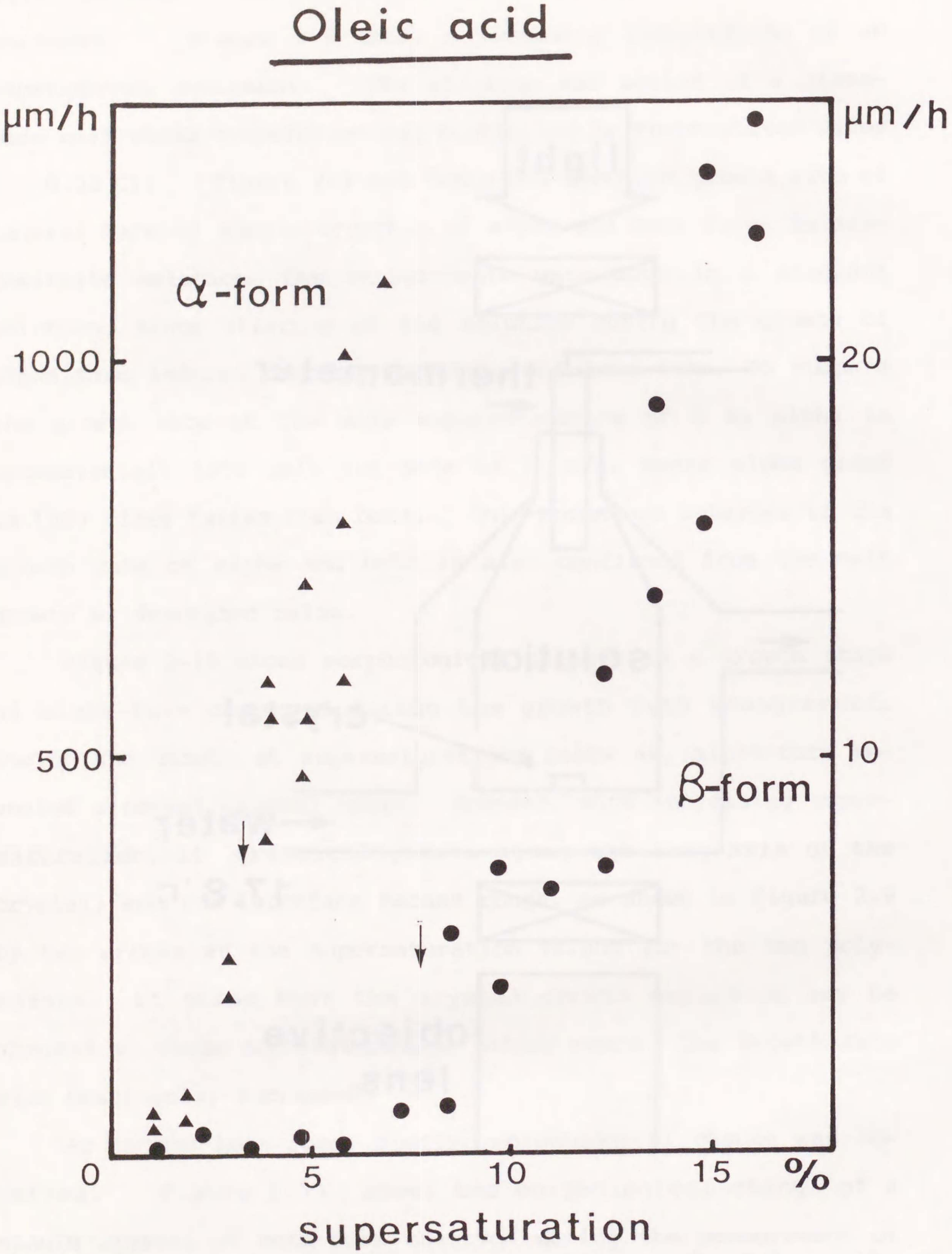


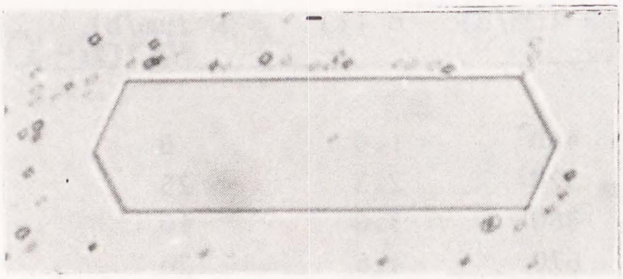
Figure 2-9 Crystal growth rate of alpha and beta polymorphs of oleic acid from acetonitrile solution as a function of supersaturation (σ).

Table 2-5
Growth rate of alpha and beta Forms of Oleic Acid from Solution Phase.

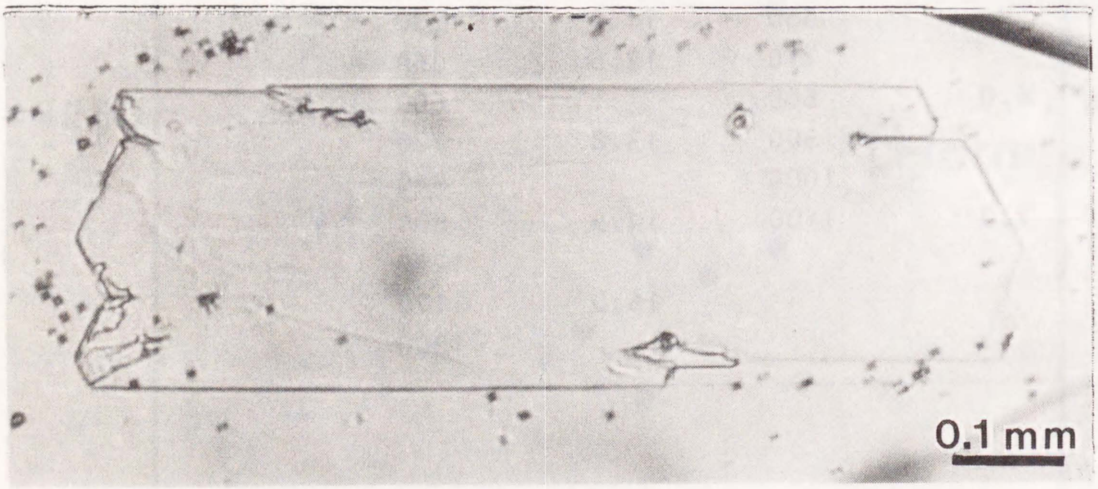
alpha		beta	
σ (%)	R (μm/h)	σ (%)	R (μm/h)
1.0	480	1.0	8
	320	2.3	25
2.0	380	3.6	10
	670	4.8	20
3.0	180	6.0	16
	240	7.2	50
4.0	390	8.4	65
	550		280
	600	9.7	210
5.0	480		360
	550	11.0	330
	710	12.5	360
6.0	600		600
	800	13.8	700
	1000		940
7.0	1100	14.9	800
			1240
		16.0	1170
			1300

Oleic acid α-form

T_s 7.0 °C



supersaturation 2.7 %



supersaturation 10 %

Figure 2-10 Morphological change of a single crystal of oleic acid alpha form in acetonitrile solution.

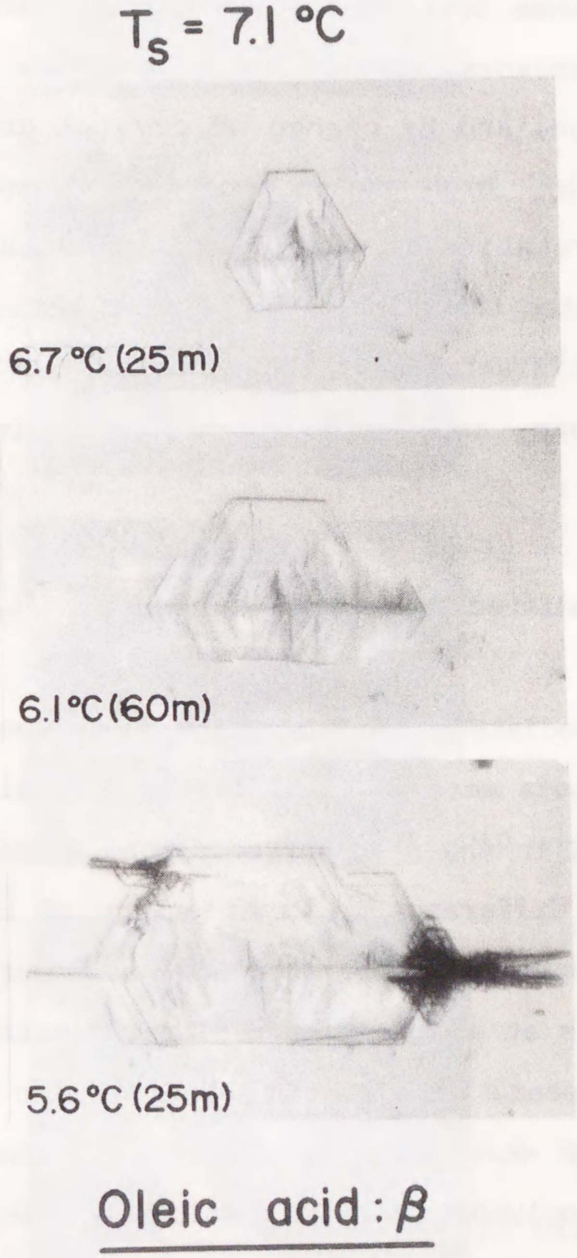


Figure 2-11 Morphological change of a single crystal of oleic acid beta form in acetonitrile solution.

rate of the face normal to b-axis (which is tentatively refereed in Figure 2-11), increased much more than that normal to the a-axis. Finally, it became to grow with a dendrite-like needle-shape. It assumes that the morphological change of beta-form was caused by an anisotropy of the growth rate which depends on supersaturation, and by change of crystal growth mechanisms which was led by high enough supersaturation. Quite different interpretation is possible, since Kaneko et al. most recently indicated that there would be two beta-forms, according to the X-ray diffractometry: the normal polyhedral shape is of stable beta-form, and dendritic needle shape is of another form.6)

d. Solution-Mediated Transformation

Solution-mediated transformation from gamma to beta and from alpha to beta were examined by the optical microscope at -5.0 C and 6.0 C in the stagnant solutions. This transformation comes from the difference in Gibbs energy of the polymorphs,7) and may be important because it could occur during the crystallization process at a slow rate. The nucleation and growth of beta at the expense of alpha or gamma in the solution with a passage of time were directly observed by the optical microscope. The polymorphic modifications of the crystals undergoing the transformation were examined by X-ray diffraction.

Figure 2-12 shows the solution-mediated transformation from alpha to beta in acetonitrile at 6 C. The amount of the alpha crystals precipitated was about 0.01 g in 10 g of solution. The solution was first saturated with respect to alpha-form. Then the beta-form crystals with a needle-shape stared to

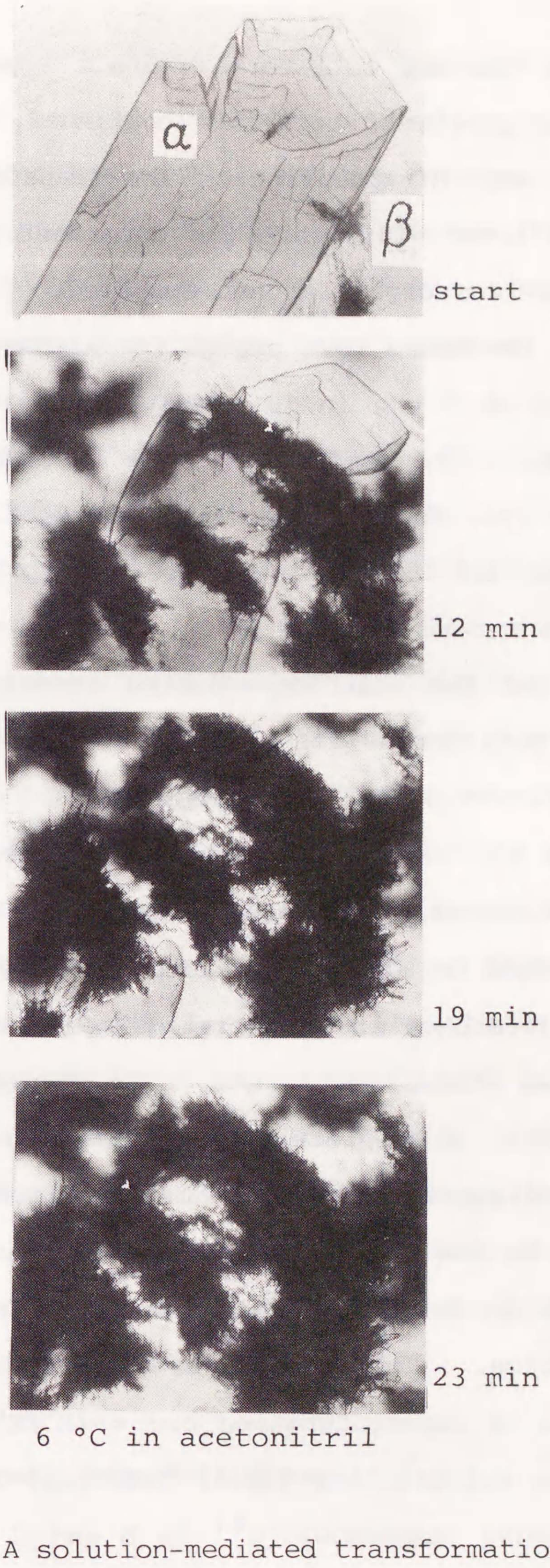


Figure 2-12 A solution-mediated transformation from alpha to beta of oleic acid in acetonitrile.

appear. Once the new beta-form crystals appeared, successive nucleation and growth of beta-form continued, while the alpha-form crystals were disappearing. The transformation was completed within 1.5hr. This rate was dependent on the amount of the first beta-form crystals that appeared. Yet it was confirmed that a few hours were enough for alpha-form even with an initial amount of 0.1 g in 10 g solution to convert perfectly into beta-form. The same process was observed for gamma→beta in decane at -5 C. The rate was much slower than alpha→beta in acetonitrile at 6.0 C. This may be due to lower temperature for gamma than for alpha.

We compared the solution-mediated transformation behavior of alpha and beta forms with that of B and C forms of stearic acid. B has lower Gibbs free energy than C below 32 C and vice versa.8) As the solid state transformation from C to B is kinetically hindered, the solution-mediated transformation is the single method to achieve the actual C→B transformation. A distinctive difference in the solution-mediated transformations between the two acids is its rate. In a stagnant solution of n-hexane at 17 C, C completely transformed to B over about two days under conditions similar to those in Figure 2-12.

This may be due to two reasons. The first one come from the difference in solubility, which results in a driving force of transformation. The driving force of the solution-mediated transformation is supersaturation σ:$\sigma = (X'/X'')-1$, in which X' and X'' are the solubilities (molar fraction) of the metastable and stable forms, respectively.8) In B and C of stearic acid, σ(C/B) at 17 C is 0.20, but σ(alpha/beta) of oleic acid at 6.0 C is 0.39. Therefore the driving force of the transformation of C→B is about a half of that of alpha → beta. This causes the different mechanisms of the solution-mediated transformations. In the case of stearic acid, the nucleation of B was less enhanced because of the smaller driving force. The growth of B is rate-determining. In oleic acid, however, the large driving force accelerated the successive nucleation of beta-form, eventually causing the higher transformation rates that were observed in Figure 2-12.

In relation to the present solution-mediated transformation from alpha to beta forms, it is worth noting that a conversion from alpha to beta in the crystal grown form the solution occurs via solution-mediation. We observed that the alpha crystals obtained from acetonitrile changed to beta in about one week at 10 C. After the conversion, the crystal was destroyed. This did not take place in the melt-grown alpha-form crystal which remained unchanged for more than several months at the same temperature. One of the reasons for this is the solution-mediated transformation via inclusions of mother solution which were incorporated in the crystal during the growth process. The newly nucleated beta crystals in the liquid inclusion can grow at the expense of alpha, and are totally transformed to beta after a long duration. So, one has to keep the solution-grown single crystals of alpha around 0 C because the occurrence and growth of beta in the liquid inclusion was significantly lowered at that temperature.

e. Crystal Growth from Melt Phase

Growth rates from melt phase were measured for alpha and beta forms as a function of supercooling(ΔT) by using a polarizing optical microscope equipped with a video system (Figure 2-

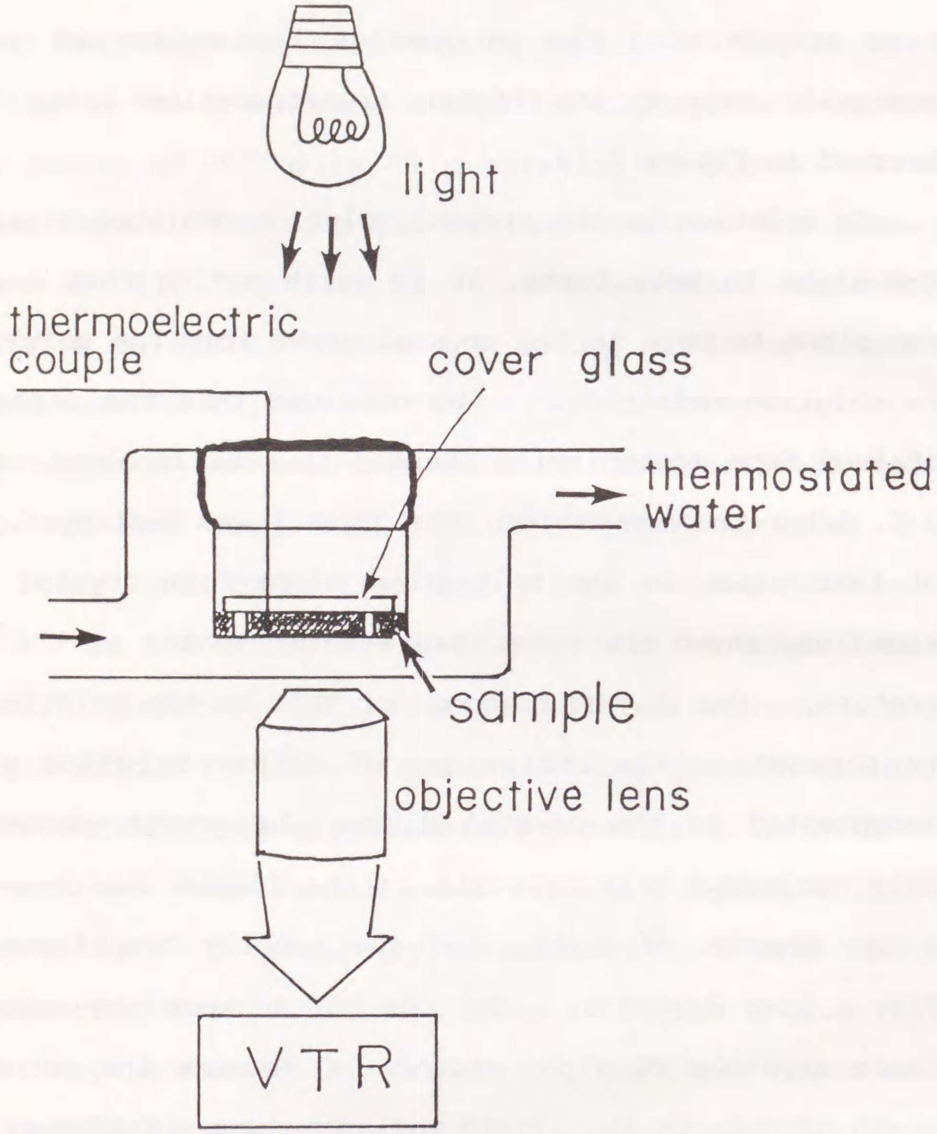


Figure 2-13 A measurement system for crystal growth rate from melt phase.

13). The molten sample was placed on a growth cell, in contact with thermostated water (± 0.02 C). The temperature of the melt was monitored with a thermocouple which was sunken in the melt. A single crystal was put in the supercooled melt. The growth rate of the crystal was measured using a video monitor. Referring to the effect of preheating of the melt, the experiments described in this section deals with the melt growth after preheating the melt at 80 C for several minutes. The precise data for the differently preheated melt will be described in section **f**.

Figure 2-14 and Table 2-6, show the growth rate of two polymorphs of oleic acid from the melt phase. Although the supercooling dependence is different, most astonishing is the growth rate; comparing at the same supercooling of 0.5 C, alpha grows ca.10^5 times faster than beta. The difference in the growth rate of this order of magnitude is fairly consistent with the data from the solution phase shown in Figure 2-9.

A morphological change was observed during the measurement of growth rates from melt phase. Figure 2-15 shows a schematic illustration of the morphological change of a single crystal of beta-form. At low supercooling, a single crystal of beta-form grew, showing the truncated lozenge shape. Whereas, with increasing supercooling, the growth rate of the face normal to b-axis increased much more than that normal to the a-axis. Eventually, it became to grow with a dendrite-like needle-shape. This suggested the same tendency of morphological change of a single crystal observed in growth from solution phase.

Figure 2-16 shows the growth rates of a- and b-faces of beta-form from the melt phase as a function of supercooling. The growth rates of the two faces crossed at 15.3 C; in the

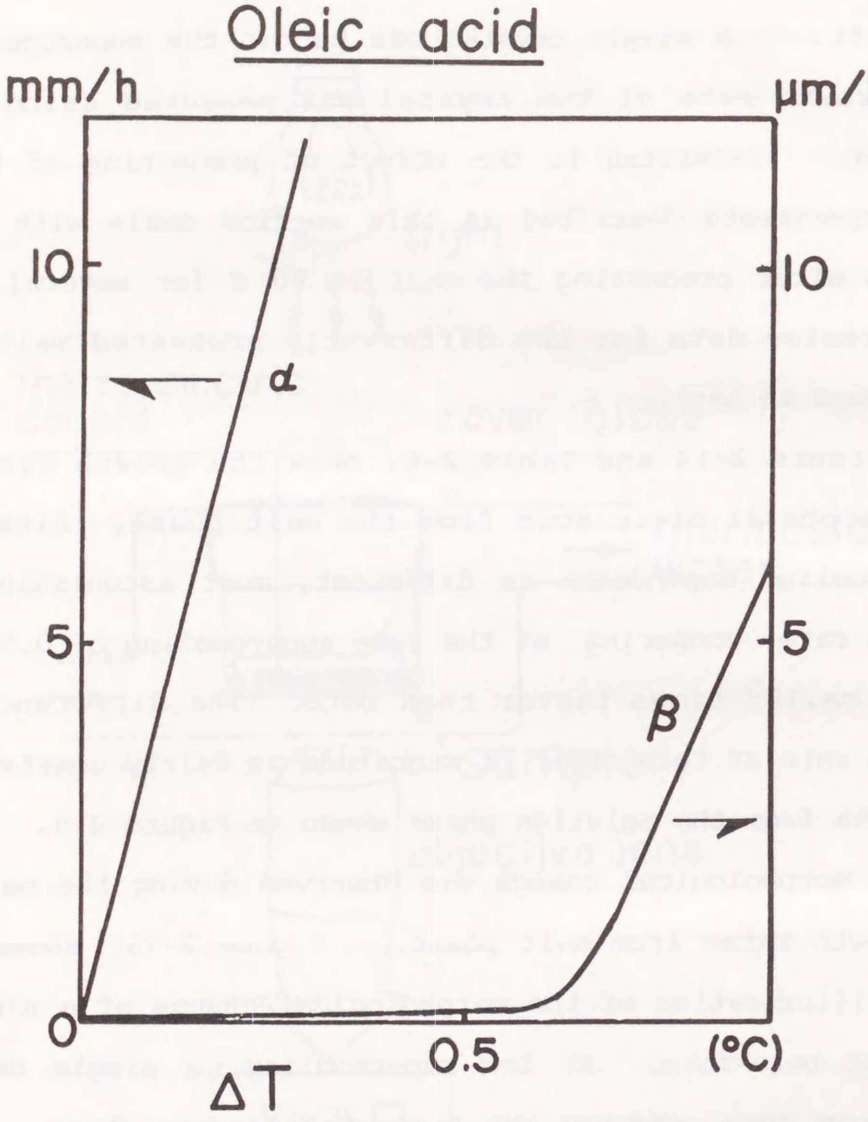


Figure 2-14 Rates of crystal growth (R) of alpha and beta polymorphs from melt phase as a function of supercooling (ΔT).

Table 2-6 Growth Rate of β-Form of Oleic Acid from Melt Phase

ΔT (^{0}C)	Growth rate (μm/h)		ΔT (^{0}C)	Growth rate (μm/h)	
	a axis	b axis		a axis	b axis
0.2	0.24	0.08	1.3	64.0	24.0
	0.08	0.11		5.33	4.46
0.3	0.77	0.05		36.0	12.0
	0.03	0.03		219.27	136.0
	0.52	0.13	1.5	90.67	74.63
	0.04	0.02		60.0	44.0
0.5	0.94	7.77		146.7	160.0
	0.15	0.05		88.0	45.3
	0.88	6.62	1.6	40.0	12.0
0.7	5.33	4.12	1.7	96.0	140.0
	7.77	4.45		75.0	172.0
	0.22	0.22		224.0	248.0
	0.44	20.44		100.0	128.0
0.8	5.33	13.33	1.9	656.0	325.0
0.9	20.88	0.89		121.0	296.0
	0.22	4.17	2.1	28.0	352.0
	1.66	3.65		120.0	240.0
	0.44	5.56	2.3	120.0	520.0
1.0	4.44	55.56		4.0	516.0
1.1	37.89	42.11		28.0	352.0
	40.0	34.0		450.0	890.0
	30.68	25.0	2.8	-	786.0
	9.33	53.33		-	1192.0
	0.66	4.67		-	890.0
1.2	80.0	148.0	3.2	-	1186.0
			3.6	-	1440.0

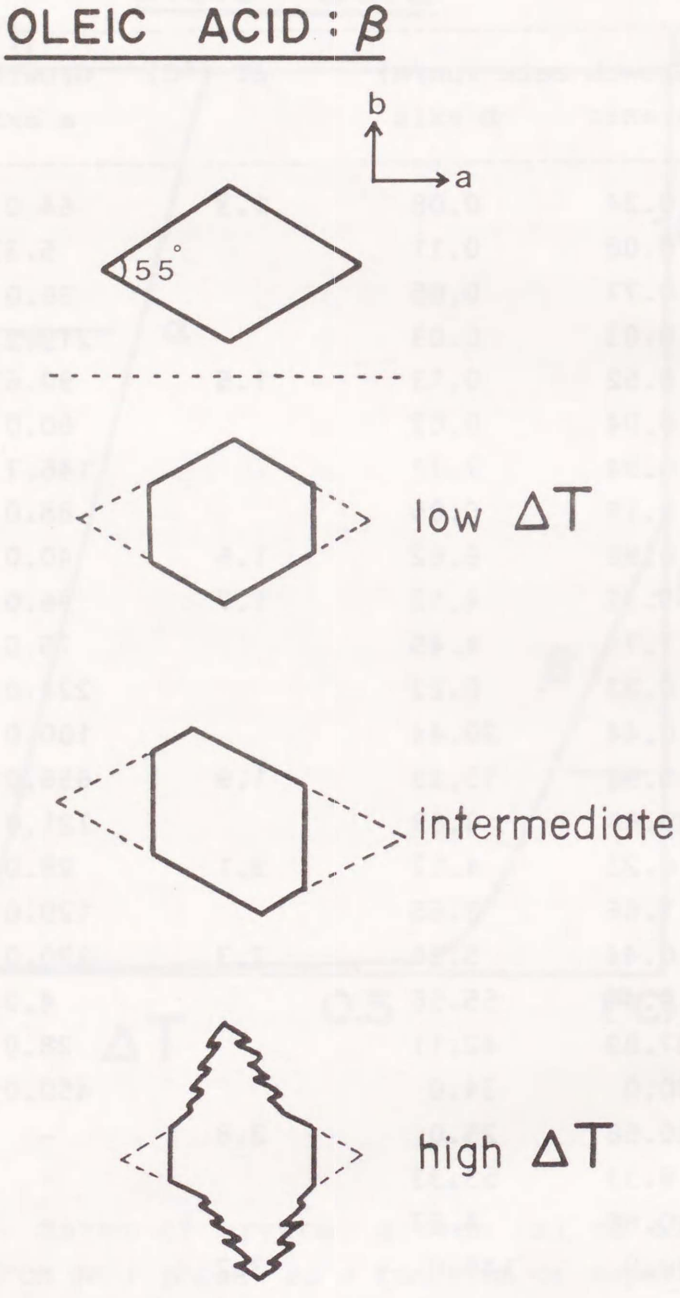


Figure 2-15 Morphological change of a single crystal oleic acid beta form in melt phase.

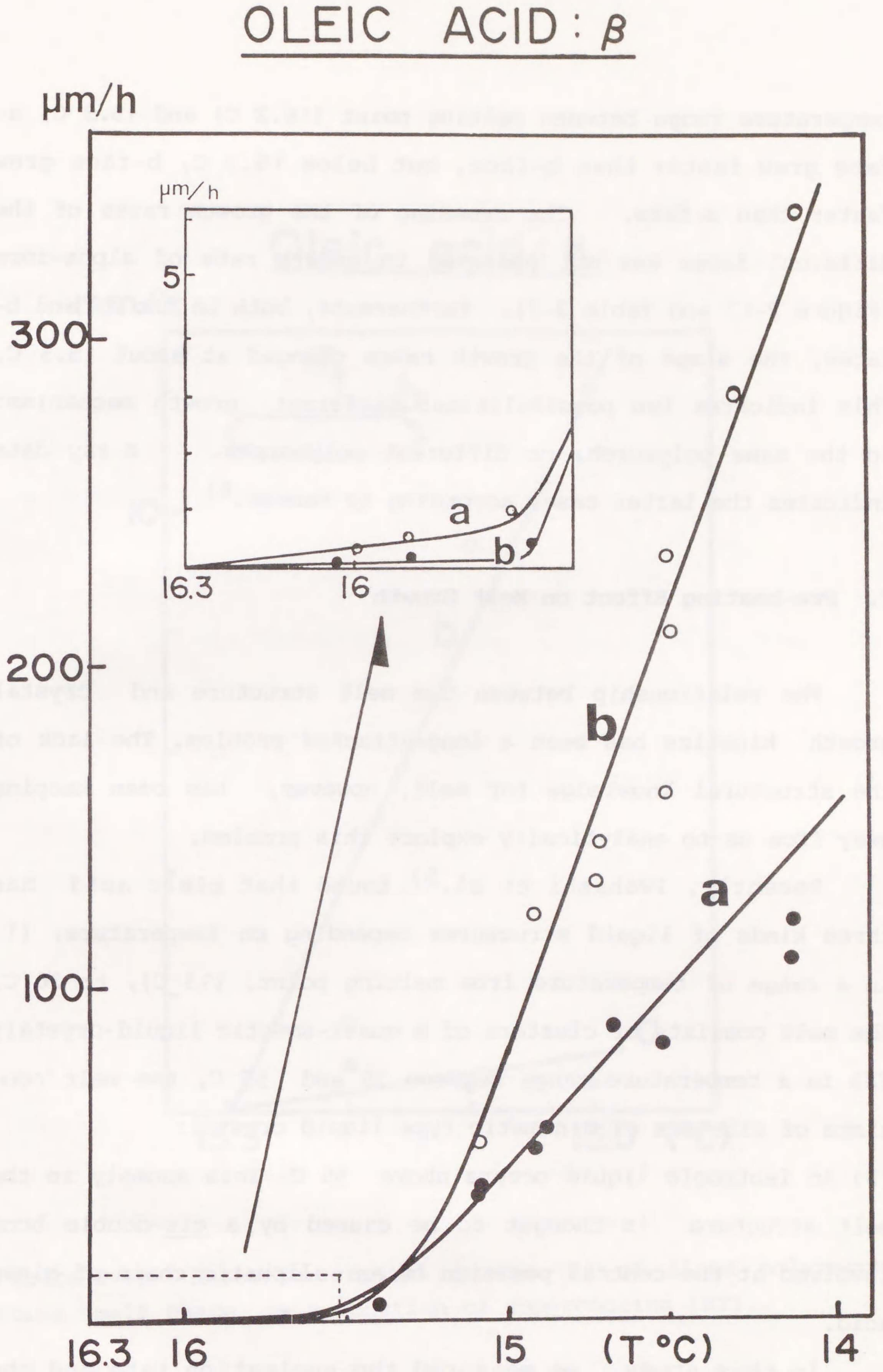


Figure 2-16 Rates of crystal growth (R) of beta polymorphs from melt phase as a function of supercooling (ΔT).

temperature range between melting point (16.2 C) and 15.3 C, a-face grew faster than b-face, but below 15.3 C, b-face grew faster than a-face. The crossing of the growth rates of the different faces was not observed in growth rate of alpha-form (Figure 2-17 and Table 2-7). Furthermore, both in the a- and b-faces, the slope of the growth rates changed at about 15.5 C. This indicates two possibilities: different growth mechanisms in the same polymorph, or different polymorphs. X-ray data indicates the latter case, according to Kaneko.[6)]

f. Pre-heating Effect on Melt Growth

The relationship between the melt structure and crystal growth kinetics has been a long-attacked problem. The lack of the structural knowledge for melt, however, has been keeping away from us to analytically explore this problem.

Recently, Iwahashi et al.[9)] found that oleic acid has three kinds of liquid structures depending on temperature: (1) in a range of temperature from melting point, (13 C), to 30 C, the melt consists of clusters of a quasi-smectic liquid-crystal: (2) in a temperature range between 30 and 55 C, the melt consists of clusters of a nematic type liquid crystal:
(3) An isotropic liquid occurs above 55 C. This anomaly in the melt structure is thought to be caused by a cis-double bond involved at the central position of an aliphatic chain of oleic acid.

In this study, we measured the nucleation rate and the growth rate of oleic acid polymorph from differently pre-heated melt phase, in order to investigate the relationship between melt structure and crystal growth mechanism.

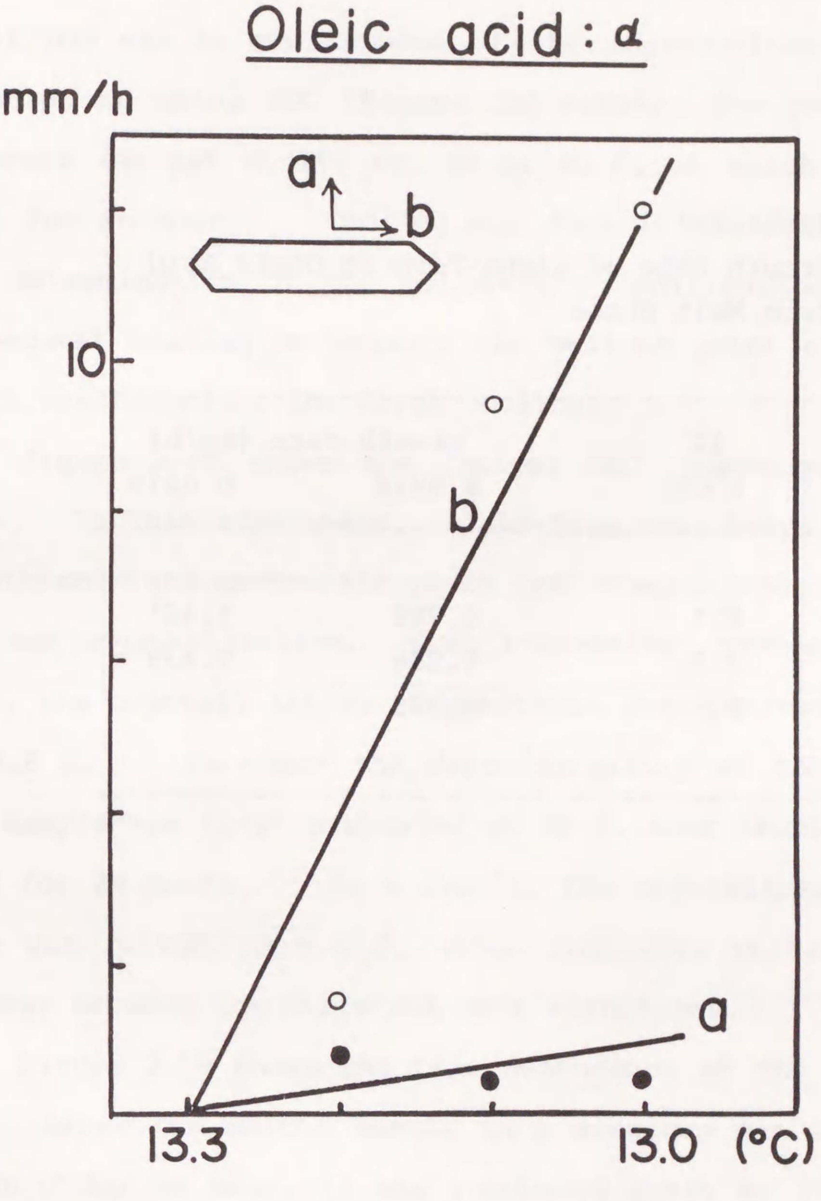


Figure 2-17 Rates of crystal growth (R) of alpha polymorphs forms melt phase as a function of supercooling (ΔT).

Table 2-7
Growth rate of alpha-Form of Oleic Acid
from Melt Phase

ΔT (^0C)	growth rate (mm/h) a axis	growth rate (mm/h) b axis
0.1	0.789	1.421
0.2	0.286	9.429
0.3	0.375	12.000

f.1 Preheating Effect on Nucleation

The nucleation rate was examined by two methods. The first one was to measurement of the supercooling for crystallization by using DSC (Rigaku DSC-8230). The preheating temperature was set at 20, 40, 60 or 80 C, at which the melt was kept for an hour. Cooling was done at the rate of 2 C/min. The determination of the polymorphic modification was done by subsequent heating to measure the melting point of the crystal which was formed at the first cooling.

Figure 2-18 shows the typical DSC thermograms of oleic acid. In this experiment, alpha-form was always crystallized. Endothermic and exothermic peaks are respectively due to melting and crystallization. With increasing preheating temperature, the crystallization temperatures were decreased from 8.5 C to 3.6 C. To check the reproducibility of this phenomenon, the sample was first preheated at 80 C, then incubated again at 20 C for 24 hours. As a result, the crystallization temperature was raised to 6.4 C, which indicates certain relaxation process between the different melt structures.

Figure 2-19 shows the time dependence of the reproducibility. After the molten sample in a aluminum pan was pre-heated at 80 C for an hour, it was incubated again at 20 C. Varying the incubation time at 20 C, we measured the crystallization temperature of alpha form with DSC. The rate of cooling was also 2 C/min. As seen in the Figure 2-19, the crystallization temperature raised suddenly at about 20 hours later.

Another method to examine the preheating effect on nucleation of oleic acid polymorph was to measure an induction time for crystallization as a function of supercooling. Figure 2-

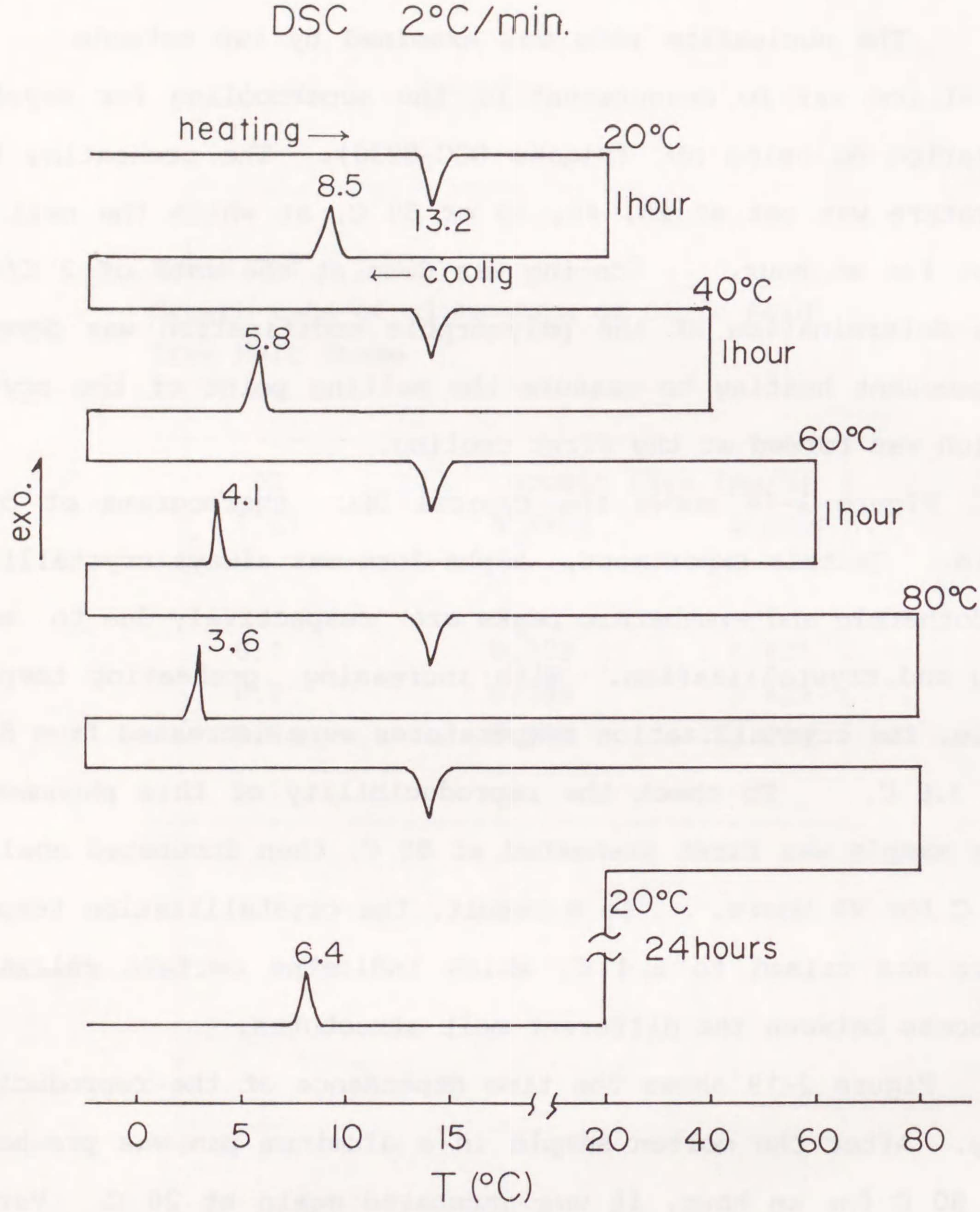


Figure 2-18 Schematic DSC thermograms of oleic acid. The rate of heating and cooling was 2 C/min.

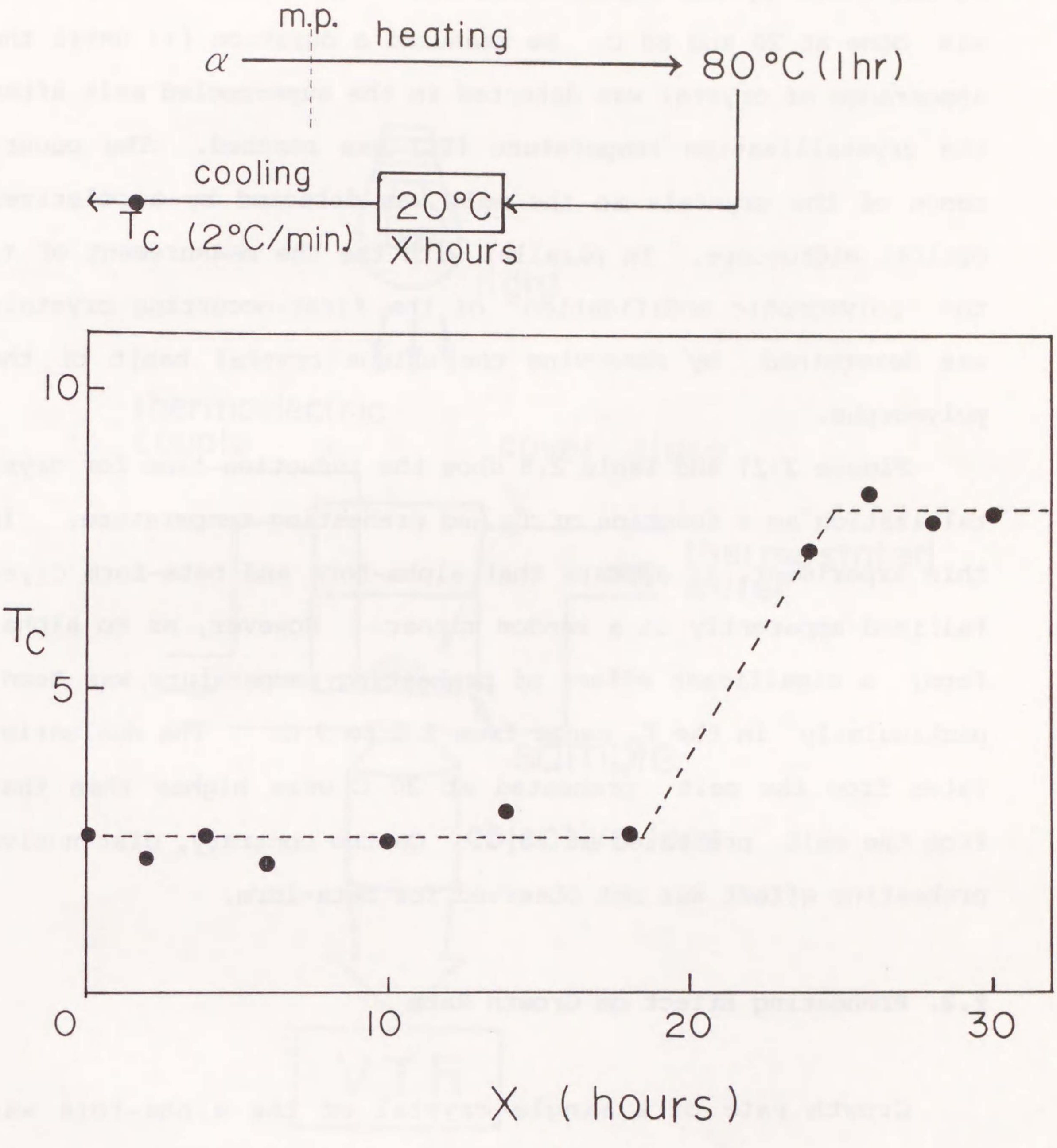


Figure 2-19 Time dependence of the diminishing of the preheating effect. The sample was first preheated at 80 C, then incubated again at 20 C. The unit of horizontal axis is the incubation time at 20 C, and of vertical one is the crystallization temperature of alpha-form by DSC with the cooling rate of 2 C/min.

20 shows the system employed for this experiment. Preheating was done at 20 and 80 C. We measured a duration (τ) until the appearance of crystal was detected in the supercooled melt after the crystallization temperature (T_C) was reached. The occurrence of the crystals in the melt was detected by a polarized optical microscope. In parallel with the the measurement of τ, the polymorphic modification of the first-occurring crystals was determined by observing the unique crystal habit of the polymorphs.

Figure 2-21 and Table 2-8 show the induction time for crystallization as a function of T_C and preheating temperature. In this experiment, it appears that alpha-form and beta-form crystallized apparently in a random manner. However, as to alpha-form, a significant effect of preheating temperature was seen; particularly in the T_C range from 3 C to 9 C. The nucleation rates from the melt preheated at 20 C were higher than that from the melt preheated at 80 C. On the contrary, distinctive preheating effect was not observed for beta-form.

f.2. Preheating Effect on Growth Rate

Growth rate of a single crystal of the alpha-form was measured as a function of supercooling of the melt which was pre-heated at different temperatures. We employed a polarizing optical microscope equipped with a video system, which is the same system as that in Figure 2-13. Preheating of the melt was done at 20 and 80 C. The method of the measurement of the growth rate was also the same as that described in <u>section 2.1-e.</u>

Figure 2-22 shows the growth rates of alpha-form as a

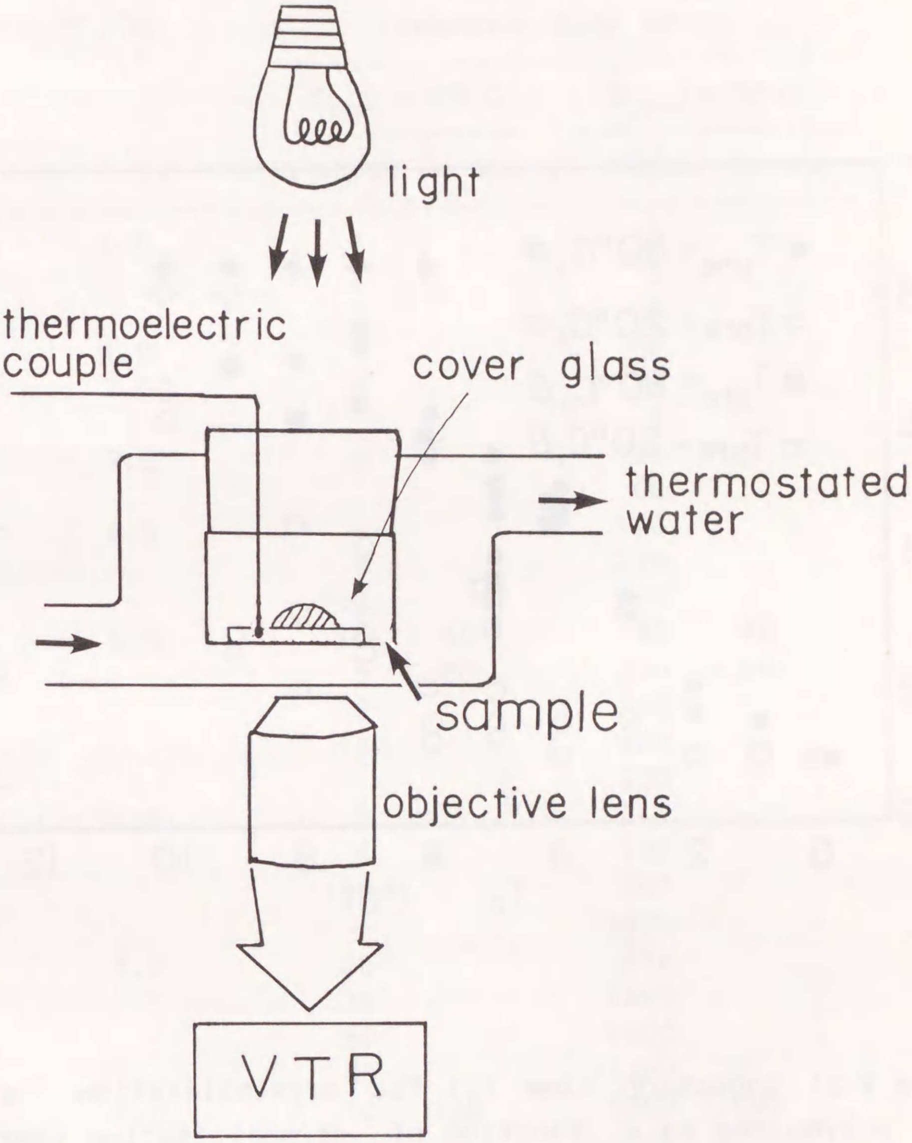


Figure 2-20 A measurement system for induction time for crystallization of oleic acid polymorphs.

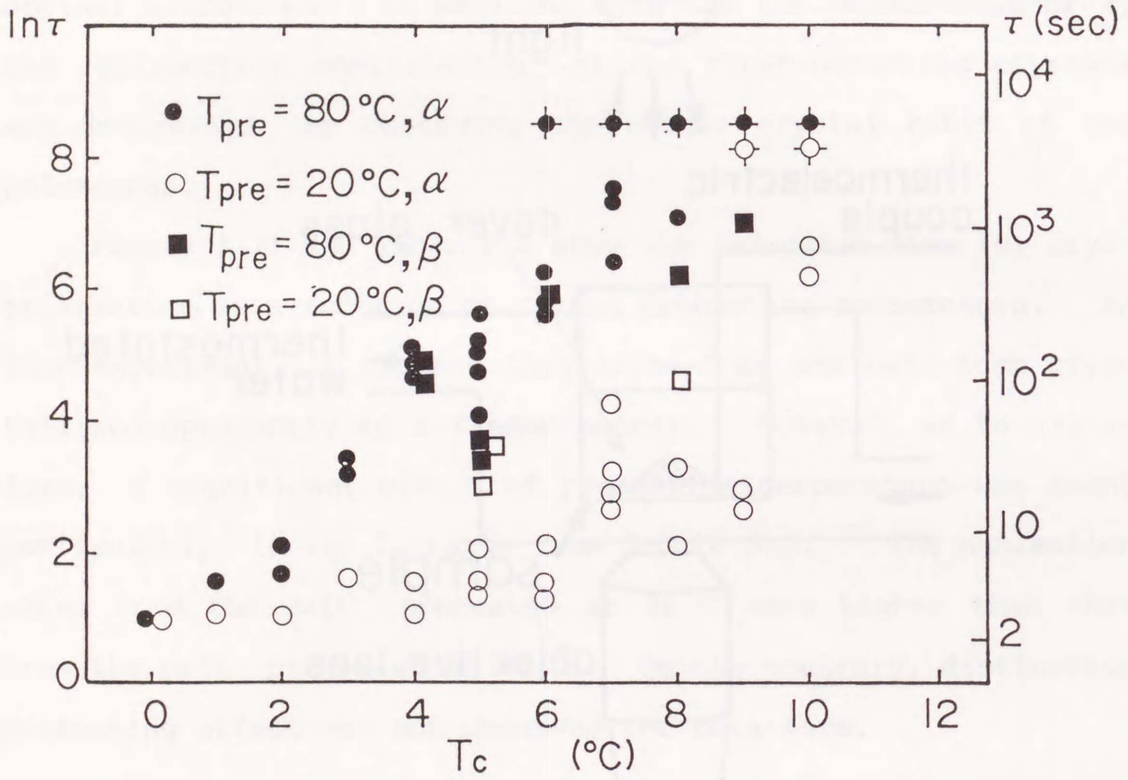


Figure 2-21 Induction time (τ) for crystallization of oleic acid polymorphs as a function of crystallization temperature (T_c). (●):alpha-form, preheated at 80 C; (○):alpha-form, preheated at 20 C; (■):beta-form, preheated at 80 C; (□):beta-form, preheated at 20 C

Table 2-8 Pre-heating effect on nucleation of oleic acid

T_C(^{0}C)	induction time (sec)			
	T_{pre} = 20 C		T_{pre} = 80 C	
	alpha	beta	alpha	beta
0.0	3		3	
	3		3	
	3		3	
1.0	4		6	
2.0	4		10	
			6	
3.0	6		30	
			60	
4.0	4		120	
	6		240	
	100		200	
5.0	10	30	90	40
	5	80	200	80
	6		240	
	10		480	
			220	
6.0	5		240	
	6		120	
	100		300	
			>3600	
7.0	20		900	
	30		1800	
	40		1200	
	80		>3600	
8.0	10	120	1200	900
	40		>3600	
9.0	20		>3600	1800
	30			
	>3600			
10.0	>3600		>3600	
	1200			
	900			

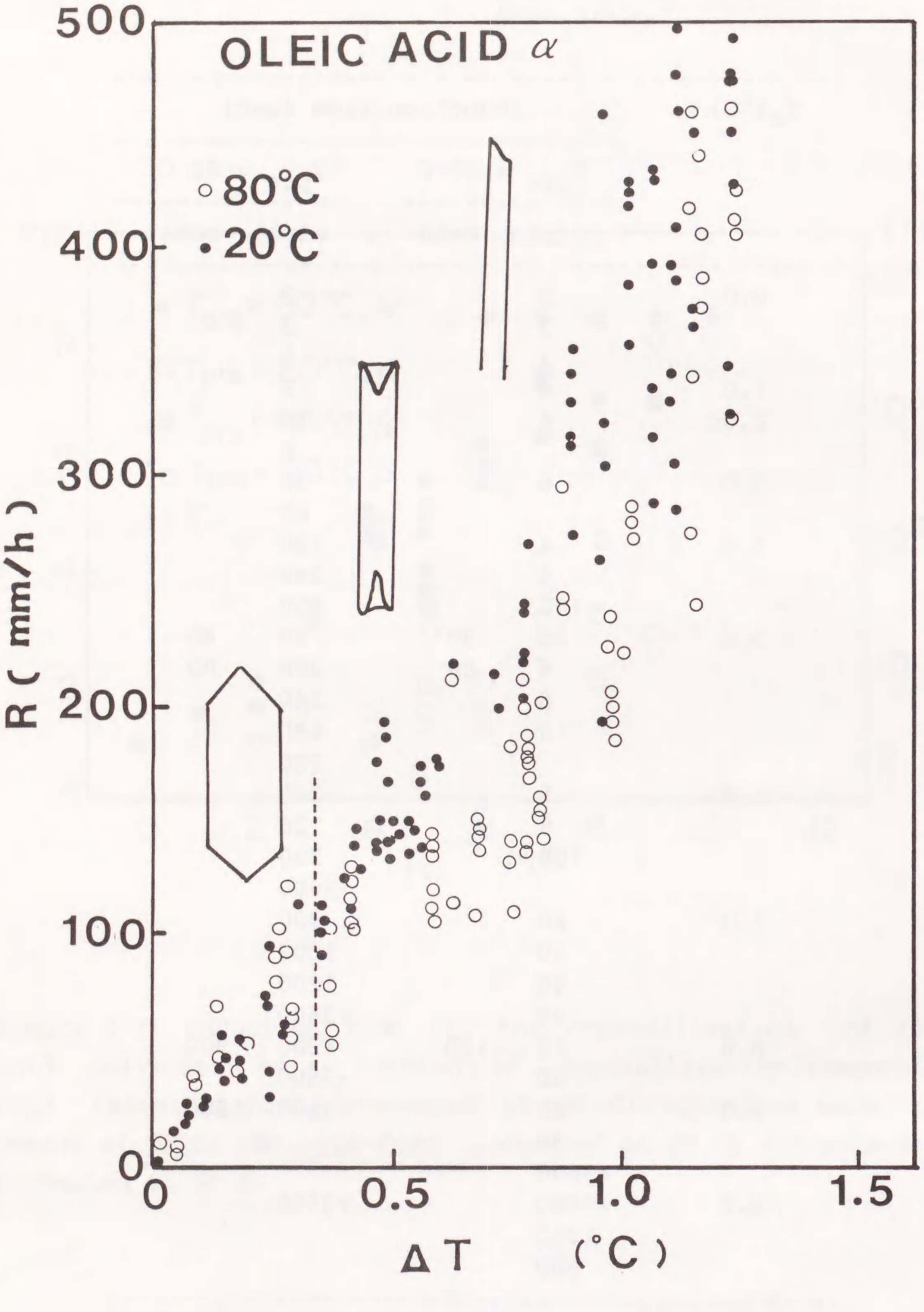


Figure 2-22 Linear growth rates (R) of single crystal of alpha-form of oleic acid from preheated melt as a function of supercooling (ΔT).
(○):pre-heated at 80 C; (●):pre-heated at 20 C.

function of supercooling. The distinctive preheating effect was not observed. The crystal shapes, observed during the measurement, were illustrated in Figure 2-22. Faceted growth was observed in the ΔT range below 0.3 C, above which the crystal habit became a needle-like shape due to morphological instability. It may be worth noting that growth rates for 80 C-preheating was a bit lower than those by 20 C-preheating, in a ΔT range above 0.5 C.

g. Discussion

g.1 Thermodynamics of Oleic Acid Polymorph

It is worthy to compare the differences in the solubility parameters of the polymorphs with those of fusion$^{1)}$ (Table 2.1-2). Gamma and beta have almost the same ΔH_d values, but the entropy term differs by 8 J/mol K. We evaluate ΔH_f and ΔS_f of gamma by adding the enthalpy (ΔH_{tr}) and entropy (ΔH_{tr}) of the gamma-alpha transformation to ΔH_f and ΔS_f of alpha: ΔH_{tr}(gamma-alpha) + ΔH_f(alpha) = 48.4 kJ/mol, ΔS_{tr}(gamma-alpha) + ΔS_f(alpha) = 170.7J/mol K. Thus, both differences for gamma and beta are in good agreement with the solubility data. In acetonitrile, ΔH_d and ΔS_d of beta-form are larger than those of alpha by 16.6 kJ/mol and 56.8 J/mol K, both of which are about 1.5 times larger than the differences in ΔH and ΔS of fusion of alpha and beta. The origin of this discrepancy is most probably due to the experimental errors in the solubility experiments. In addition, acetonitrile/oleic acid may be far from ideal-solution conditions due to a remarkable difference in their molecular structures. This may be reflected in a larger difference between fusion and solubility data.

We calculated T' = $\delta(\Delta H_d)/\delta(\Delta S_d)$ for alpha-beta using the solubility and melting data. This T' value is very important because T' may be equivalent to the crossing point of the Gibbs energies of the two forms. This was found to be true for B and C of stearic acid[10]. The solubility data of alpha and beta of oleic acid yields T'=19.1 C, whereas also be attributed to the non-ideal solution of the acetonitrile/oleic acid system mentioned above.

Based on the G-T relationship of Figure 2-7 of alpha and beta of oleic acid, it is reasonable to expect that the metastable forms may crystallize more frequently than the most stable beta-form, according to an Ostwald step rule. This has been observed in solution and melt growth as described above.

g.2 Crystallization Behavior in Oleic Acid Polymorphism

The anomalous behavior of the polymorph-dependent growth rate of alpha and beta has been exhibited in two aspects: difference in growth rate between alpha and beta, and preheating effect of melt phase in of melt phase in nucleation from the melt phase.

As to the first problem, the influence of the crystal morphology and condition of environment around the growing crystal surface, like diffusion or convection, may be regardless, since the crystal shape of alpha and beta do not differ appreciably. Instead, it seems that the influence of the molecular structure might be most decisive. The conformation of aliphatic chain differs each other; disordered omega-chain in alpha-form, and all-trans conformation in beta-form. Accordingly, the aliphatic chain packing expressed in the subcell packing and olefin conformation of alpha and beta differ to a greater extent. It seems that the beta form is most closely packed. Hence, the form having most closely packed aliphatic chain may need larger activation energy for crystallization than the form having loosely-packed chain as in alpha, resulting in the slower crystallization rate.

As to the preheating effect, a reproducible and distinctive effect on the nucleation was observed in the alpha-form of oleic acid. This supports Iwahashi's hypothesis which maintains a possibility of existence of three structures in the melt phase. Our results suggest that at least two phases may exist in the melt. The two phases seem to be able to transform reversibly. Since the melt preheating at 20 C accelerated the nucleation more than the preheating at 80 C, the melt structure formed at 20 C may be more ordered than that at 80 C. In the case that clusters having ordered structures exist in the melt, they may be easier to covert to nuclei.

In equation 1.4, the molecular clustering may influence the nucleation in two ways; it increases J (the rate of nucleation) through the pre-exponential term in which the reaction steps from the monomers to the critical nuclei are diminished. On the contrary, N_o (monomer concentration per unit medium volume) and, accordingly, the actual supersaturation ratio (in case of the solvent crystallization) might be decreased, hence J decreases due to the stabilization of the molecular clusters. The overall nucleation rate is determined by two conflicting influences. In the solvent crystallization, two opposing factors may become comparative. It appears that, in the melt crystallization, the latter effect might be regardless, since the decrease in the monomer concentration is negligible. Hence the nucleation may be enhanced.

Although the preheating process significantly effected the nucleation of alpha-form, beta-form was not influenced remarkably. This result possibly originates in the difference of molecular structures between the two polymorphs as discussed above. The alpha-form reveals conformational disordering in the omega-chain segment. This "interfacial melting" assumes molecular fluidity at the omega-chain segment due to an introduction of the *cis* double bound. Namely, the alpha-form contains a rigidly packed delta-chain and a "liquid-like" omega-chain which is located at the lamellar interface in the dimerized crystalline structure. No "interfacial melting", however, occurred in the aliphatic chain of the beta-form, which has all-*trans* aliphatic chains both in the omega- and delta-chains.

The difference in the molecular structure, especially in the "interfacial melting", seems to cause polymorph dependence on the preheating effect. Although detailed crystalline structure have not been obtained in alpha- and beta-form, we estimate that beta-form has higher density than alpha-form. It can be speculated that alpha-form, having closer structures to the melt, would be more sensitive to the melt structure than the beta-form.

We may further discuss heterogeneous nucleation, which is enhanced due to catalytic effects of the foreign substance as shown in Figure 1-8. Oleic acid molecules may chemically interact through carboxyl groups, when growth units are monomeric species. The carboxyl groups may interact with ionic crystals, surfactant agencies in a micellar solution, and even with metal surface. Accordingly, it is possible to induce the heterogeneous nucleation on these surfaces which leads not only to the enhanced nucleation but also the orientational effect of the crystalline phase if the substrate forms a particular shape. Hence, we have to recognize a possibility that the preheating may affect the interaction with substrate which may be a contaminated substance or glass walls of the growth cell. To confirm this, further experiments are needed.

Finally, a contrast that the preheating remarkably affected the nucleation of the alpha-form but not of alpha-form, may recall certain ordering of melt molecules at solid-liquid interface.[11,12) The ordering of melt molecules at the solid-liquid interface may diminish the difference in structures of the bulk melt operating in the crystal growth process.

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2.2 Erucic Acid

The present section mainly describes crystallization mechanisms in polymorphic modifications of erucic acid. In doing so, prerequisite are molecular and structure properties of the erucic acid polymorphs. In the first, these properties are reviewed in brief.

a. Structural Aspects of Erucic Acid Polymorphs

We have clarified complicated polymorphism of erucic acid by thermal1) and X-ray diffraction methods1-3) and spectroscopic analysis.4) Four polymorphs, alpha, alpha$_1$, gamma and gamma$_1$, were discovered. Figure 2-23 shows the transformation circuit of the four polymorphs. From the melt or solution phases, alpha$_1$-form first crystallizes, then transforms either to gamma$_1$ or to alpha. A polymorphic conversion from alpha$_1$- or gamma$_1$-forms to alpha-form was very slow in the solid state due to some steric hindrance. Alpha-form is obtained via the transformation from gamma$_1$-form (alpha$_1$-form). Slow crystallization from solution also produces alpha-form. Once alpha-form occurs, it transforms to gamma-form in the solid state on cooling. On moderate heating, gamma$_1$- and gamma-forms transform to alpha$_1$- and alpha-form at 9.0 C and -1.0 C, respectively. Furthermore, alpha-form transforms to alpha$_1$-form at 31.2 C, which is just below the melting point of alpha$_1$(34.0 C).

The crystal structures of gamma- and gamma$_1$ forms were determined by using four circle X-ray diffractometer.2,3) Figure 2-24 and Table 2-9 show the crystal structure and crystal data of gamma-form, respectively. The monoclinic unit cell

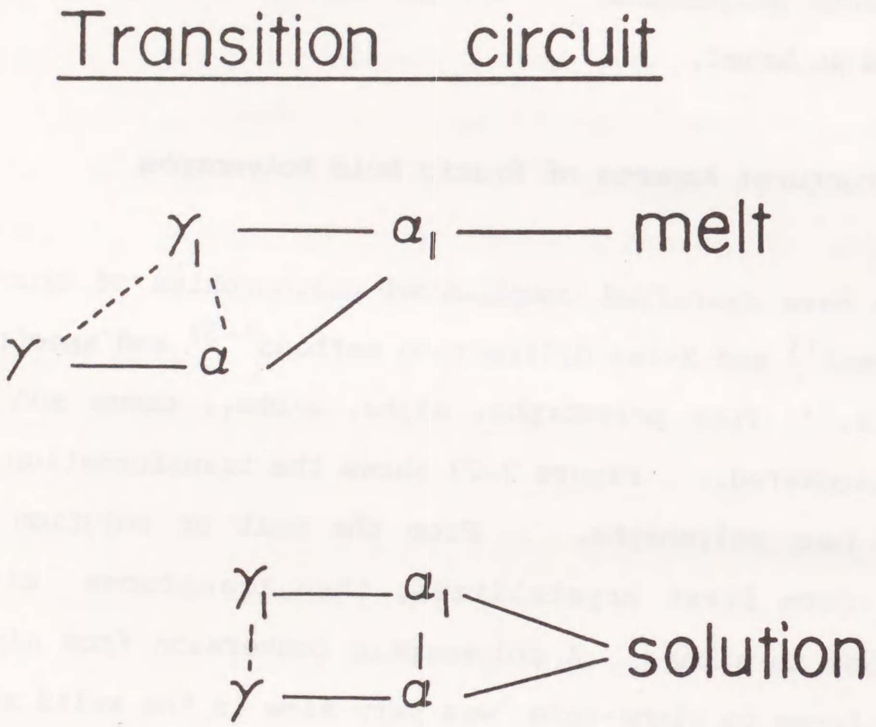

Figure 2-23 A transformation circuit of four polymorphs of erucic acid.

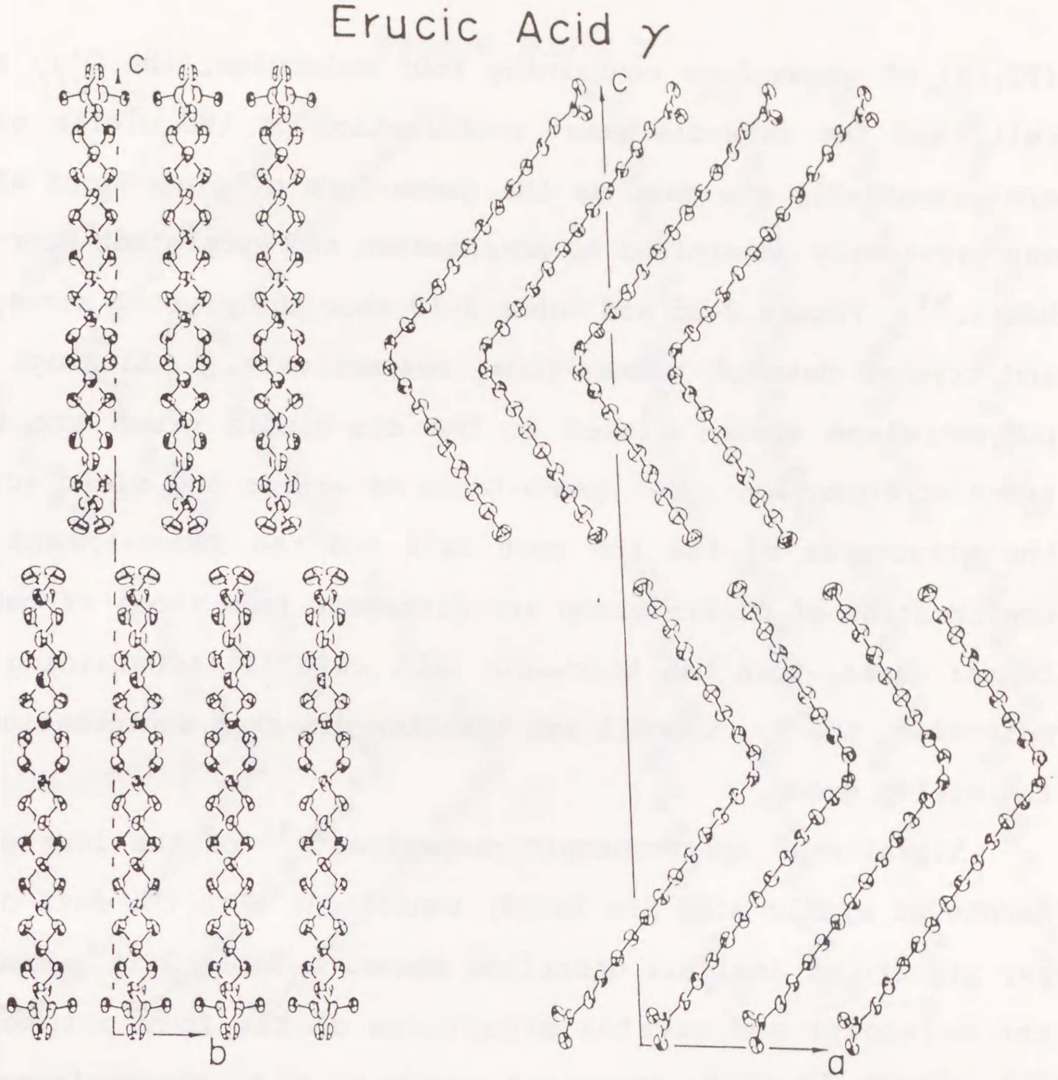

Figure 2-24 Crystal structure of gamma-form of erucic acid. (From Ref. 2,3.)

Table 2-9 Crystal Data of Eurcic Acid Gamma-form.

gamma phase of erucic acid
cis-$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$,
Molecular weight: 338.57
Triclinic: $P2_1/a$
a=9.564 b=4.683 C=49.49 A
β=93.65 degree
V=2198 A^3
z=4
D_x=1.02 gcm^{-3}
Temperature 233 K

(From Ref. 3.)

($P2_1/a$) of gamma-form containing four molecules, the $O'_{//}$ subcell, and the skew-cis-skew' conformation of the olefin group are essentially the same as the gamma-form of oleic acid which was previously determined by Abrahamsson and Ryderstedt-Nahringbauer.5) Figure 2-25 and Table 2-10 show the crystal structure and crystal data of gamma$_1$-form, respectively. Although the polymethylene chains linked to the cis-olefin group are all-trans conformation like gamma-forms of erucic and oleic acids, the structures of the the unit cell and the subcell, and the conformation of olefin group are different from those of gamma-forms: gamma$_1$-form has triclinic unit cell ($P\bar{1}$) containing two molecules, the $T_{//}$ subcell and the skew-cis-skew conformation of the olefin group.

Vibrational spectroscopic properties2,4) of the four polymorphs of erucic acid are fairly consistent with the data of X-ray structural analysis described above. Table 2-11 summaries the molecular and crystal structures of the four polymorphs obtained from the spectroscopic method. As to the conformation of the polymethylene chains, alpha- and alpha$_1$-forms revealed conformational disordering in their omega-chains, keeping the delta-chains ordered. Hence, the reversible transformations of gamma-alpha and gamma$_1$-alpha$_1$ were of a kind of order-disorder transformation as observed between gamma- and alpha-forms of oleic acid.6,7) The conformation of the olefin group and subcell structure of alpha and alpha$_1$ were almost identical. The same properties were observed in gamma and gamma$_1$.

The X-ray diffraction patterns of the four polymorphs and their relative intensities are shown in Figure 2-26 and Table 2-12, respectively. In the case of alpha-gamma transformation, the short spacing spectra changed drastically by cooling alpha

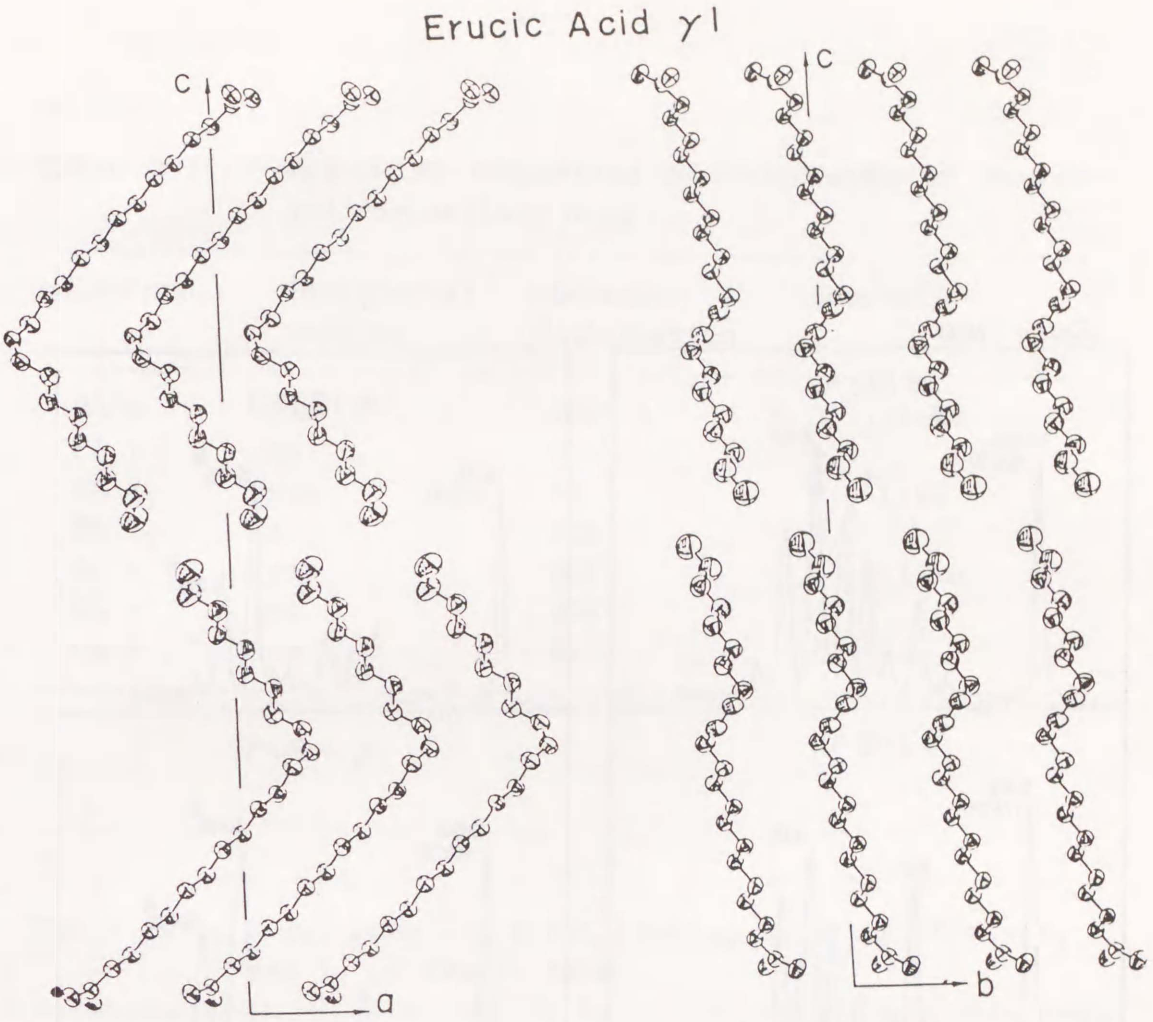

Figure 2-25 Crystal structure of gamma$_1$-form of erucic acid. (From Ref. 2,3.)

Table 2-10. Crystal Data of Eurcic Acid Gamma$_1$-form.

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gamma$_1$ phase of erucic acid
cis-$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$,
Molecular weight: 338.57
Triclinic: $P\bar{1}$
a=5.462 b=5.197 C=44.16 A
α=91.20 β=90.85 γ=117.32 degree
V=1113.2 A^3
z=2
D_x=1.01 gcm^{-3}
Temperature 266 K

(From Ref.3.)

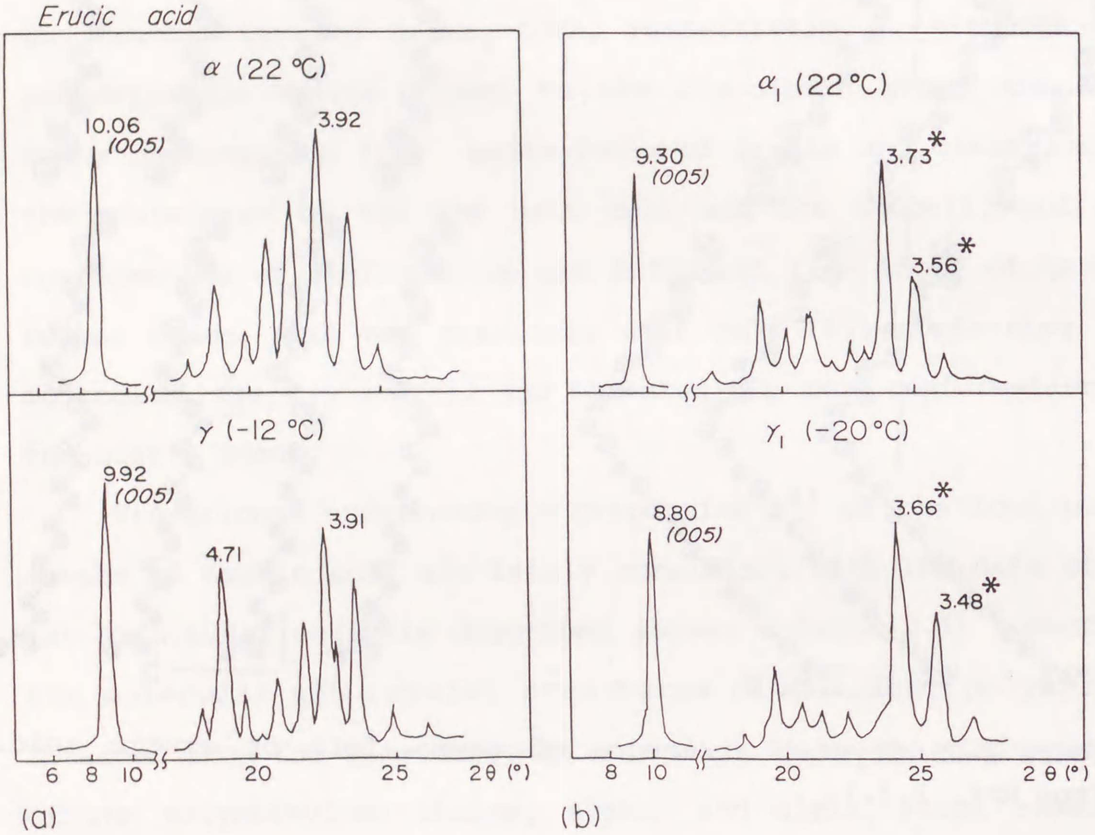

Figure 2-26 X-ray diffraction patterns of (a), alpha- and gamma-forms and (b), alpha$_1$- and gamma$_1$-forms of erucic acid (unit, Angstrom).

Table 2.11 Molecular Properties of Polymorphs of Erucic and Peroselinic Acid

polymorph	interfacial melting	olefinic conformation	subcell
EA α	yes	SCS'	$O'_{//}$-like
EA γ	no	SCS'	$O'_{//}$
EA α_1	yes	SCS	$T_{//}$-like
EA γ_1	no	SCS	$T_{//}$
OA α	yes	SCS'	$O'_{//}$-like
OA γ	no	SCS'	$O'_{//}$
OA β	no	SCS	//-lile

Table 2-12. X-ray Long and Short Spacing Spectora of α,γ,α_1 and γ_1 of Erucic Acid

	Long spacing	Short spacing
α	50.3(vs)	3.74(s), 3.92(vs), 4.11(s) 4.30(s), 4.37(m), 4.46(w)
γ	49.6(vs)	3.68(w), 3.91(vs), 4.04(s) 3.73(s), 4.26(m), 4.71(s)
α_1	46.5(vs)	3.40(W), 3.56(s), 3.73(vs) 3.93(w), 4.04(w), 4.21(m) 4.40(m), 4.60(m)
γ_1	44.0(vs)	3.30(w), 3.48(ws), 3.66(vs) 3.77(m), 3.98(w), 4.18(m) 4.32(m), 4.55(m)

Unit, Angstrom.
vs,very strong; s,strong; m,medium; w,weak.

below -3 C (Figure 2-26a), and the long spacing value was decreased by 0.7 A. In contrast, subtle changes were detectable between alpha$_1$ (22 C) and gamma$_1$, which was kept at -20 C for one hour(Figure 2-26b). On cooling, two major peaks (denoted by *) looked unchanged, although their lattice parameters were decreased. The long spacing was largely changed from 46.5 A (alpha$_1$, 22 C) to 44.0 A (gamma$_1$, -20 C).

Comparing with the polymorphs of oleic acid, the powder X-ray diffraction patterns (Figure 2-26) of gamma- and alpha-forms of erucic acid are almost identical to gamma- and alpha-forms of oleic acid (Figure 2-3), respectively: three strong peaks of alpha-form ; 4.30 A, 3.92 A and 3.74 A, and three strong peaks of gamma-form; 4.71 A, 3.91 A and 3.73 A. No superposition of the long spacing spectra with higher indices gave rise to a confusion in the short spacing spectra. Therefore, the subcell structures of gamma and alpha of erucic and oleic acids must be the same. As to gamma-forms of the two acids, the detailed X-ray structural analyses have proved it. As to the long spacing, alpha-form is longer than gamma-form by 0.7 A. This difference is a bit smaller than those of oleic acid; 43.3 A (alpha) and 41.9 (gamma). It is inferred that the increase in the long spacing of alpha-form of oleic acid may be due primarily to the disordering in the omega-chain. This assumption can also be applied to two couples of polymorphs of gamma/alpha and gamma$_1$/alpha$_1$ of erucic acid; the interlamellar distance of the disordered form is longer than that of the ordered form.

The melting point of alpha$_1$-form (34.0 C) is close to the crystal which Craven$^{8)}$ determined as a triclinic structure with the lattice parameters of a=9.88 A, B=5.17 A, C=47.8 A, alpha=102^0, beta=91^0 and gamma=87^0. This structure gives rise to long spacing of alpha of the present study. Therefore, it is concluded that alpha$_1$-form is equivalent to the crystal examined by Craven.

As to gamma$_1$-form, its X-ray diffraction short spacing pattern does not differ from alpha$_1$-form, being singly decreased by 0.04-0.06 A. This reduction must be a simple lattice constriction due to different temperatures examined. However, the long spacing of gamma$_1$-form is shorter than alpha$_1$-form by 2.5 A, which is appreciably large compared to the difference between gamma- and alpha-forms. Hence, one may assume that the transformation from alpha$_1$-form to gamma$_1$-form is accompanied with the decrease in the chain inclination with respect to the basal surface, the change in the subcell structure being rather small. In addition, the disordering in the omega-chain is introduced, as spectroscopic data indicate.$^{3)}$ The peculiar thermal hysteresis exhibited in alpha$_1$-gamma$_1$ transition may be related to the lattice displacements and the molecular re-ordering.

To conclude the structural properties in the polymorphism of erucic acid, four polymorphs were identified. Two of them, gamma- and alpha-forms, revealed the same features as gamma- and alpha-forms of oleic acid, undergoing the transformation characterized by the conformational disordering in the omega-chain. The other two forms, gamma$_1$- and alpha$_1$-forms, also revealed the order-disorder transformation like gamma-alpha, but neither form revealed the complete similarity in the crystal structures to gamma- and alpha-forms, and to the third form, beta, of oleic acid as well. The successive solid-state transformations resulted in the single melting of the high-temperature form of alpha$_1$. In the case of oleic acid, double melting of alpha-and beta-forms was observed. From the similarities or dissimilarities between oleic and erucic acids, it was clarified that the polymorphism of one cis-double bonded unsaturated acid significantly depends on the position of the double bond in the aliphatic chain.

b. Morphology

Single crystals of alpha and alpha_1 forms were grown from acetonitrile solutions around 18 C.1) Figure 2-27 shows the morphology of the single crystals. Alpha-form revealed an elongated hexagonal shape, whereas alpha_1-form had a rhombohedral shape. The crystal habit of alpha-form, which is the same as that of alpha-form of oleic acid, also suggests the equality of the subcell structure between the two alpha-forms. This is because, in such as long-chain compounds, the crystal habits of basal faces reflect the subcell structures. The interplanar angles of the lateral faces of alpha_1-form are 55^0 and 125^0 which are the same values of beta-form of oleic acid (figure 2-2). This means a similarity in the subcell structure, yet dissimilarities were seen in three properties: (a) growth forms of crystal are rhombohedral in alpha_1-form, but needle-like in beta-form; (b) the X-ray diffraction short spacing spectra are largely different, and (c) there is no conformational disordering in oleic acid beta-form. Hence, one may conceive that the detailed molecular structures of alpha_1- and beta-forms would be very different.

The crystals of alpha and alpha_1 of erucic acid were employed for the measurement of solubility and kinetics of crystal growth.

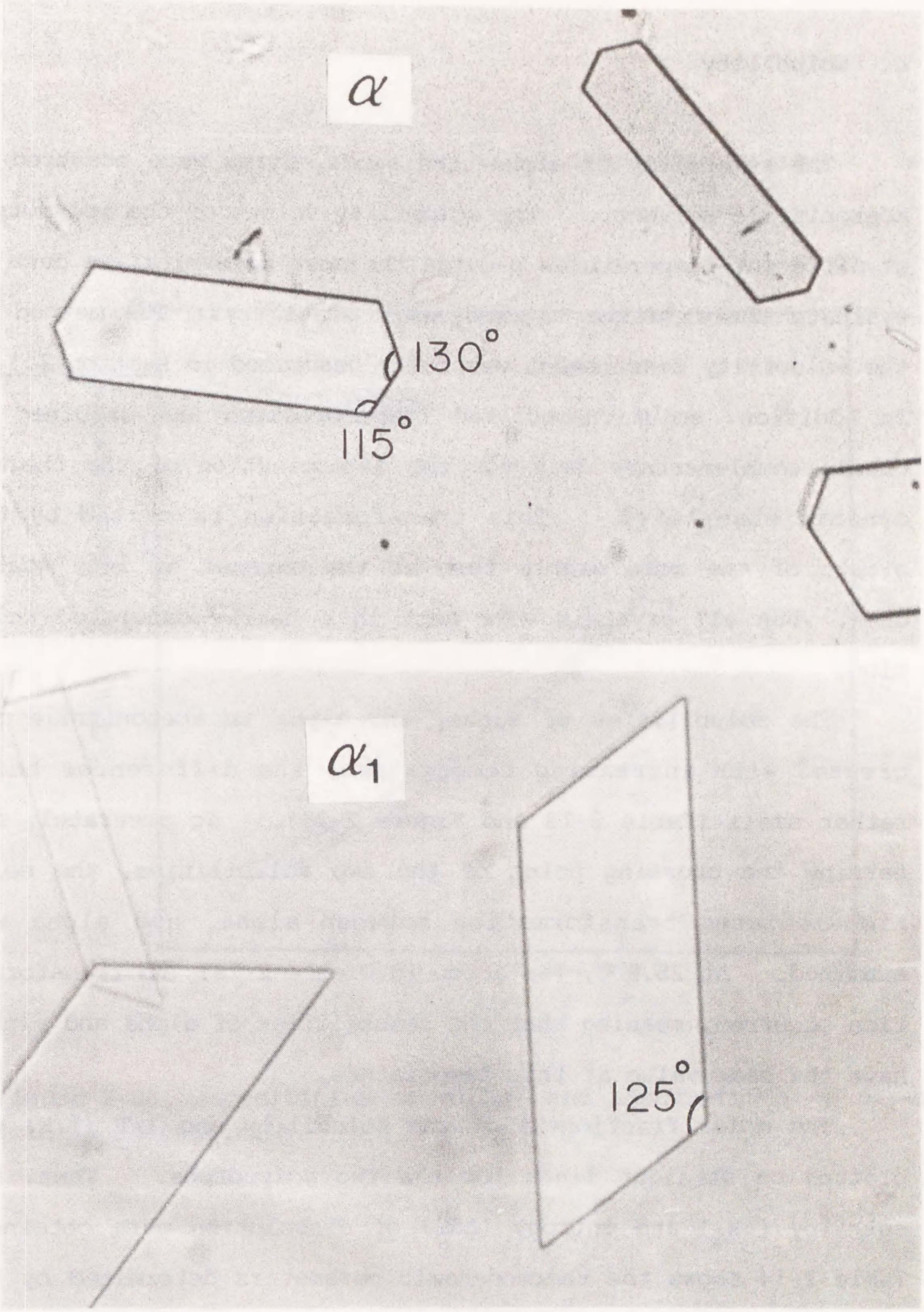

Figure 2-27 Single crystals of alpha- and alpha$_1$-forms of erucic acid grown from acetonitrile.

c. Solubility

The solubility of alpha- and $alpha_1$-forms were measured in acetonitrile solvent. The solubility values of the polymorphs at different temperatures provide the most determinative data to evaluate the relative thermodynamic stability. The method of the solubility measurement was fully described in section 2.1.b. In addition, solution-mediated transformation was examined to obtain complementary data for the determination of the thermodynamic stability. This transformation is caused by the growth of the more stable form at the expense of less stable ones, when all crystals were kept in a nearly saturated solution.

The solubilities of $alpha_1$ and alpha in acetonitrile increased with increasing temperature, the differences being rather small (Table 2-13 and Figure 2-28). To accurately determine the crossing point of the two solubilities, the solution-mediated transformation between $alpha_1$ and alpha was examined. At 25.9 C, (an arrow in Figure 2-28), no transformation occurred, meaning that the solubilities of alpha and $alpha_1$ have the same value at this temperature.

The molar fraction(X) of the solubility and 1/T (1/K) was plotted on straight lines for the two polymorphs. Then, the enthalpy (ΔH_d) and entropy (ΔS_d) of dissolution were obtained. Table 2-14 shows the thermodynamic parameters determined by the solubility and solid-state transformation. As to alpha and $alpha_1$, the solubilities (Figure 2-28) clearly show that $alpha_1$ is more stable above 25.9 C, and vice versa below that temperature. The solid-state transformations are some-what complicated. The reversible transformations of gamma-alpha and $gamma_1$-

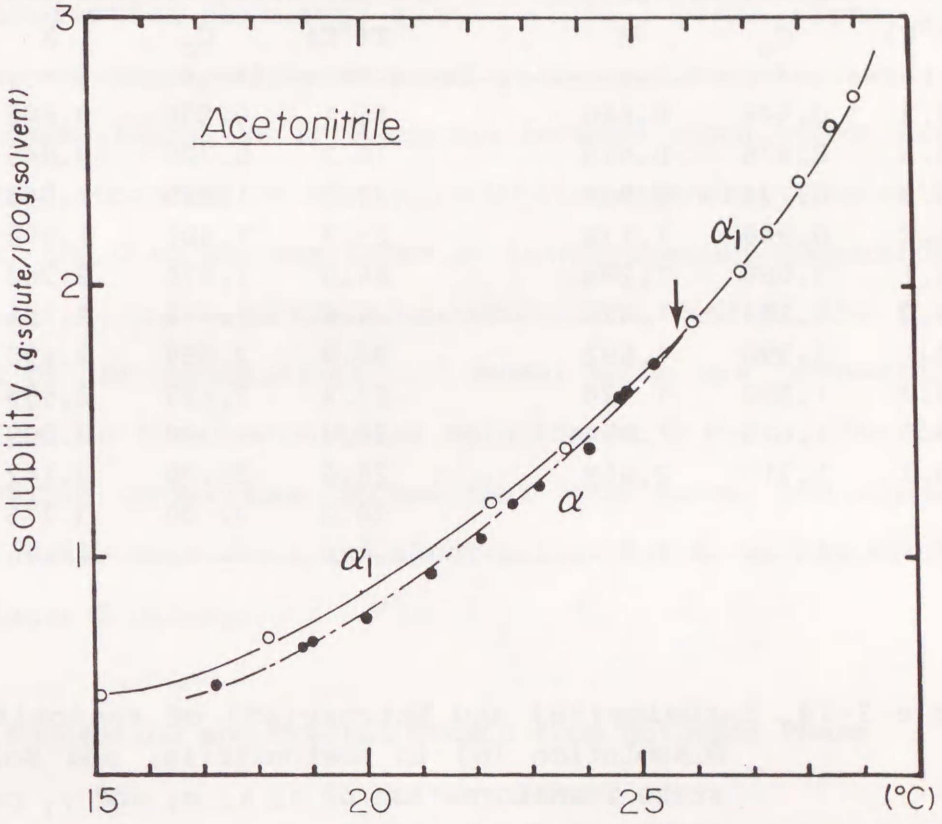

Figure 2-28 Solubilities of alpha- and alpha$_1$-forms of erucic acid in acetonitrile.

Table 2-13 Solubilities of alpha and alpha$_1$-forms of Erucic Acid in Acetonitrile: C_o (g solute/100g solvent) and X (molar fraction)

alpha			alpha$_1$		
T(^{0}C)	C_o	X	T(^{0}C)	C_o	X
17.2	0.545	0.660	15.1	0.500	0.606
18.8	0.675	0.818	18.2	0.700	0.845
20.0	0.774	0.937	22.3	1.200	1.045
21.2	0.940	1.138	23.7	1.400	1.694
22.1	1.069	1.294	26.0	1.870	2.262
23.2	1.259	1.524	26.6	1.952	2.361
24.1	1.398	1.692	26.9	2.059	2.490
24.7	1.590	1.924	27.4	2.199	2.659
24.8	1.615	1.954	28.0	2.390	2.889
25.3	1.710	2.069	28.6	26.00	3.142
			29.0	27.00	3.275

Table 2-14. Enthalpy(ΔH) and Entropy(ΔS) of Fusion(f), Dissolution (d) in Acetonitrile, and Solid-state Transformation of α, γ, α_1 and γ_1 polymorphs of Erucic Acid

	T (°C)	ΔH (kJ/mol)	ΔS (J/mol K)
α_1 (f)	34.0	51.0	166.2
α_1 (d)	-	94.5	264.9
α (d)	-	106.6	305.1
Solid-state Transformation			
$\alpha \rightarrow \alpha_1$	31.2	5.4	17.6
$\gamma_1 \rightarrow \alpha_1$	9.0	8.9	31.6
$\gamma \rightarrow \alpha$	-1.0	8.8	32.3

$alpha_1$ mean that $alpha_1$ is more stable than gamma above -1.0 C.

Consequently, crystal Gibbs energies (G) of the four polymorphs against temperature (T) may be drawn in a range of temperature from -20 to 34 C (Figure 2-29) in a qualitative manner. According to this G-T relationship, one may argue the following transformation pathways: $gamma_1$-$alpha_1$, gamma-alpha, alpha→ $alpha_1$ and $gamma_1$→alpha occurred in the solid state, among which only $gamma_1$→alpha is irreversible because $alpha_1$→alpha actually occurred according to the solubility relationship (Figure 2-28). Hence, the G values may cross at transformation temperatures at heating of the reversible transformation, and at the crossing point of the solubilities. $Gamma_1$→alpha was irreversible as examined in the acetonitrile solution at 2 C. The two irreversible conversions proved that both $gamma_1$ and $alpha_1$ are less stable than gamma and alpha below 25.9 C, as illustrated in the lager G values.

d. Nucleation and Crystal Growth from Solution Phase

d.1 Nucleation from Solution Phase

Occurrence of alpha and $alpha_1$ was measured from acetonitrile solution as a function of supersaturation (σ). The relative concentration of each polymorph was counted for first appearing 50 crystals in solution whose supersaturation was controlled by varying the solute concentration. The value of supersaturation, σ, was calculated according to the solubility data. In prior to the measurement, the sealed solution in a glass-made cell was heated up to 80 C in order to achieve complete dissolution. T_c was kept at a constant temperature (17.8

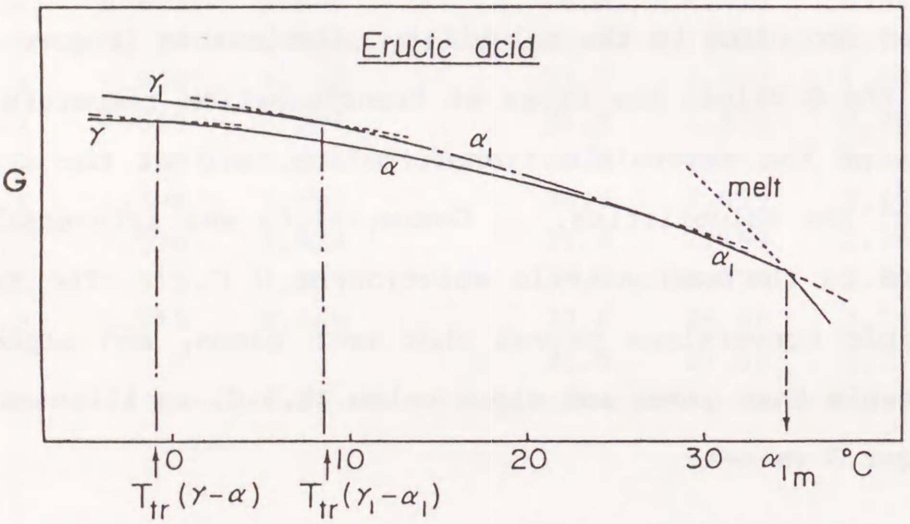

Figure 2-29 A relationship between Gibbs energy (G) and temperature (T) of gamma$_1$-, alpha$_1$-, gamma- and alpha-forms of erucic acid.

C) maintained by thermostated water running around the growth cell which was placed on a stage of a polarized optical microscope (Figure 2-30). At 17.8 C, alpha-form is more stable than alpha_1. The determination of polymorphic modification of first appearing crystals was done by observing the unique crystal habit.

The results were shown in Figure 2-31 and Table 2-15. In the lower supersaturation range, the occurrence of the most stable alpha-form was mostly observed. However, in the supersaturation range above 11%, metastable alpha_1-form occurred predominantly. The relative concentration of the two forms has the same value at around σ=11%.

The occurrence is primarily governed by nucleation of crystal, as the successive growth may be less determinative. Hence, the nucleation function of alpha and alpha_1 at 17.8 C obtained from Figure 2-31 may be drawn in Figure 2-32. A simple nucleation function can be written as[9)]

$$J = K_0 \exp \left\{ \frac{C' \gamma^3}{(kT)^3 (\ln\beta)^2} \right\} \qquad [2.2\text{-}1]$$

in which K_0 a kinetic parameter, C' a parameter including a shape factor of a crystal nucleus and molecular volume, kT thermal factors, γ a crystal-solution interfacial energy and β supersaturation ratio ($\beta = X/X_S$; X, molar fraction of the actual solution and X_S; molar fraction in the saturated solution).

According to the difference in the solubilities of alpha and alpha_1, one obtains the difference in the β values between alpha and alpha_1. Therefore, J_α must be larger than $J_{\alpha 1}$ at lower β values. At larger β values, however, $J_{\alpha 1}$ exceeds J_α

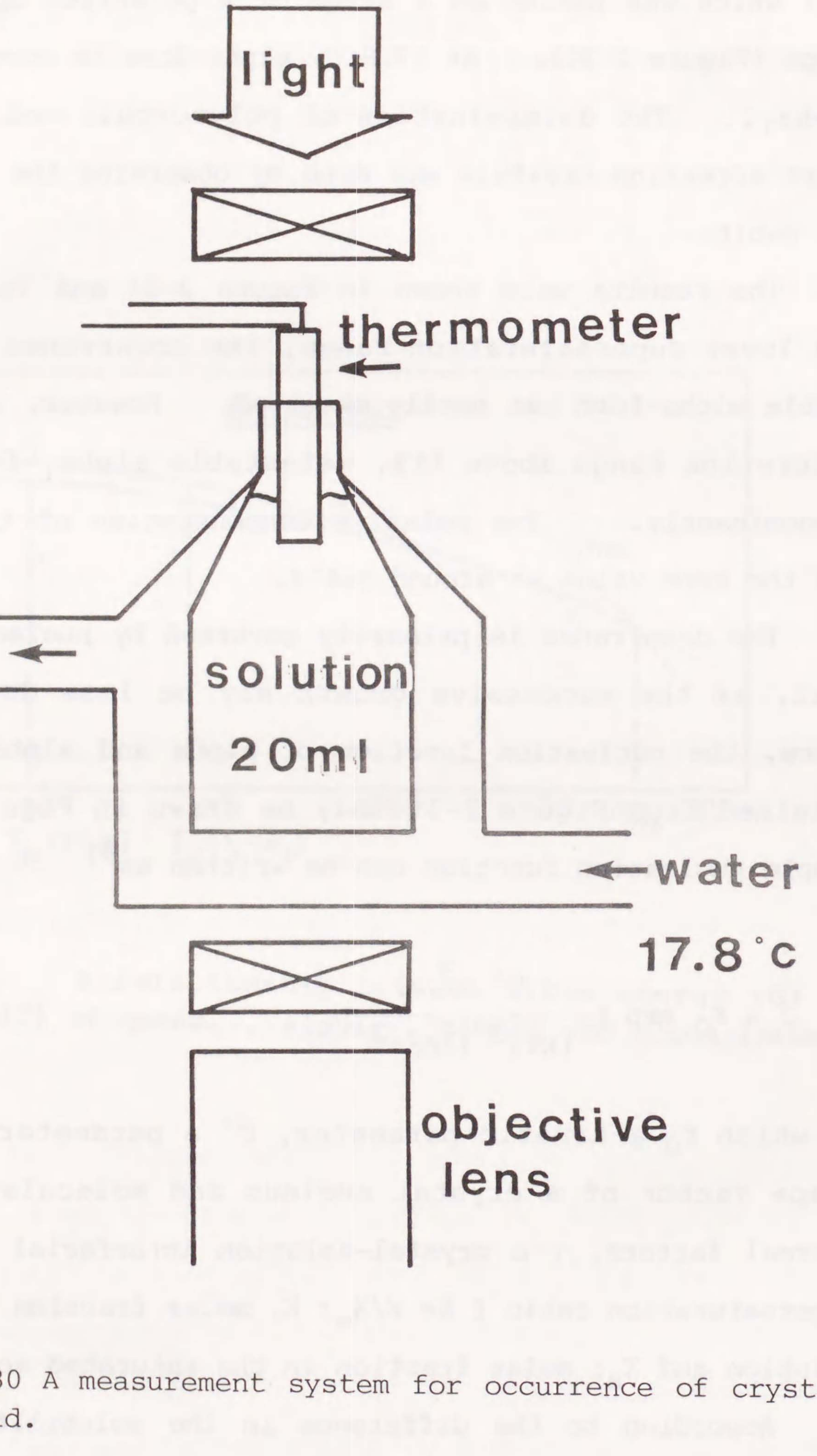

Figure 2-30 A measurement system for occurrence of crystals of erucic acid.

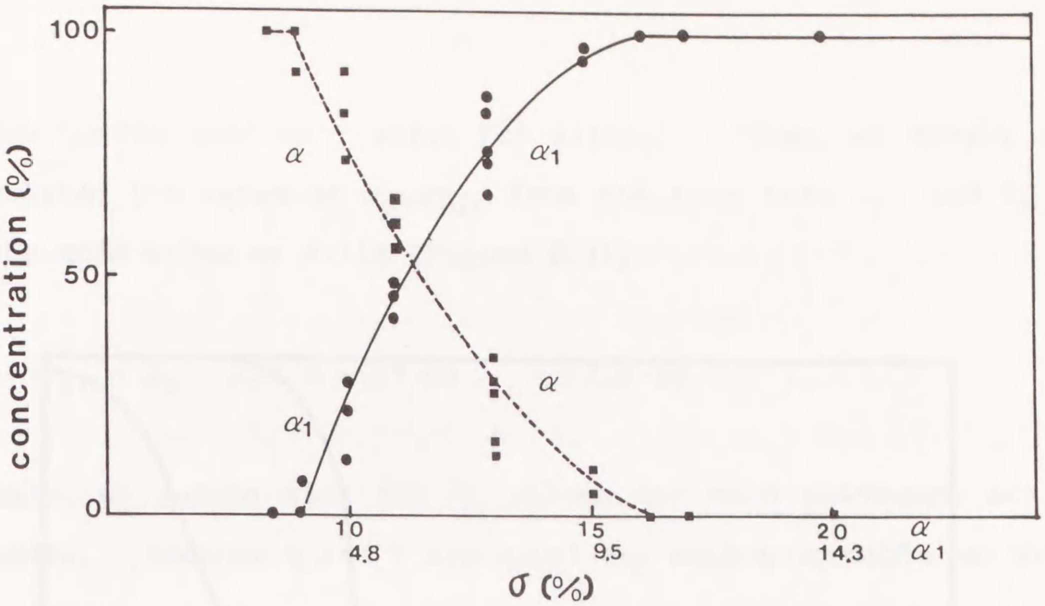

Figure 2-31 Relative concentration of occurred crystals of alpha- and alpha$_1$-forms of erucic acid from acetonitrile solution.

Table 2-15 Occurrance of α and α$_1$ Polymorphs of Erucic Acid at 17.8 C as a Function of Supersaturation (σ) in Acetonitrile

σ(α) (%)	σ(α$_1$) (%)	concentration (%) α	concentration (%) α$_1$	σ(α) (%)	σ(α$_1$) (%)	concentration (%) α	concentration (%) α$_1$
5.0	0.1	0	0	13.0	7.6	42	58
		0	0			38	62
		0	0			34	66
7.0	2.0	0	0			30	70
		0	0			28	72
		0	0	15.0	9.5	12	88
8.0	3.0	100	0			12	88
		100	0			10	90
		100	0	16.0	10.5	0	100
9.0	3.8	100	0			0	100
		92	8			0	100
		90	10	17.0	11.4	0	100
10.0	4.8	90	10			0	100
		80	20			0	100
		76	24	20.0	14.3	0	100
11.0	5.7	74	26			0	100
		64	36			0	100
		56	44				

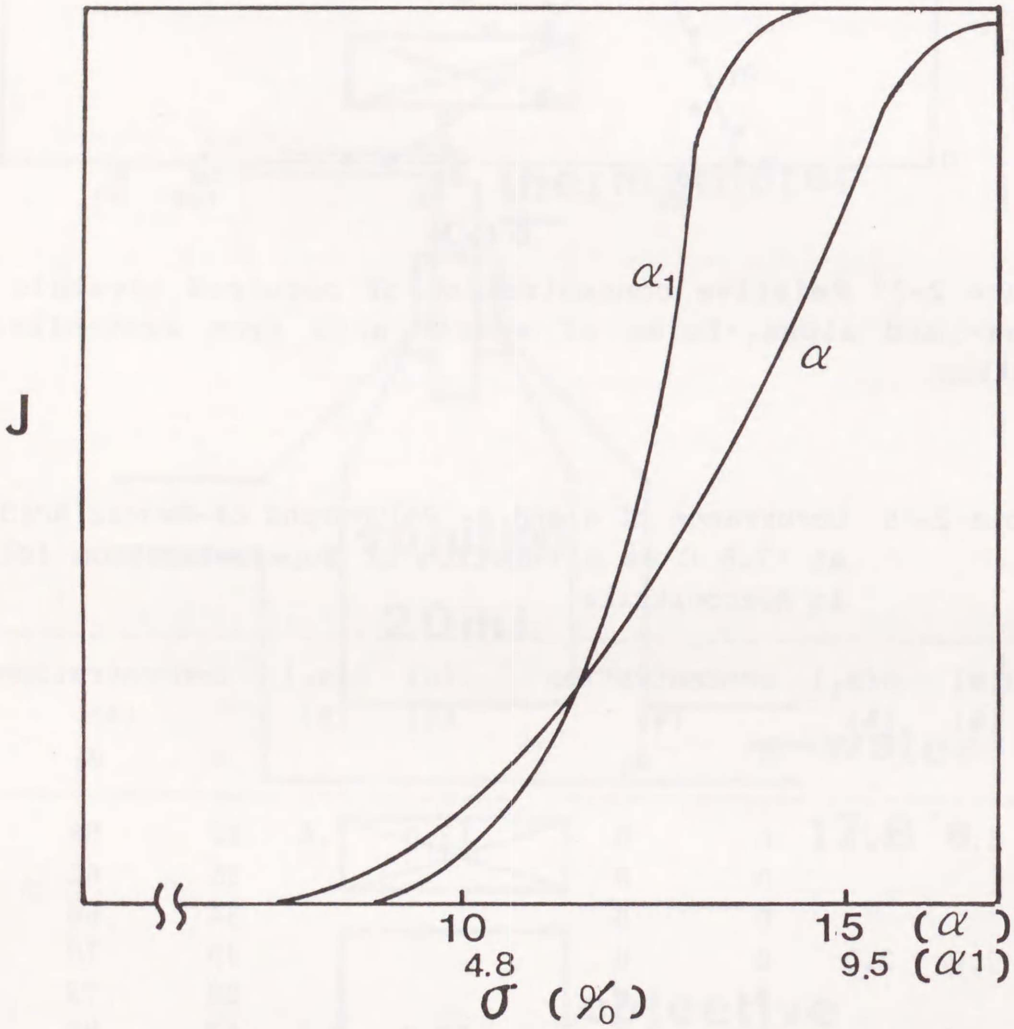

Figure 2-32 Nucleation functions (J) of alpha- and alpha$_1$-forms of erucic acid in acetonitrile at 17.8 C.

due to the smaller γ value for alpha_1. Then, we simply calculated the ratio of $\gamma_{\alpha 1}/\gamma_{\alpha}$, from the fact that $J_{\alpha 1}$ and J_{α} had the same value at σ=11% (Figure 2-31):

$$J_{\alpha 1} = J_{\alpha} \quad (\text{at } \sigma = 11\ \%) \qquad [2.2\text{-}2]$$

Here, we assume that the K_o values for each polymorph are the same. Because k and T are equal for each polymorph, we have

$$\frac{C'_{\alpha 1}\ \gamma_{\alpha 1}^{\ 3}}{C'_{\alpha}\ \gamma_{\alpha}^{\ 3}} = \frac{(\ln \beta_{\ \alpha 1})^2}{(\ln \beta_{\ \alpha})^2} = 0.282 \qquad [2.2\text{-}3]$$

where the value of 0.282 were calculated from solubilities. It could be assumed that the shape factors of alpha- and alpha_1 were the same, since the crystal shape of the two polymorphs did not differ appreciably. However, the molecular volumes may be different. In the case of gamma_1- and gamma-forms, the ratio of molecular volumes are 1.013. If we give the rational maximum value of 1.04 for the ratio of molecular volumes of alpha_1- and alpha-forms, we have

$$\gamma_{\alpha 1} / \gamma_{\alpha} = 0.638 \qquad [2.2\text{-}4]$$

if we calculate as the C' values are the same in the two polymorphs, the ratio of the γ is

$$\gamma_{\alpha 1} / \gamma_{\alpha} = 0.655 \qquad [2.2\text{-}5]$$

In this calculation, the difference of γ values between alpha- and alpha_1-forms is too large in a quantitative manner.

Hence, we assume that the the nucleation rate J dose not depend only on the surface energy and supersaturation. Certain influences of heterogeneous nucleation may be relevant, although quantitative treatment are very difficult.

This result may be discussed in terms of Ostwald's law of stages$^{10)}$, which predicts that metastable state tends to first appear under non-equilibrium conditions. Recent consideration on the crystalline polymorphism showed$^{11)}$ that the polymorph which has the higher rate of occurrence can vary with the supersaturation, and depends not only on the surface energy. As a result, the preferable occurrence of the metastable polymorph can be rather limited in a narrow range of supersaturation, as seen in azo disperse dye$^{12)}$ and in the present case.

d.2 Crystal Growth from Solution Phase

The rate of crystal growth from solution phase were measured for alpha- and alpha$_1$-forms as a function of supersaturation (σ) by using a polarized optical microscope equipped with a video system. The measurements were carried out in a stirred acetonitrile solution (Figure 2-33). A single crystal was put in the supersaturated solution in a glass-made growth cell whose temperature was controlled by thermostated water (± 0.02 C). The temperature of the solution was monitored with a thermocouple which was sunken in the solution. We measured the growth rate along the b axis for each polymorph. The supersaturation was varied by changing the temperature, keeping the solute concentration constant.

Figure 2-34 and Table 2-16 shows the dissolution and growth rates of alpha-form at the saturation temperatures (σ = 0 %)

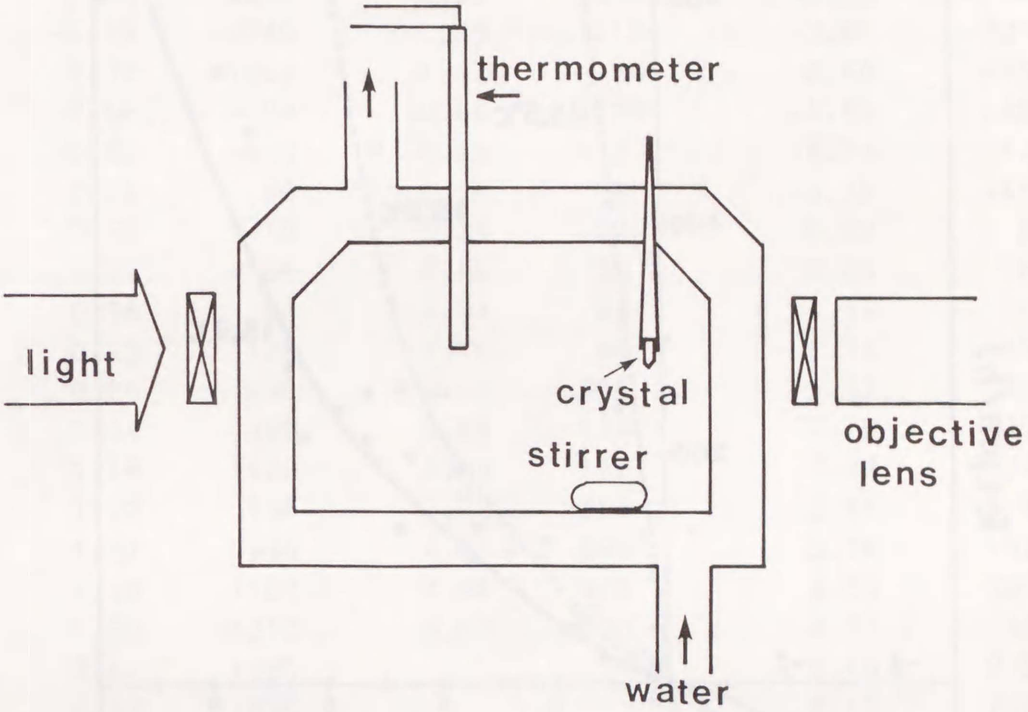

Figure 2-33 A measurement system for rate of crystal growth.

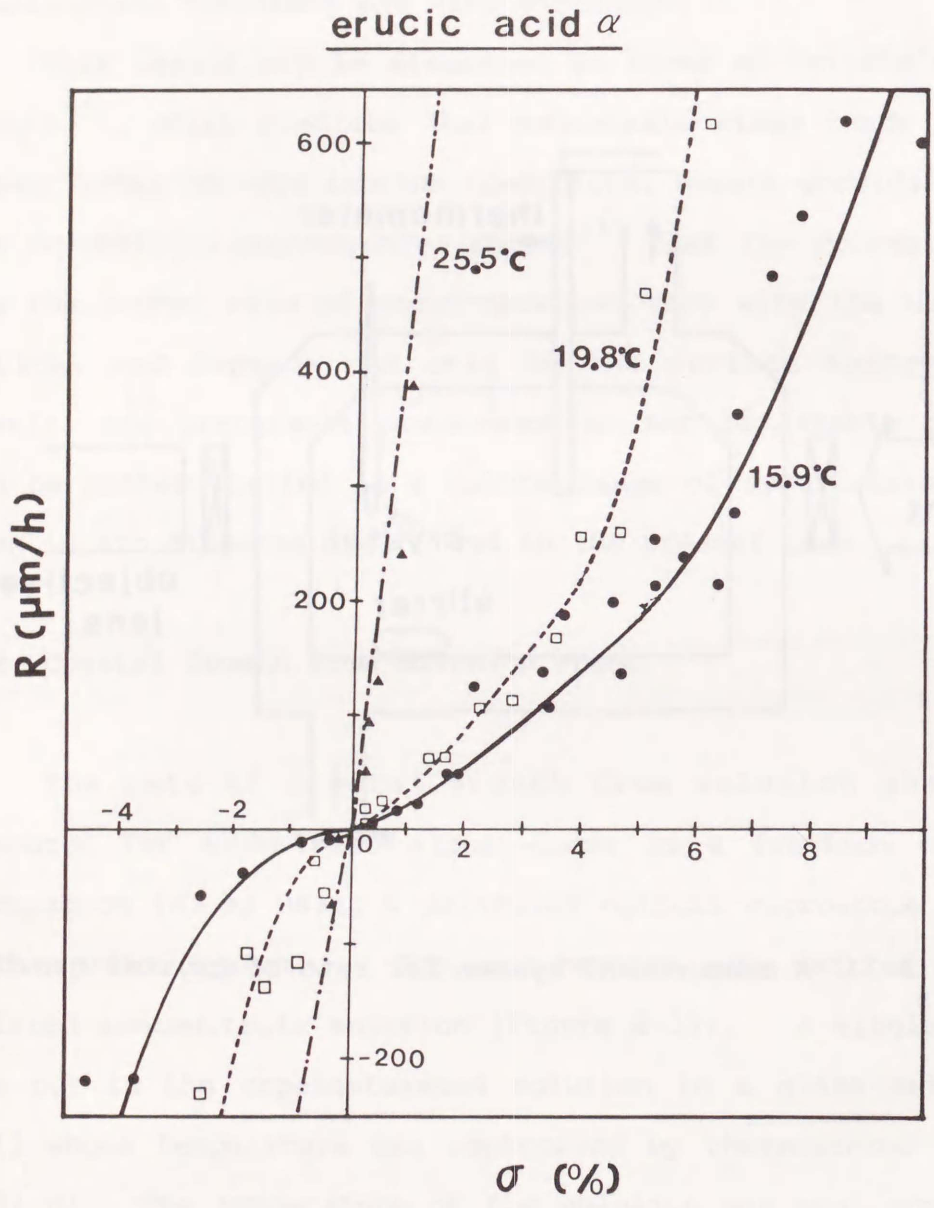

Figure 2-34 Rates of crystal growth (R) of alpha-form in acetonitrile as a function of supersaturation (σ).

Table 2-16 Growth and Dissolution Rates of Alpha-form form Solution

Ts = 22.5 C		Ts = 19.8 C		Ts = 15.9	
σ (%)	R (μm/h)	σ	R	σ	R
-1.96	-2270	-2.51	-230	-4.65	-220
-1.55	-1340	-1.85	-112	-3.61	-220
-1.38	-1650	-1.42	-136	-2.66	-55
-0.68	-780	-0.84	-121	-1.87	-38
-0.55	-640	-0.66	-32	-0.74	-12
-0.22	-68	0.24	8	-0.32	-16
-0.55	-10	0.24	24	0.29	8
0.27	51	0.48	32	0.88	16
0.38	96	1.34	64	1.17	20
0.55	125	1.70	64	1.74	55
0.86	240	2.26	115	1.92	55
1.04	390	2.88	124	2.19	130
1.18	620	3.66	168	3.36	146
1.22	940	3.93	260	3.41	116
1.41	930	4.65	270	3.76	182
1.50	1100	4.99	470	4.54	200
1.69	1210	6.00	620	4.77	146
2.41	1450			5.16	215
2.43	1830			5.16	260
2.85	2770			5.75	240
3.39	3290			6.24	215
3.82	4820			6.59	280
				6.59	370
				7.21	480
				7.66	540
				8.51	630
				9.90	600

were 25.5, 19.8 and 15.9 C. The value was changed by changing the crystallization temperature. Although the dispersion of data is remarkable, some tendency can be drawn; (a) the growth rate increases with increasing temperature and supersaturation. (b) There are turning points in the supersaturation dependence of the growth rate as indicated by arrows. These turning points correspond to the changing points in the morphology of the single crystal: from the unique polyhedral habit, as shown in Figure 2-27, to needle-like shape.

Figure 2-35 and Table 2-17 shows the growth rates of alpha- and alpha$_1$-forms around 25.5 C where the solubility difference between the two forms are very small (Figure 2-28). The two forms of erucic acid grew more or less in the same manner.

e. Solution Mediated Transformation

Solution mediated transformation rate from alpha$_1$-form to alpha-form was measured at 16 C, at which temperature alpha-form was more stable. Powder crystals, mixed at the ratio of alpha$_1$/alpha = 90 / 10, were put in the stirred solution which was saturated with respect to alpha$_1$-form at this temperature. Every two hours after the transformation initiated, the precipitants were taken out from the solution. We measured the concentration of polymorphs from the intensities of X-ray diffraction long spacing spectra of each form. The result is shown in Figure 2-36 and Table 2-18. The transformation finished over 20 hours. This rate was much slower than that of oleic acid (section 2.1d). In the case of oleic acid, the rate-determining factor was nucleation of beta-form due to the big difference of the solubilities between alpha- and beta-form. However,

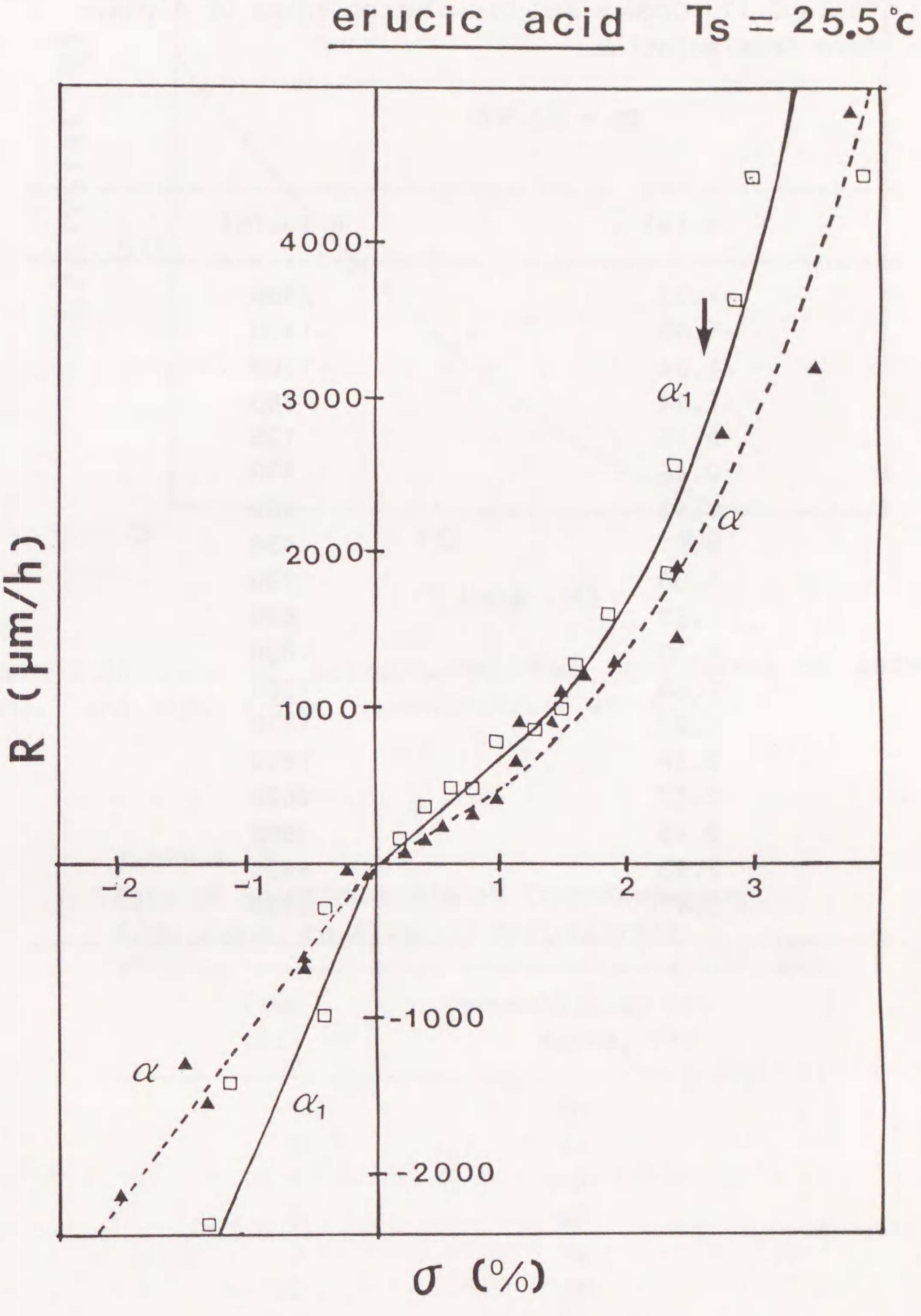

Figure 2-35 Rates of crystal growth (R) of alpha-form and alpha$_1$-forms in acetonitrile at 25.5 C as a function of supersaturation (σ).

Table 2-17 Growth and Dissolution Rates of Alpha$_1$-form from solution

Ts = 22.5 C

σ (%)	R (μm/h)
-1.23	-2400
-1.09	-1430
-1.34	-1000
-1.34	-290
0.16	125
0.46	330
0.62	480
0.81	480
1.00	730
1.33	820
1.50	1000
1.68	1200
1.87	1570
2.29	1840
2.37	2620
2.78	3600
2.93	4430
3.87	4430

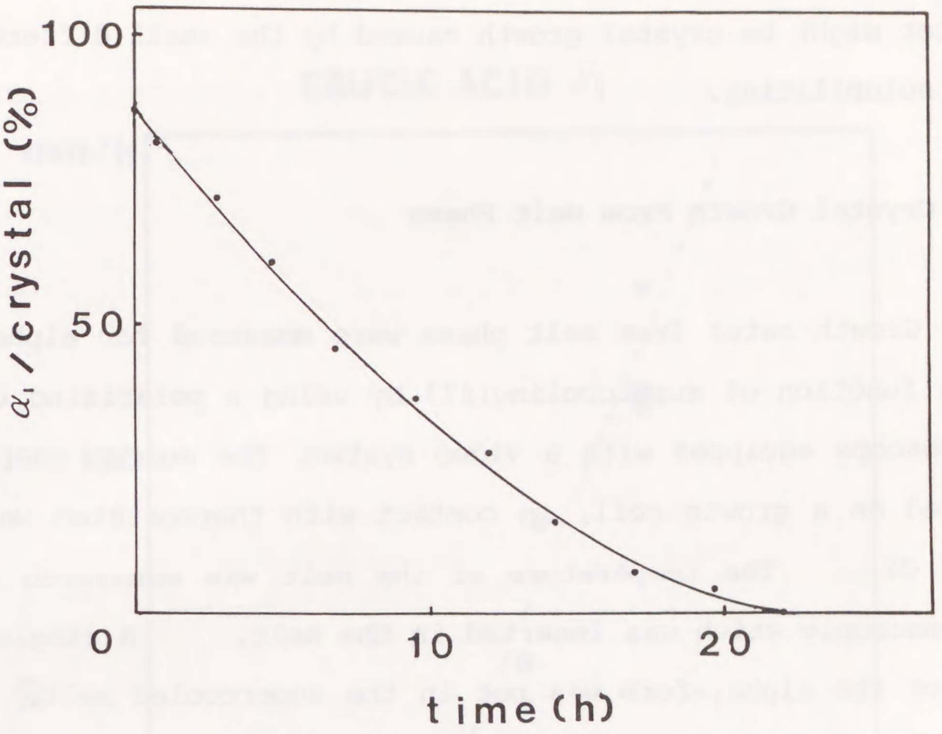

Figure 2-36 Rate of solution-mediated transformation between alpha$_1$- and alpha-forms in acetonitrile at 16.0 C.

Table 2-18
Rate of Solution-mediated Transformation
from alpha$_1$ to alpha in Acetonitrile

Time (hr)	Concentration of alpha$_1$ (%)
1	78
3	72
5	60
7	46
9	36
12	28
14	15
17	8
20	6
22	0

in the case of alpha- and alpha$_1$-forms, the rate-determining factor might be crystal growth caused by the small difference in the solubilities.

f. Crystal Growth From Melt Phase

Growth rates from melt phase were measured for alpha$_1$-form as a function of supercooling(ΔT) by using a polarizing optical microscope equipped with a video system. The molten sample was placed on a growth cell, in contact with thermostated water (± 0.02 C). The temperature of the melt was monitored with a thermocouple which was inserted in the melt. A single crystal of the alpha$_1$-form was put in the supercooled melt. The measurement of the growth rate of the crystal was carried out on a video monitor.

Figure 2-37 and Table 2-19 shows the growth rate of alpha$_1$-form of erucic acid from the melt phase. This rate is of the same order of magnitude as that of oleic acid alpha-form (Figure 2-14). This means that the growth rate of oleic acid beta-form is extraordinarily slow.

g. Discussion

Table 2-20 summarizes kinetic parameters of melt and solution growth of the polymorphs of erucic acid compared with oleic acid. Some thermodynamic parameters are also shown. Both kinetic coefficients and the dependence on supercooling or supersaturation are polymorph-dependent, but most manifest were the kinetic coefficients of alpha- and beta-forms of oleic acid; namely the difference range from 400 (solution growth) to 10^5

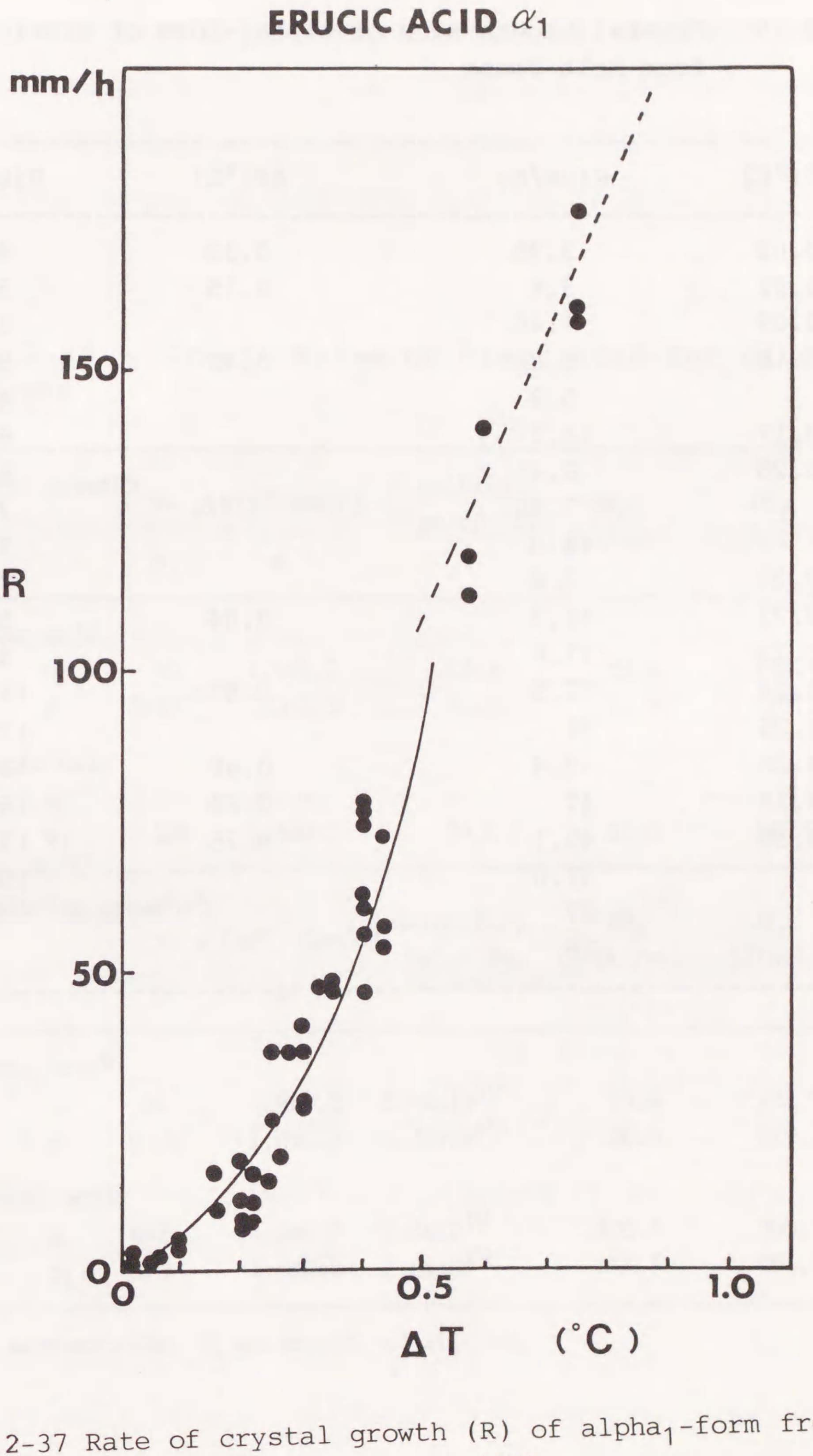

Figure 2-37 Rate of crystal growth (R) of alpha$_1$-form from melt phase as a function of supercooling (ΔT).

Table 2-19 Crystal Growth Rate of Alpha$_1$-form of Erucic Acid from Melt Phase

ΔT(°C)	R(μm/h)	ΔT(°C)	R(μm/h)
0.02	3.75	0.33	46.9
0.02	3.4	0.35	38.8
0.05	1.45		37.5
0.10	3.8	0.40	55.3
	5.9		46.1
0.17	16.3		60.9
0.20	8.45		61.9
	7.90		74.2
	16.0		77.6
0.21	7.8		78.1
0.22	11.3	0.44	53.6
0.23	11.6		56.3
0.24	12.5	0.57	112.5
0.25	36		118.4
0.26	18.4	0.60	140
0.28	37	0.60	160
0.30	40.1	0.75	176
	37.0		158
	27		
	28		

Table 2-20 Growth Rates of Oleic Acid and Erucic Acid Polymorphs

<Melt growth>	$V= p(\Delta T)^n$ (mm/h) p	 n	melting point(°C)	ΔH_f (kJ/mol)	ΔS_f (J/mol/K)
Oleic acid					
α	40	1.0±0.2	13.3	39.6	138.4
β	3×10^{-4}	1.0±0.3	16.2	51.9	179.3
Erucic acid					
α	–	–	–	–	–
α_1	181	1.5±0.2	34.0	51.0	166.2
<Solution growth>^{a}	$V= p'(\sigma)^{n'}$ (μm/h) p'	 n'	solubility $\left(\frac{\text{molecules}}{\text{cc solution}}\right)$	ΔH_d (kJ/mol)	ΔS_d (J/mol/K)
Oleic acid^b					
α	34	1.9±0.5	3.48×10^{19}	59.4	222.9
β	0.09	1.0±0.2	2.32×10^{19}	76.0	279.7
Erucic acid^c					
α	642	1.0±0.2	2.6×10^{19}	106.5	305.1
α_1	810	1.0±0.2	2.6×10^{19}	94.5	264.9

a) acetonitrile, b) quiescent, c) stirred.

(melt growth). It appears that no thermodynamic parameters such as melting point or solubility may account for this anomaly. There are some indications that oleic acid beta-form has the largest crystal density and entropy of fusion. Hence, we speculate some kinetic influences due to molecular structural properties the details of which, however, are presently lacking.

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2.3 Petroselinic Acid

Petroselinic acid stands on a unique position in a series of cis-monounsaturated fatty acids, since its polymorphic properties are quite different from other molecules. This is undoubtedly due to its position of a double bond closer to -COOH group.

In the first, structural properties of petroselinic acid polymorphs are described. Thereafter, crystallization and transformation behavior is explained.

a. Physical Properties of Petroselinic Acid Polymorphs

Polymorphic behavior of petroselinic acid was examined with differential scanning calorimetry,1) X-ray diffractometry,1,2) vibrational spectroscopy2,3) and optical microscopy1). Two polymorphs were observed: a high-melting (HM) form and a low-melting (LM) form. The transformation circuit is shown in Figure 2-38. The two forms always crystallized at the same time from the melt and the solution phase. The LM form transforms slowly to the HM form in the solid state above 17.8 C. On rapid heating of the LM form crystals, it melts at 28.5 C. HM form has melting point at 30.5 C. The enthalpy of fusion (ΔH_f) and the entropy of fusion (ΔS_f) of the HM form are 47.5 kJ/mol and 156.4 J/mol, respectively. 1)

To obtain short and long spacings of powdered samples, X-ray diffraction patterns (Rigaku, Cu-Kα : λ=0.1542nm , Ni-filter) were employed. Figure 2-39 and Table 2-21 show the X-ray diffraction short spacing patterns of the two forms of petroselinic acid and the B form of stearic acid.7) There are

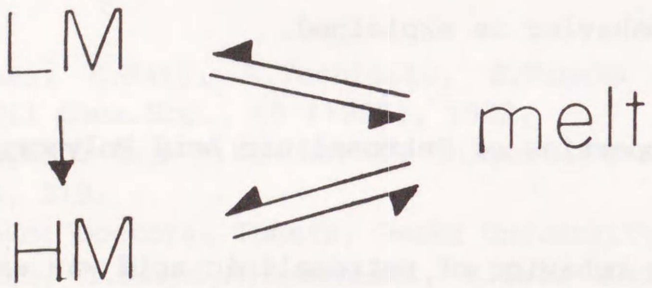

Figure 2-38 Polymorphism and phase transitions in petroselinic acid.

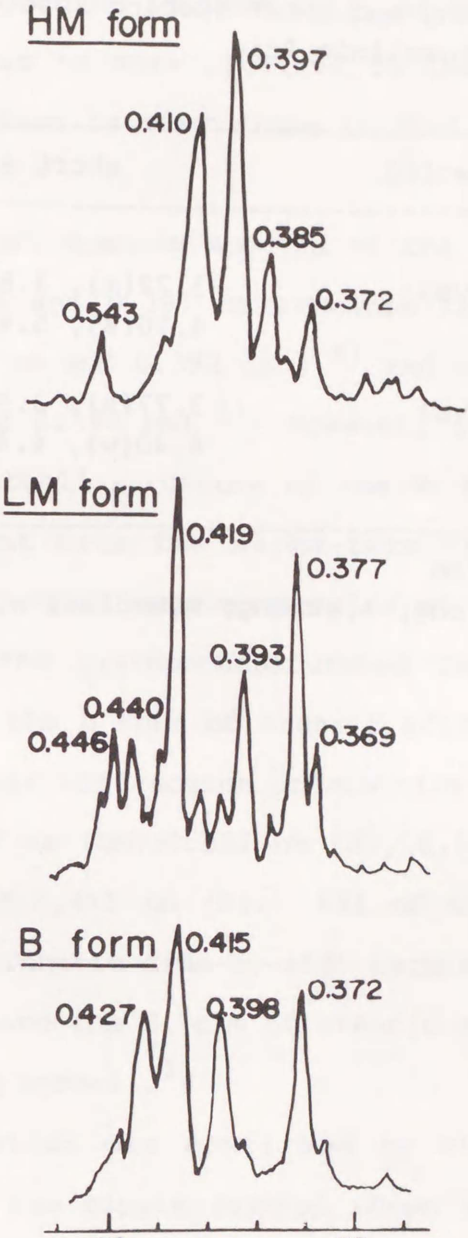

Figure 2-39 X-ray diffraction short spacing spectra of HM form and LM form of petroselinic acid, and B form of stearic acid. (unit, nm)

Table 2-21 X-ray Long and Short Spacing Spectora of LM Form and HM Form of Petroselinic Acid

	long spacing	short spacing
HM	44.0(vs)	3.72(m), 3.85(m), 3.97(vs) 4.10(s), 5.43(w)
LM	41.4(vs)	3.77(s), 3.93(m), 4.19(vs) 4.40(w), 4.46(w)

Unit, Angstrom
vs, very strong; s,strong; m,medium; w,weak.

distinctive differences between the two polymorphs of petroselinic acid. Long spacing, $d_{(001)}$=4.14 nm of the HM form is shorter than that of the LM form $d_{(001)}$=4.40 nm, meaning that the long-chain axis is more inclined in the HM form, and that $d_{(001)}$ of the LM form is very close to that of stearic acid B, 4.38 nm.

As to the short spacing spectra of the HM form, two strong peaks at 0.410 nm and 0.397 nm resemble those of erucic acid alpha-form (0.411 nm and 0.392 nm),4) and of oleic acid alpha-form (0.412 nm and 0.394 nm).5) However, it was recently confirmed that the subcell structure of the HM form of petroselinic acid was different from the alpha-form of oleic and erucic acids.6) The XRD short spacing spectra of the LM form did not resemble any of the cis-monounsaturated fatty acids, instead rather similar to the B form of stearic acid; major short spacing values and their diffraction intensities are quite similar. For example, 0.377 nm (LM):0.372 nm (B), 0.393 nm (LM):0.398 nm (B), 0.419 nm (LM):0.415 nm (B). All of these properties indicate the same aliphatic side-by-side packing of the LM form of petroselinic acid and the B form of stearic acid which is packed according to an $O\perp$ subcell.7)

This speculation was confirmed by the polarized FT-IR spectra taken for the single crystal shown in Figure 2-40. We detected the typical Davidov splitting at 1473 cm^{-1} and 1463 cm^{-1} bands with a polarization direction along the a-axis2) (bisectrix of 75^0), and at 731 and 720 cm^{-1} bands with a polarization direction along the b-axis, which are characteristic of the $O\perp$ subcell for the CH_2 scissoring and CH_2 rocking modes, respectively. The fine structural analysis using 4-cycle X-ray diffraction of the LM form of petroselinic acids confirmed that

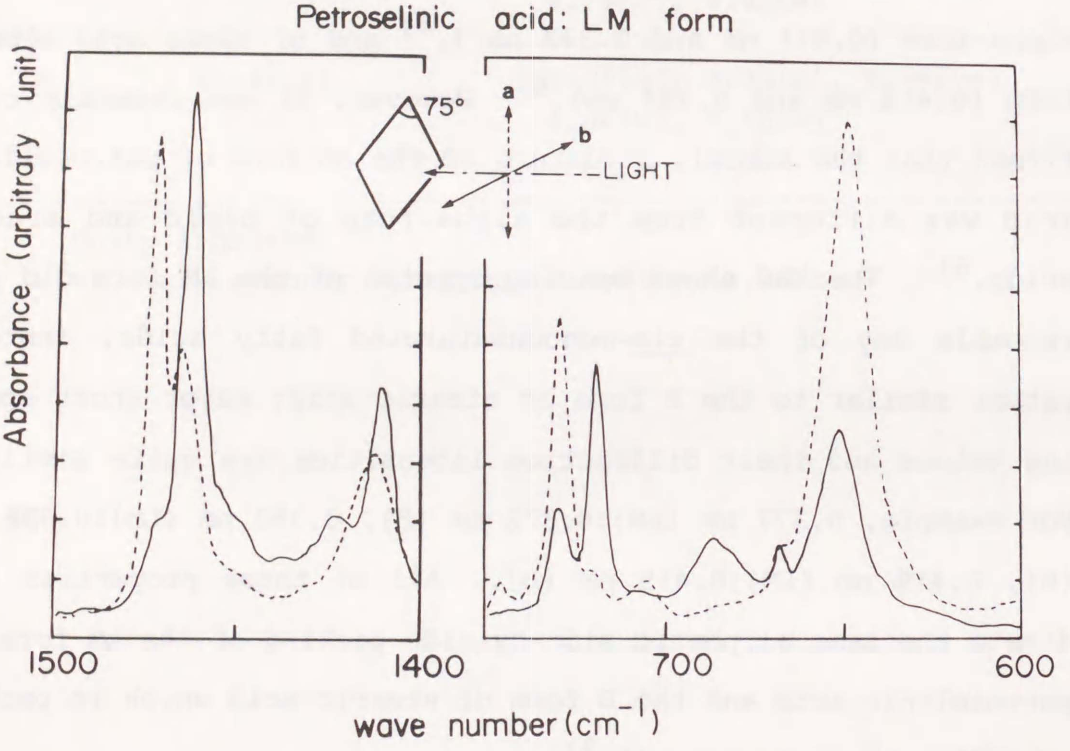

Figure 2-40 Polarized FT-IR spectra of the LM form of petroselinic acid taken at 15 C. (From Ref.1.)

the LM form is exactly packed according to the $O_\perp$ packing.[2,3]

Figure 2-41 and Table 2-22 show a crystal structure and the structural data of LM form,[2] which was determined by Kaneko et al.[2] The structure of LM form belongs to a double-layered polytype structure, orth II, previously found in monoclinic n-alkanes[8,9] and stearic acid B form.[10] Eight molecules in a unit cell form two bimolecular layers related each other by a two-fold c-screw axis. The two hydrocarbon chains linked to the cis olefin group assume all-trans conformation constructing the $O_\perp$ type subcell. This subcell structure is found for the first time in cis-monounsaturated fatty acids.

As to the HM form, the crystal structure was obtained by means of single crystal X-ray diffraction analysis.[6] The crystal data were summarized in Table 2-23. The hydrocarbon chains also assume all-trans conformation, but the subcell structure was more complicated; the omega-chain was in $M_{//}$, but a twisted $O_\perp$ arrangement was observed in the delta-chain. The olefinic C_{11}-C_{12}=C_{13}-C_{14} conformation of the two asymmetric molecules in a unit cell are expressed by the internal rotation angles of (91^0, cis, 130^0) and (137^0, cis, -119^0).[6] Namely, two different conformations, SCS-type and SCS' type, were constructed alternatively in the unit cell.

Table 2-24 summarizes the major molecular properties observed in the two forms of petroselinic acid together with those of other cis-unsaturated fatty acids. As to the LM form, a single crystal analysis[2,3] clarified that the olefinic conformation is expressed by the internal rotation angles of (157^0, cis, -160^0) which far differ from those having the conformations of skew-cis-skew (SCS) (erucic acid gamma_1,[2,3]) or skew-cis-skew' (SCS') (oleic acid gamma[11], erucic acid gamma[2,3]).

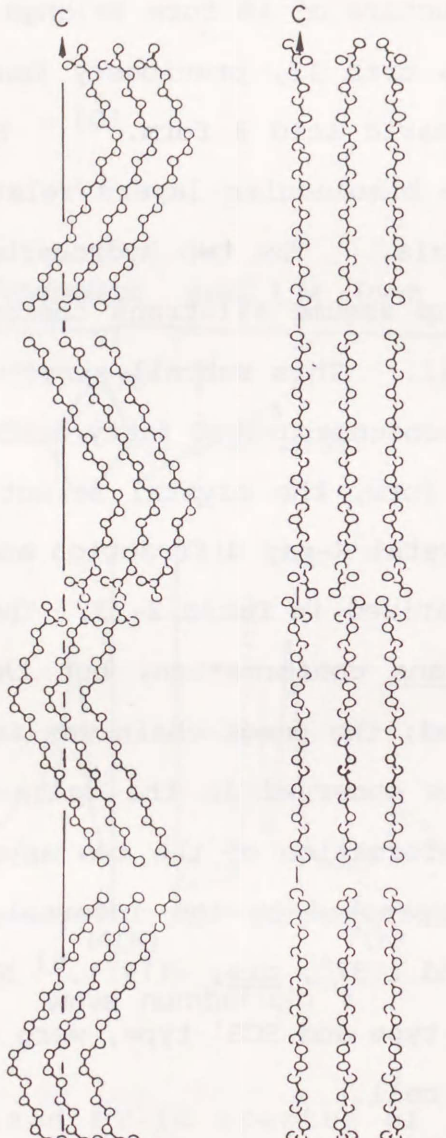

Figure 2-41 Crystal structure of LM form of petroselinic acid. (From. Ref.2,3.)

Table 2-22
Crystal Data of Petroselinic acid LM Form

---

low melting phase of petroselinc acid
<u>cis</u>-$CH_3(CH_2)_{10}CH=CH(CH_2)_4COOH$
Molecular weight:284.84
Orthorhombic:Pbca
a=7.31 b=5.656 c=88.01 A
Z=8
Dx=1.06gcm^{-3}
Temperature 263K

(From Ref.2.)

Table 2-23
Crystal Data of Petroselinic Acid HM Form

high melting phase of petroselinic acid
cis-$CH_3(CH_2)_{10}CH{=}CH(CH_2)_4COOH$
Molecular weight:284.48
Triclinic:$P\bar{1}$
a=5.359 b=8.874 c=41.390 A
$\alpha=90.490^0$ $\beta=89.12^0$ $\gamma=113.81^0$
Z=8

(From Ref.2.)

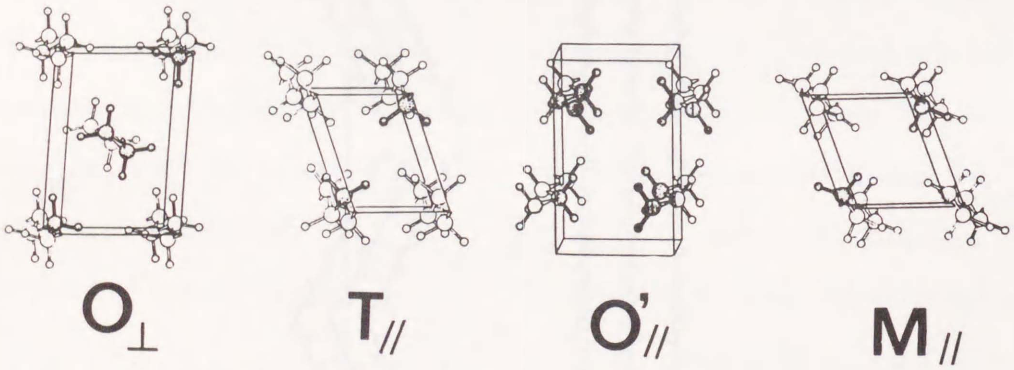

Figure 2-42 Four typical subcells.

Table 2-24 Molecular Properties of Polymorphs in <u>cis</u>-Monounsaturated Fatty Acids

Acid	Polymorph	ω-chain	Δ-chain	olefinic conformation	subcell	$T_{tr}{}^{a}$(°C)	$H_{tr}{}^{a}$(kJ/mol)
Palmitoleic	γ	7	9	SCS'[b]	$O'_{//}$	-18.4 (→ α)	7.5
Oleic	γ	9	9	SCS'	$O'_{//}$	-2.2 (→ α)	8.8
Asclepic	γ	7	11	n.i.[c]	$O'_{//}$	-15.0 (→ α)	7.8
Erucic	γ	9	13	SCS'	$O'_{//}$	-1.0 (→ α)	8.8
Erucic	γ_1	9	13	SCS[d]	$T_{//}$	9.0 (→ α_1)	8.9
Oleic	β	9	9	SCS	//-like	-	-
Petroselinic	LM	12	6	(157,cis,-160)	$O_{\perp}$	-	-
Petroselinic	HM	12	6	91,cis,130 137,cis,119	$O_{\perp}+M_{//}$	-	-
Methyl oleate	Low	9	-	SCS	$T_{//}$-like	-	-
Methyl oleate	High	9	-	SCS'	$T_{//}$-like	-	-

(a) Polymorphic transformation. (b) <u>Skew</u>-<u>cis</u>-<u>skew</u>'. (c) Not identified. (d) <u>Skew</u>-<u>cis</u>-<u>skew</u>.

We assume that there would be a specific relationship between the olefinic conformation and the subcell structure in the cis-monounsaturated fatty acids, since SCS' is observed in the crystals having the $O'_{//}$ subcell (Figure 2-42), whereas $T_{//}$ reveals in the crystals with the SCS conformation. Hence, we infer that the peculiar olefinic conformation of the LM form might enable a total of the aliphatic chain to be packed according to the $O_{\perp}$ subcell.

From this, it is worth noting that the polymorphism of petroselinic acid stands on a specific position in the structural properties of cis-monounsaturated fatty acids. In the first, the LM form belongs to an orthorhombic system, both the unit cell and the subcell. Secondly, there is no order-disorder transformation in the HM and LM form, in contrast to other four cis-monounsaturated fatty acids. The absence of the interfacial melting in the LM and HM forms were confirmed by (a) there was no DSC peaks,1) which are indicative of the order-disorder transformation like gamma-alpha, for the LM and HM forms, (b) Raman and FT-IR spectra also indicated no characteristic change. Third peculiarity is a unique olefinic conformation which may stabilize the $O_{\perp}$ subcell packing as discussed above. In the end, the easiness of the solid-state transformation from the $O_{\perp}$-packed LM form to the complicated HM form. These properties may be ascribed to the position of the cis-double bond, omega-12.

The order-disorder transformation always occurred in the acids whose omega-chain length is shorter than or the same as the delta-chain length. In the ordered gamma phase, the shorter omega-chain may induce a modified molecular vibrational mode at the methyl-sided chain segment which is somewhat different from that in the interior chain portion involving -$(COOH)_2$- in a dimerized unit cell. This was reflected in the X-ray diffraction structure data using the single crystal which proved that the thermal factors at the omega-chain in gamma-form of erucic acid were remarkably larger than those at the delta-chain.[2,3) Hence, on heating, many gauche conformations may be introduced only in the omega-chain segment, transforming to interfacially melted alpha-form, in prior to the melting of the whole molecule.

We estimated that 30-40% of hydrocarbons in the omega-chain in the alpha-form may be in the gauche conformation, by comparing the entropies per CH_2 for the gamma-alpha transformation and fusion of alpha-form of oleic acid to that of fusion of n-alkane crystal having all-trans conformation.12,13) In the longer omega-chain, however, the molecular motion may be hindered like in the interior chain portion. Therefore, "interfacial melting" may not occur in the two forms of petroselinic acid.

Finally, we assume that the effect of parity, even or odd, of the position of the double bond may also be influential. This problem arises, because (a) the order-disorder transformation occurred in the odd-number positioned unsaturated acids (Table 2-24), and (b) the melting points of triacylglycerols 14) and ΔH_{tr} of major chain transformation of phosphatidylcholines,15) both of which have cis-monounsaturated fatty acyl chains, significantly depend on the parity of the double bond position. This phenomenon must be related to the fact that the parity of the carbon numbers of the saturated fatty acids remarkably influences the melting behavior; namely the methyl end packing mode differs between odd- and even-numbered acids, stabilizing the total of the crystal cohesive energy in a different way. Accordingly, this influence may also occur in the cis-monounsaturated fatty acid where the acyl chain is separated into two parts by the cis double bond. The parity of the omega-chain varies with the position of the double bond. The melting point of an odd-numbered saturated acid with (2n+1) carbons is lower than that of an even-numbered saturated fatty acid with (2n) carbons, because of the less stable CH_3 end group packing in the odd-numbered acid. The odd-numbered omega-chain in the cis-monounsaturated fatty acids may behave like the odd-numbered saturated fatty acid, presumably causing the "interfacial melting".

b. Occurrence of HM and LM

The crystallization from the melt was examined in a wide range of crystallization temperature (T_c) (Figure 2-43 and Table 2-25). The two forms always crystallized at the same time. At T_c=-196 C, the concentration of the HM form was approximately 15 %. With increasing T_c, the concentration of the LM form decreased. The rate of melt-crystallization was extremely lowered at T_c= 26 C, where the LM and HM forms crystallized at the same ratio. Hence, it was impossible to obtain the single polymorph of petroselinic acid by simply chilling the melt. The melt growth of the HM form was singly possible above 28.5 C.

Using a polarizing optical microscope, we observed the dissolution and growth process of the single crystal, by fluctuating the solution temperature around the equilibrium condition. As to the metastable form, we completed the experiment before the more stable form started to crystallize.

We grew the single crystals of the two forms. Figure 2-44a

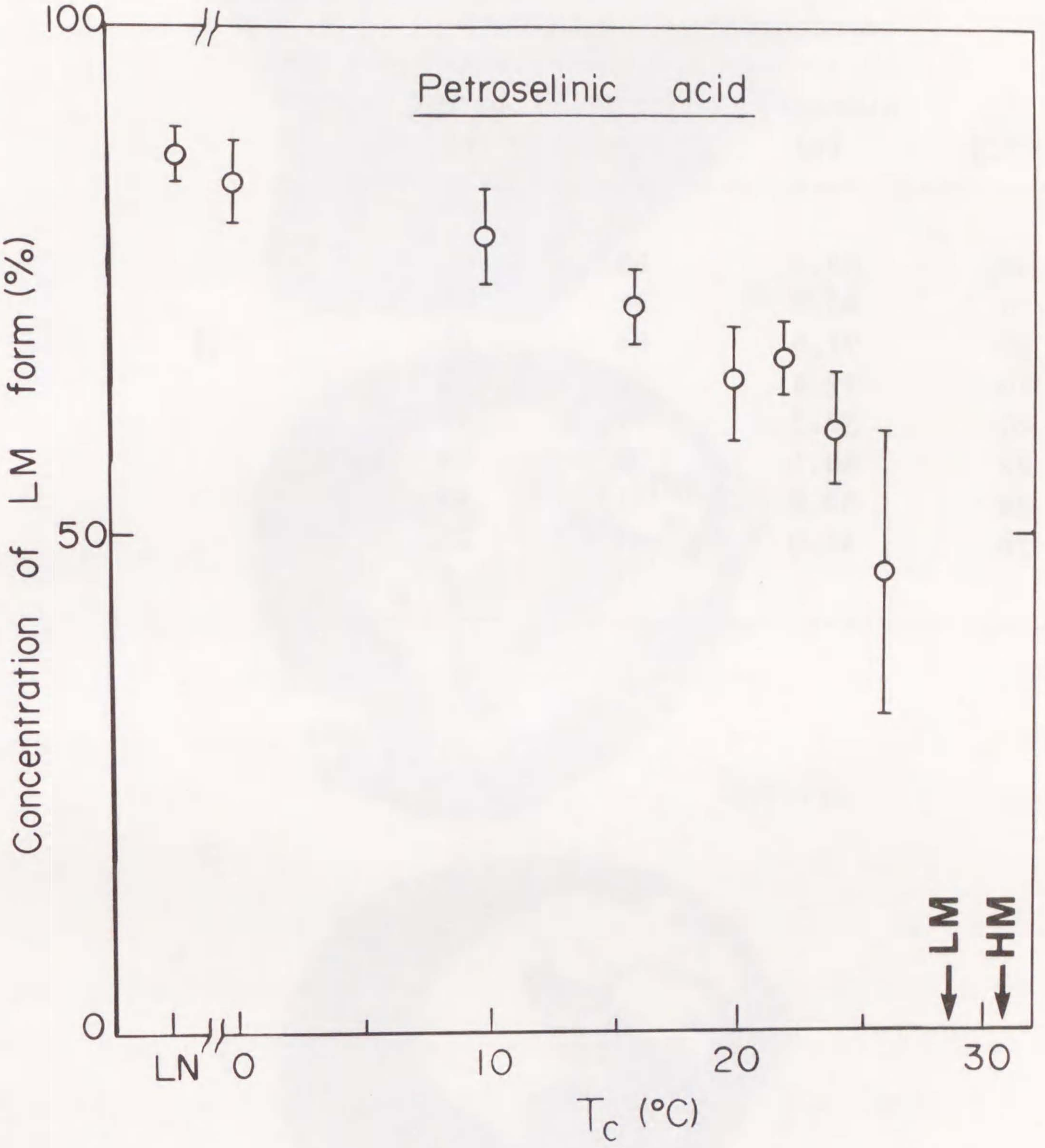

Figure 2-43 Melt crystallization of HM form and LM form of petroselinic acid. Two arrow indicate the melting points of the two polymorphs.

Table 2-25 Occurrance of LM Form and HM Form of Petroselinic Acid from Melt Phase

T_C (^{0}C)	concentration of LM form average (%)	highest (%)	lowest (%)
LN_2	89.3	92	85
0	86.7	91	81
10	77.6	84	75
16	72.6	76	69
20	57.7	71	49
22	68.1	73	64
24	59.6	71	49
26	44.0	67	25

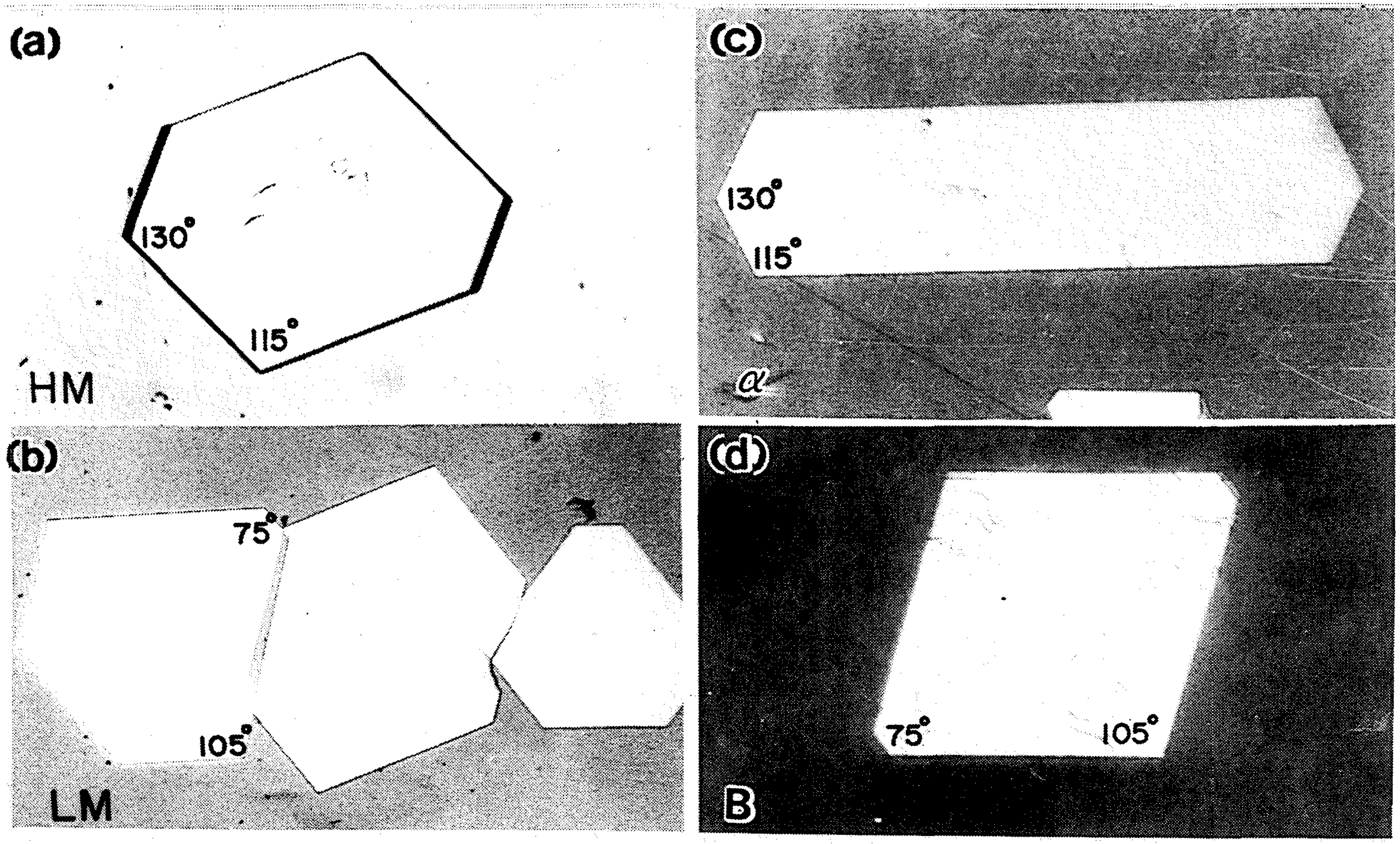

Figure 2-44 Single crystals of two polymorphs of petroselinic acid, (a) and (b), and α-form of oleic acid (c), and B form of stearic acid (d).

shows single crystals of the HM form grown form the melt at 29 C. The same single crystal also could be grown from acetonitrile solutions at 20 C. The LM form was grown from acetonitrile solution around 15 C (Figure 2-44b). It is worthy to note that, while growing the single crystals of the LM form from acetonitrile solutions, the HM form also crystallized, particularly when the solute concentration was raised. This is due to rather small differences in the solubility between the HM and LM forms, and also in nucleation rate of crystal.

The single crystals of the two forms showed plate-like shape, the basal plane assuming parallel to dimerized bi-molecular lamella. The interplanar angles between the lateral faces are 130^0 and 115^0 in the HM form, and 105^0 and 75^0 in the LM form. The interplanar angles of the HM form are the same as those of the alpha-form of oleic, asclepic, erucic and palmitoleic acids (Figure 2-44c). The difference in the crystal morphology between Figure 2-44a and Figure 2-44c may be ascribed to a difference in growth rates of the lateral planes, presumably reflecting the differences in the molecular structures. The crystal shape of the LM form showed no similarity to the three forms of oleic acid. Conspicuously, this crystal shape is identical to that of the B form of stearic acid, Figure 2-44d. This indicates a similarity in an aliphatic side-by-side packing between the two forms.

Using the single crystal of the LM form, we measured its melting point under the polarizing optical microscope. We put the single crystal on a glass cell whose temperature was rapidly changed by thermostated water. Soon after the single crystal of the LM form was put at 29 C, it rapidly melted (Figure 2-45b), and the HM form was nucleated and grew at the expense of the LM

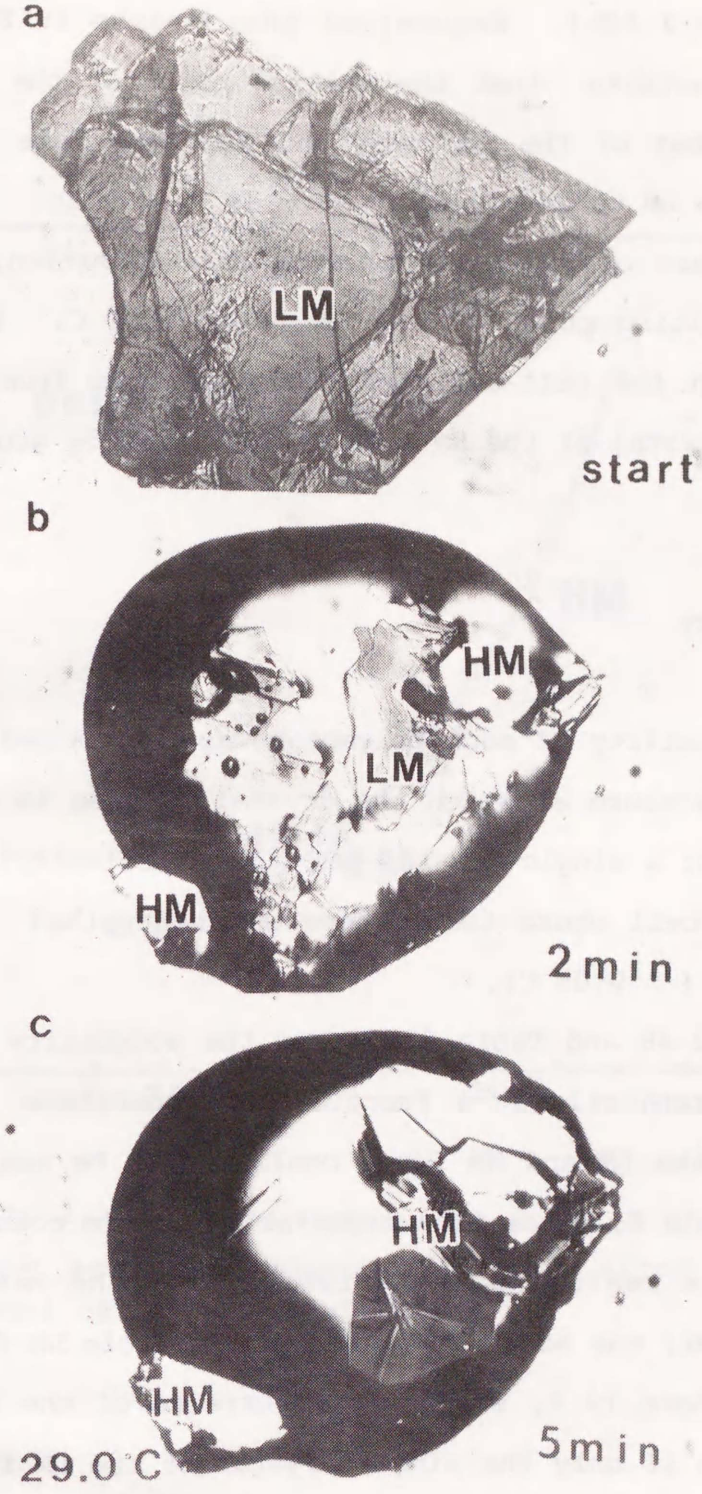

Figure 2-45 Melt-mediated transformation from LM form to HM form of petroselinic acid, just after the single crystal of the LM form was put at 29.0 C (a), crystallization of HM form at the expense (melting) of LM form (b), and crystal growth only of HM form (c).

form (Figure 2-45c). Sequential photographs in Figure 2-44 clearly demonstrate that the melting point of the LM form is lower than that of the HM form, and that the rate of a melt-mediated from LM to HM transformation is very high. By changing the temperature of the melt-mediated transformation, we determined the melting point of the LM form, 28.5 C. We observed that, through the melt-mediated transformation from LM to HM, the single crystal of the HM form could easily be grown from the melt phase.

c. Solubility

The solubility of each polymorph was determined by measuring the temperature at which the crystal became in equilibrium with solution: a single crystal was put in acetonitrile solution in a growth cell whose temperature was controlled by thermostated water (± 0.05 C).

Figure 2-46 and Table 2-26 show the solubility of the two forms in acetonitrile as a function of temperature. The solubilities of the LM and HM forms could easily be measured separately at 10-19 C, since the more stable LM form occurred rather slowly, while measuring the solubility of the metastable HM form. However, the solubility of the metastable LM form was not measurable above 19 C, since the occurrence of the HM form was enhanced even if only the single crystal of the LM form was put in the solution.

Hence, we examined a solution-mediated transformation between the HM and LM forms, to precisely determine a crossing temperature of the two solubilities, as exhibited in erucic acid.$^{4)}$ The crystal powders of the two forms were added in

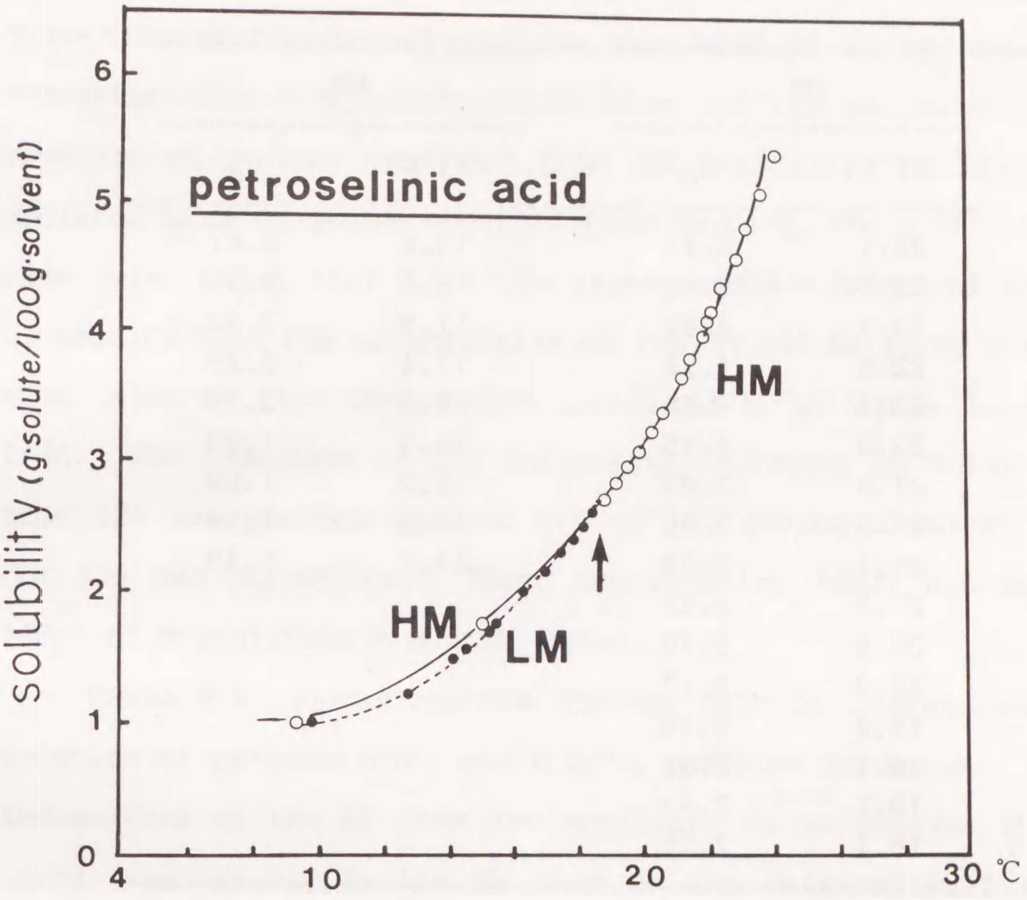

Figure 2-46 Solubilities values of HM form and LM form of petroselinic acid in acetonitrile. An arrow indicates a crossing point of the two solubilities.

Table 2-26 Solubilities of HM and LM Polymorphs of petroselonic Asid in Acetonitrile C_o(g solute/100g solvent)

HM		LM	
T(^{0}C)	C_o	T(^{0}C)	C_o
24.1	5.41	18.4	2.61
23.6	5.10	18.1	2.51
23.1	4.82	17.8	2.42
22.8	4.58	17.4	2.29
22.3	4.35	16.9	2.14
22.0	4.15	16.3	1.99
21.8	3.97	15.2	1.69
21.5	3.80	14.5	1.59
21.1	3.65	14.1	1.49
21.9	3.55		
20.5	3.39		
20.2	3.23		
19.8	3.08		
19.4	2.95		
19.1	2.83		
18.7	2.71		

nearly saturated acetonitrile solution. While keeping the temperature of solution constant and prohibiting solvent evaporation, the crystal-suspended solution was stirred to induce the growth of more stable form at the expense of less stable one. A direction of the transformation was determined by observing characteristic X-ray diffraction long spacing patterns of the crystals which were taken out from the solution. The solution-mediated transformation occurred from LM to HM above 18.7 C, and vice versa below 18.7 C. No transformation occurred at 18.7 C, meaning that the solubilities of the HM and LM forms have the same value at this temperature indicated by an arrow in Figure 2-46. The logarithm of the solubility expressed by molar fraction (X) was plotted against 1/T (K^{-1}), giving straight lines for the two polymorphs. Then, the enthalpy (ΔH_d) and entropy (ΔS_d) of dissolution were calculated.

Table 2-27 summarizes the thermal data of fusion and dissolution of petroselinic, oleic acid and stearic acids. No fusion data of the LM form are available to us because it converts irreversibly to the HM form by the melt-mediated transformation. From Table 2-27, it follows that all of the thermal parameters of petroselinic acid are larger than those of oleic acid, except for beta-form of oleic acid whose enthalpy and entropy values of fusion are larger than those of the HM form of petroselinic acid. T_m and ΔH_f of alpha'-form of asclepic acid are 13.8 C and 39.8 kJ/mol, respectively,16) both of which are close to those of oleic acid alpha-form and smaller than those of the two forms of petroselinic acid. This may be attributed to more dense molecular packing in petroselinic acid caused by shifting the position of cis-double bond toward the -COOH group. As to beta-form of oleic acid, its molecular pack-

Table 2-27 Melting Point (T_m, C)), Enthalpy (ΔH, kJmol^{-1})) and Entropy (ΔS, JK^{-1}mol^{-1}) of Fusion (f)*, Dissolution (d) (in Acetonitrile) of HM and LM Forms of Petroselinic Acid, and Forms of Oleic Acid and C Form of Stearic Acid

Petroselinic acid				Oleic acid				stearic acid			
	T_m	ΔH	ΔS		T_m	ΔH	ΔS		T_m	ΔH	ΔS
HM(f)	30.5	47.5	156.4	α(f)	13.3	39.6	138.4	C	69.6	61.3	178.8
LM(f)	28.5	-	-	β(f)	16.2	51.9	179.3				
HM(d)	-	87.4	253.6	α(d)	-	59.4	222.9				
LM(d)	-	91.8	268.6	β(d)	-	76.0	279.7				

*)From Ref.1.

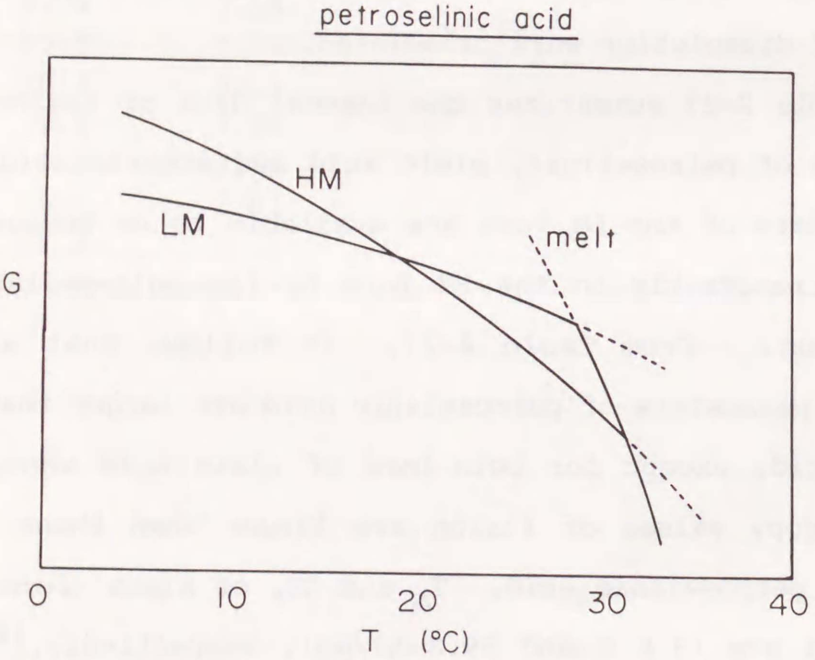

Figure 2-47 A qualitative illustration of Gibbs free energies of HM form, LM form and melt of petroselinic acid against temperature.

ing may be extraordinarily dense.

It is interesting to compare the LM form to the B form of stearic acid. Neither solubility datum in acetonitrile nor fusion datum can be obtained for B, since B is sparingly soluble in acetonitrile and irreversibly transforms to C on heating. So, we evaluated $\Delta H_f(B)$ as 65.6 kJ/mol by adding $\Delta H_{tr}(B \rightarrow C) = 4.3$ kJ/mol to $\Delta H_f(C)$.17) Similarly, by adding the difference in ΔH_d between the LM and HM forms, 4.4 kJ/mol, to $\Delta H_f(HM) = 47.5$ kJ/mol, we evaluated $\Delta H_f(LM) = 51.9$ kJ/mol, which is quite smaller than the estimated value of $\Delta H_f(B)$ of stearic acid. This is due to unstable molecular packing caused by the cis-double bond, irrespective of its position.

The thermodynamic stability was confirmed by the solubility data and the melt-mediated transformation, as drawn in a qualitative relationship between Gibbs free energy (G) and temperature (G-T relationship) in Figure 2-47.

d. Rates of Polymorphic Transformation

The rate of transformation in a crystalline state from the LM to HM forms was measured at various temperatures above 18.7 C (Figure 2-48 and Table 2-28). The initial sample was formed by chilling the melt at liquid N_2 temperature, containing ca.85 % of the LM form (see Figure 2-25). The concentration of the LM form was decreased with increasing time for incubation and increasing temperature for transformation, as measured by intensity ratios of the characteristic X-ray diffraction long spacing spectra. No solid-state transformation was detectable from HM to LM below 18.7 C.

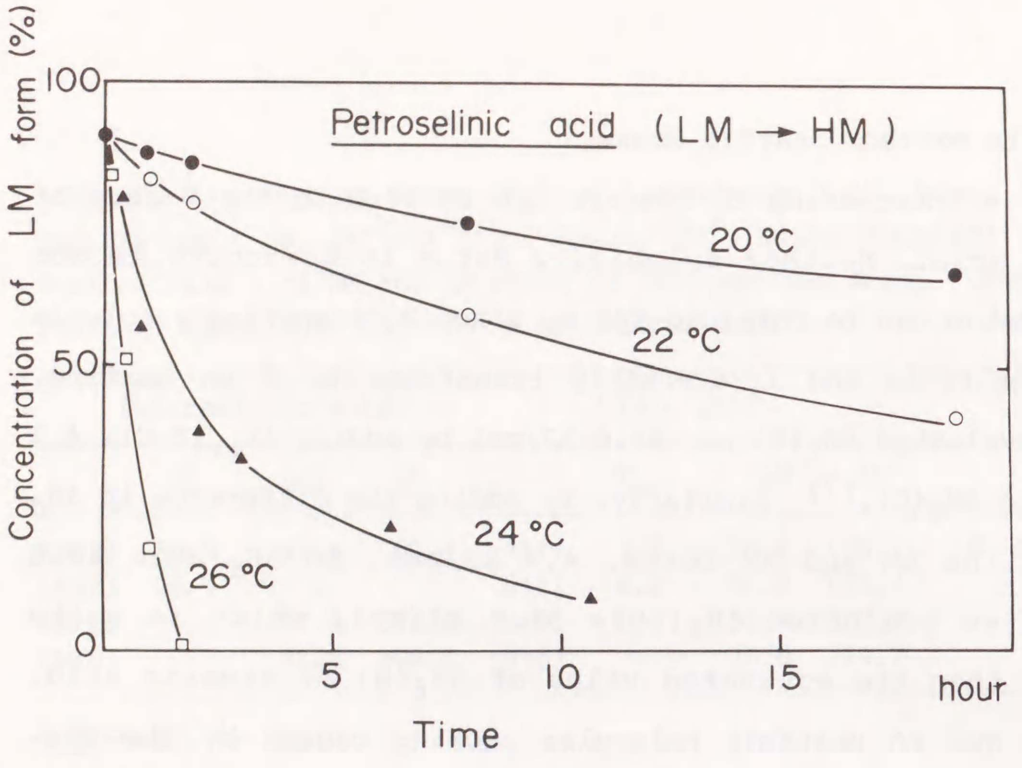

Figure 2-48 Solid-state transformation from LM form to HM form.

Table 2-28 Rates of Transformation from LM Form to HM Form in Solid-state

Time	Concentration of HM Form (%)			
	20^0C	22^0C	24^0C	26^0C
1min	90	90	90	90
10min	90	90	84	84
30min	90	90	79	51
1h	87	82	56	18
2h	85	79	38	-
3h	83	72	32	-
6h	76	65	22	-
8h	74	58	-	-
18h	66	43	-	-
20h	65	41	-	-

e. Discussion

According to this G-T relationship (figure 2-47), one may argue the following transformation pathways; above 18.7 C from LM to HM via the solid-state, solution-mediated and melt-mediated transformations, all of which were observed in the present study. Below 18.7 C, however, the transformation from HM to LM was observed only via solution-mediation, since no solid-state transformation was observed from HM to LM. Hence, the transformation feature between the two forms of petroselinic acid may be characterized by the irreversible solid-state conversion from LM to HM and its easiness above 18.7 C (Figure 2-48). This is compared to two examples; from B to C in stearic acid occurred at 54 C although two G values cross at 32 C,18) and from alpha to alpha$_1$ in erucic acid occurred at 31.2 C while 25.9 C was the crossing point of the two G values.4)

The reversibility in the solution-mediated transformation comes from its lower activation energies in the growth of the more stable form at the expense (dissolution) of the less stable one.19) By contrast, the irreversibility in the solid-state transformation is primarily ascribed to steric hindrance associated with the changes in the molecular structures. In the present case, we presume the steric hindrance due to olefinic conformation, subcell packing, methyl end packing and -COOH conformation. As to the subcell packing (Figure 2-42), the LM and HM forms are in the $O_{\perp}$ and $O_{\perp}+M_{//}$ subcells, respectively. However, the conversion in the subcell does not solely justify the irreversibility from HM to LM, since from $O_{\perp}$ to $T_{//}$ occurs irreversibly in beta'($O_{\perp}$) $\rightarrow$ ($T_{//}$) in tristearoylglycerol,20) but reversibly in the A'($T_{//}$) $\rightarrow$ C'($O_{\perp}$) in tridecanoic acid.21)

The reversibility or irreversibility in the polymorphic transformation of saturated fatty acids are discussed in terms of collective rotational movement of the aliphatic chains and conformational gauche-trans conversions.[22] In petroselinic acid, however, the transformation properties may be explained not only by the aliphatic chain interactions, but also by olefinic interactions.

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2.4 Asclepic Acid

As a third positional isomer of cis-octadecenoic acid, this section describes the polymorphism of asclepic acid, which has been analyzed for the first time in the present study.

a. Thermodynamics stability of Asclepic Acid Polymorphs

Polymorphic behavior of asclepic acid was examined by differential scanning calorimetry,$^{1)}$ X-ray diffractometry and optical microscopy. Alpha, alpha', gamma and two metastable forms were confirmed. Figure 2-49 show the transition circuit of the five polymorphs. High-temperature metastable form and alpha'-form crystallize at the same time by rapid melt cooling. With decreasing temperature, alpha'-form and high-temperature metastable form transformed to alpha-form at about -4 C, and to low-temperature metastable form at about -37 C. A subsequent transformation occurred between alpha-form and gamma-form at about -18 C by further cooling. On heating, a successive transformations occurred; from gamma to alpha at -15 C, from alpha to alpha' at -1.4 C and low-temperature form to high temperature form at about -37 C. The high- temperature metastable form further transformed to alpha' below the melting point of alpha'-form (13.8 C). The enthalpy values of transformation, were 7.8 kJ/mol (gamma-alpha), enthalpy of fusion of alpha'-form was 0.7 kJ/mol (alpha-alpha') and 39.8 kJ/mol.$^{1)}$

The crystal Gibbs free energy relationship of the five polymorphs are depicted in Figure 2-50. To conclude there are

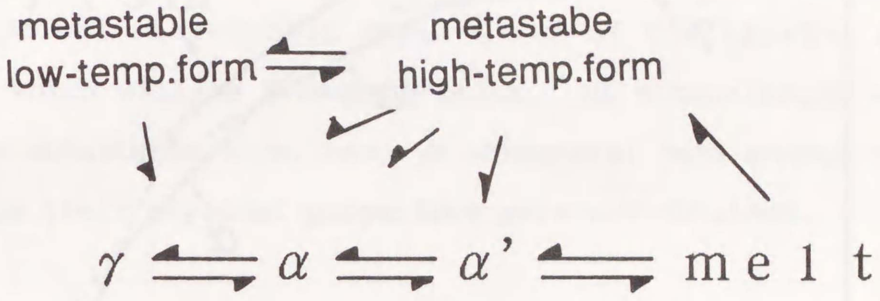

Figure 2-49 Transformation circuit of the five polymorphs of asclepic acid.

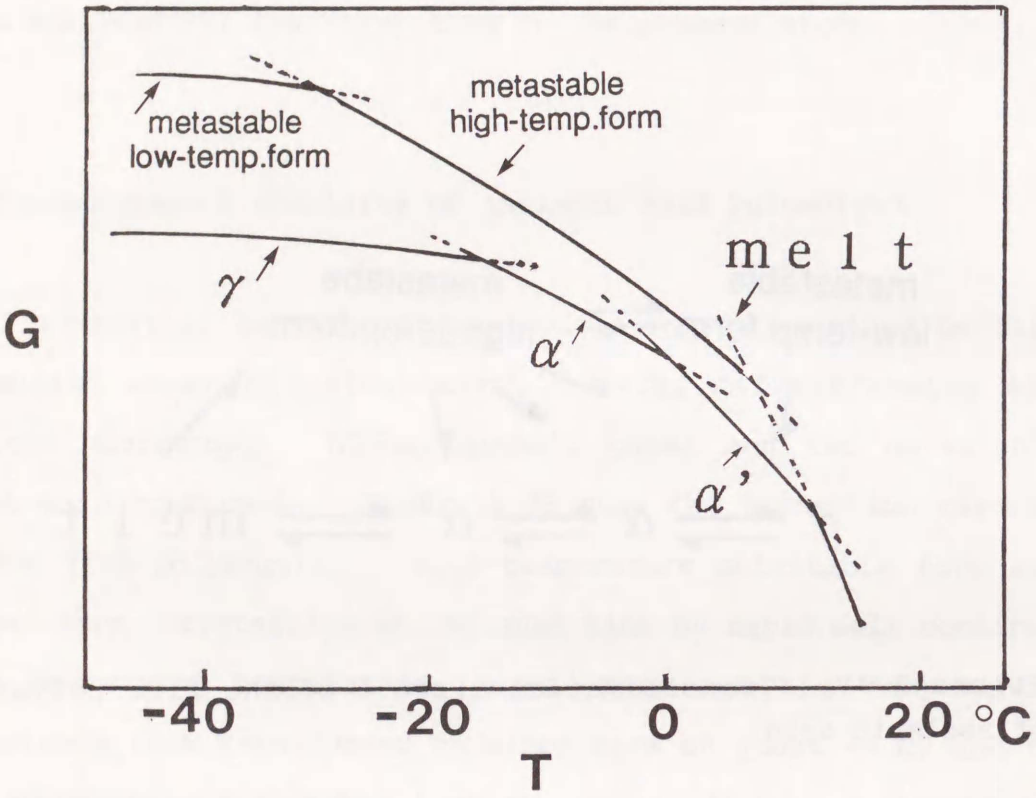

Figure 2-50 Crystal Free energy relationship of asclepic acid polymorphs.

more stable forms. The transformation temperature between the two metastable forms is about -37 C. As to the thermodynamically stable polymorphs, three forms were isolated, exhibiting reversible transformations at -15.0 C and -1.4 C, and alpha'-form melts at 13.8 C.

The nomenclature of the polymorphs was given to the three stable forms, gamma, alpha and alpha', in accordance with oleic,[2)] erucic[3)] and palmitoleic[4)] acid, taking into account the ΔH_t values and the experimental data of X-ray diffraction and optical microscopic observation of the crystal shape as well, which will be discussed below. No nomenclature was given to the metastable form, because structural data enough to characterize their physical properties were not obtained.

b. X-Ray Diffraction

The crystallization procedure using the X-ray diffraction apparatus was done in the following manner. The molten sample (>80 C) was mounted on a sample holder made of copper. It was rapidly solidified, and attached to a thermostat in a chilled room whose temperature was below 0 C. The thermostat was then set up on the X-ray goniometer, rapidly sealed and evacuated by a vacuum pump. The temperature of the sample was raised or lowered with resistance heating and liquid N_2 reservoir of the thermostat, being accurate within ±1 C.

Figure 2-51 shows the X-ray diffraction short and long spacing spectra of alpha-form taken at -10 C, and gamma-form at -40 C. The thermal annealing was carried out to obtain the two forms as follows; cooling the melt to -130 C, heating again to 10 C, and then cooling again to -10 C and -40 C. Table 2-29

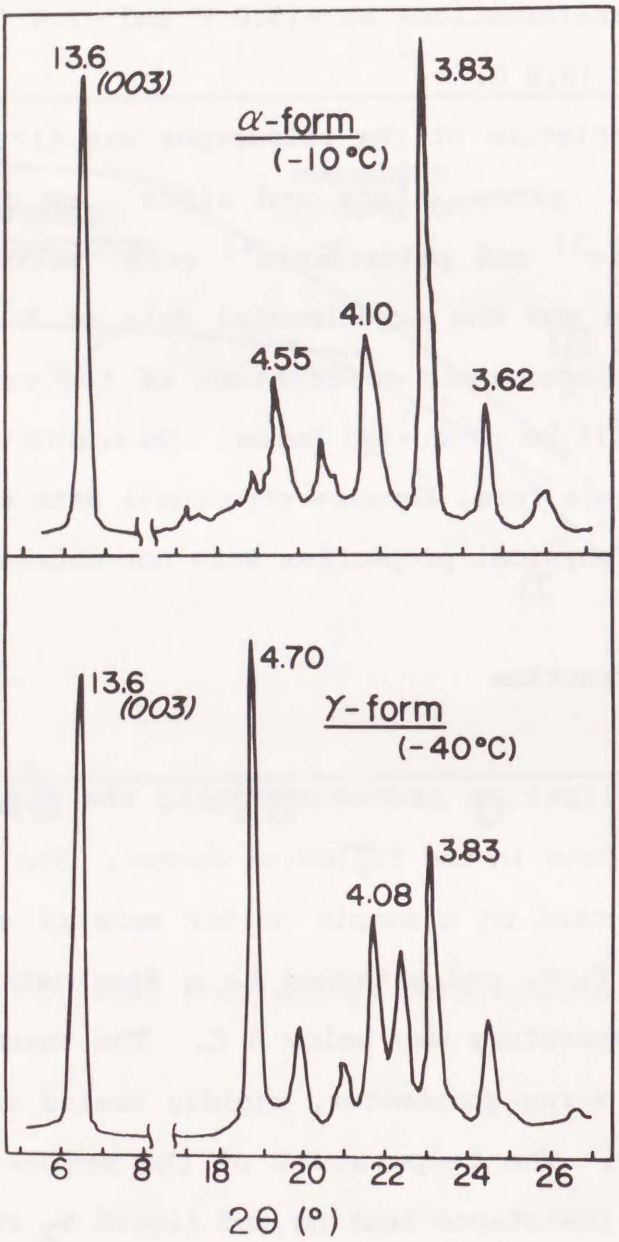

Figure 2-51 X-ray diffraction spectra of α-form and γ-form of asclepic acid.

Table 2-29

X-ray diffraction long and short spacing spectra (A) of α-form and γ-form of asclepic acid.

	long spacing	short spacing*
α-form	40.8	3.43(w), 3.62(m), 3.83(vs), 4.10(s) 4.27(m), 4.55(s)
γ-form	40.8	3.63(w), 3.83(s), 3.80(m), 4.08(s) 4.23(w), 4.46(m), 4.70(vs)

*) vs: very strong, s: strong, m: medium, w: weak.

summarizes the long and short spacing values. The two forms have the same long spacing value of 40.8 A. The reversible conversion between gamma-form and alpha-form was observed in the X-ray diffraction pattern around -15 C. However, no appreciable change was detectable between alpha-form and alpha'-form, both in the short and long spacing spectra.

The data of X-ray diffraction patterns of alpha-form and gamma-form are quite identical to alpha-form and gamma-form of oleic acid.$^{2)}$ Particularly, the strong peaks of 3.83 A in alpha-form and 4.70 A in gamma-form are the same in the two acids. This means that the subcell packings are identical for each modification; $O'_{//}$ in gamma-form$^{5-7)}$ and $O'_{//}$-like in alpha-form.$^{8)}$

As to the metastable forms, the rapid crystallization revealed specific X-ray diffraction long spacing pattern. Figure 2-52 shows the pattern taken at -130 C, at which temperature the molten sample was chilled using liquid N_2. The major pattern is of gamma-form; particularly only gamma-form was seen in the short spacing spectra. However, weak long spacing spectra were observed, corresponding to a long spacing value of 35.4 A. This spectra disappeared around -30 C upon heating, and did not appear after the annealing process. Therefore, we concluded that these spectra correspond to one of the metastable forms.

The occurrence of the metastable forms were observed in the transformation behavior and the X-ray diffraction patterns, when the sample was first crystallized from the melt phase. Due to rapid transformation to the more stable forms, no structural information about the metastable forms was available, except for the fact that the low-temperature metastable form has the long spacing value of 35.4 A, which is quite short compared to gamma-

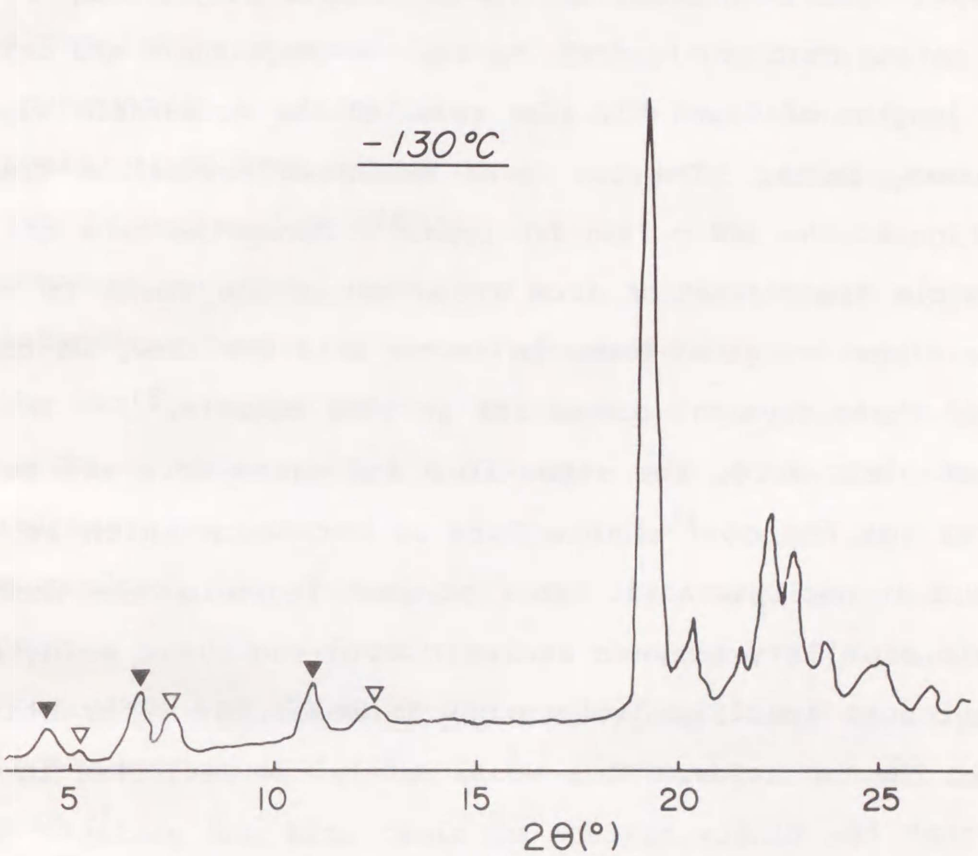

Figure 2-52 X-ray diffraction spectra of asclepic acid after chilling the melt at -130 C. Long spacing spectra: γ-forms (▼) and a metastable form (▽).

form and alpha-form of asclepic acid (40.8 A), and also to all polymorphs of oleic and petroselinic acids.2,9) A transformation around -37 C is caused by conversion from one metastable form to another metastable form. The details of this transformation may be clarified with certain methods which can follow the rapid rate of irreversible transformation.

As to the occurrence of the metastable polymorphs, it is worth noting that erucic acid, having the omega-chain and delta-chain lengths of 9 and 13, also revealed the metastable alpha_1 and gamma_1 forms. The two forms exhibited reversible transformation of the order-disorder type.3) Since the rate of irreversible transformation from these metastable forms to more stable alpha- or gamma-forms in erucic acid was slow, we could analyze their physical properties in some details.3) In the case of oleic acid, the alpha-form and gamma-form are metastable, yet the most stable form is beta-form which is not observed in asclepic acid. This contrast regarding the thermodynamic stability between asclepic acid and oleic acid, may suggest some specific differences in molecular interactions between the two acids. This would partly be reflected in the fact that the binary mixture of oleic acid and asclepic acid forms a eutectic system, implying sparing molecular interactions between the two acids in the crystalline state.10)

Finally, the alpha-alpha' transformation with the small values of ΔH_t and ΔS_t was observed for the first time in asclepic acid. No appreciable change in the X-ray diffraction patterns was observed. We infer that the transformation would be related to subtle changes in the aliphatic chain structures, which will be analyzed with more sensitive instruments in the future.

c. Morphology

Single crystals of the three stable forms, alpha', alpha and gamma, were grown from acetonitrile or decane solvents in order to observe the crystal shape with a polarized optical microscope.

Figure 2-53 shows the morphology of single crystals of alpha-form and gamma-form of asclepic acid taken at -2 C in acetonitrile solution and at -20 C in decane solution, respectively. Alpha-form exhibited an elongated hexagonal shape with interplanar angles of 130^0 and 115^0. The same morphology was observed for alpha'-form at 2 C in acetonitrile solution. The interplanar angles of gamma-form were 90^0. The crystal shape of the two forms are exactly the same as the corresponding polymorphs of oleic acid.11) The same shape of the alpha-form crystal was also observed in erucic acid.3) Combining the crystal morphology, the thermal data and the X-ray diffraction short spacing spectra, we conclude that the polymorphic transformation from gamma-form to alpha-form in asclepic acid is identical to those observed in oleic acid,2,11) palmitoleic acid4) and erucic acid.3)

d. Discussion

It is interesting to compare the thermodynamical parameters of the gamma-alpha transformation of oleic acid, erucic acid, palmitoleic acid and asclepic acid (Table 2-30). There is a systematic relationship between the length of the omega-chain and transition temperature (T_t), enthalpy (ΔH_t) and entropy (ΔS_t) of the gamma→alpha transformation. In oleic acid and

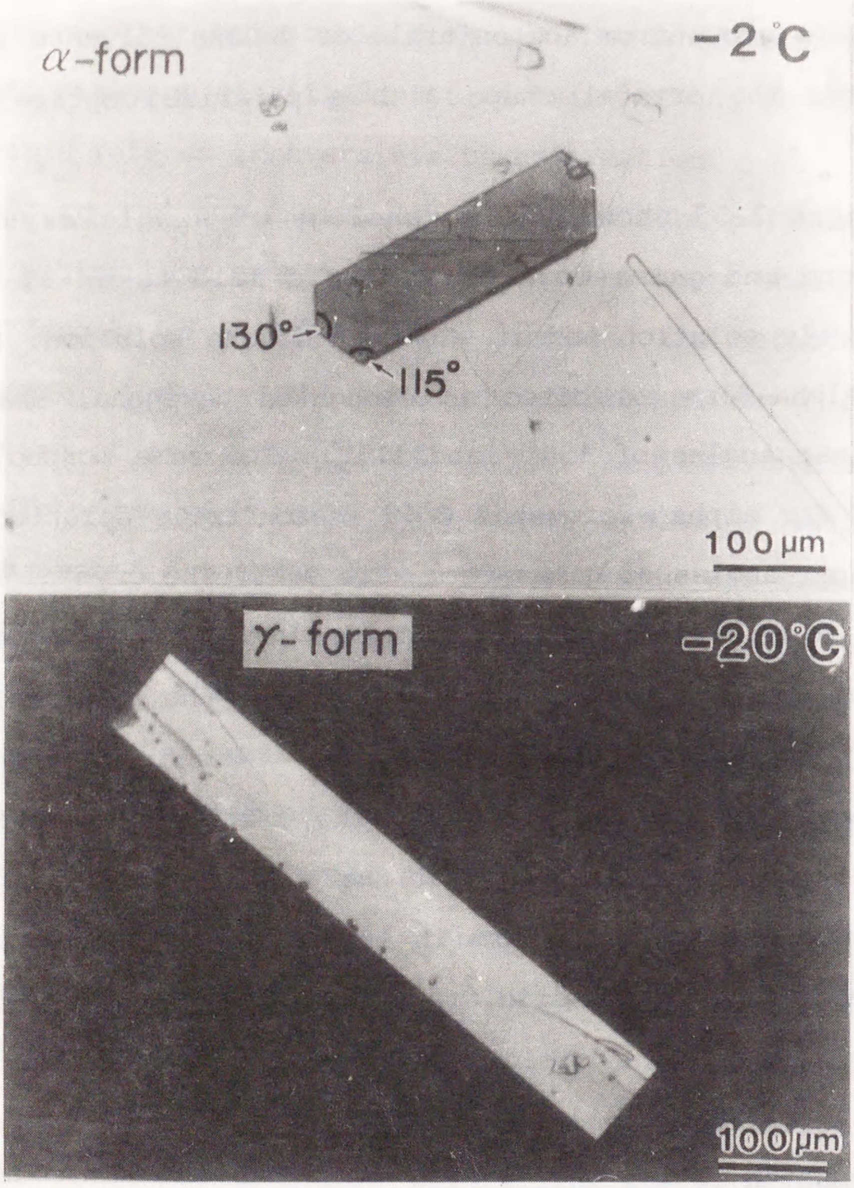

Figure 2-53 Single crystal morphology of α-form and γ-form of asclepic acid.

Table 2-30

Thermal parameters of polymorphic $\gamma \rightarrow \alpha$ transformations in oleic acid, erucic acid, palmitoleic acid and asclepic acid

unsaturated fatty acid	ω-chain	Δ-chain	transformation parameters* T_t	ΔH_t	ΔS_t
erucic acid	9	13	-1.0	8.8	32.3
oleic acid	9	9	-2.2	8.8	32.5
asclepic acid	7	11	-15.0	7.8	30.2
palmitoleic acid	7	9	-18.7	7.5	29.5

*)T_t: transformation temperature (C), ΔH_t: transformation enthalpy ($kJmol^{-1}$), ΔS_t: transformation entropy ($JK^{-1}mol^{-1}$).

erucic acid with the omega-chain length of 9, the T_t values are -2.2 C and -1.0 C, respectively. The ΔH_t value is the same, 8.8 kJ mol^{-1}, yielding the ΔS_t values of 32.5 J $K^{-1}mol^{-1}$ for oleic acid and 32.3 J $K^{-1}mol^{-1}$ for erucic acid. The values of oleic acid and erucic acid for each polymorphic modification are quite similar. Palmitoleic acid and asclepic acid with the omega-chain length of 7 also showed an excellent similarity to each other; T_t is -18.7 C for palmitoleic acid and -15.0 C for asclepic acid. Similarly, the ΔH_t and ΔS_t values have quite similar values in the two acids. This relationship is consistent with the molecular properties of the gamma-alpha transformation.

As repeatedly described in the preceding sections, the gamma-alpha transformation is of an order (gamma)-disorder (alpha) type, accompanied by a conformational disordering in the omega-chain segment. Hence, we characterized the gamma-alpha transformation "interfacial melting".6) Upon heating, the omega-chain may be disordered before a total of the crystal lattice is melted. This disordering is ascribed to unstable molecular motions in the omega-chain, caused by the existence of the double bond placed either at the central portion, in the case of oleic acid, or closer to the methyl end group, in the case of erucic acid, palmitoleic acid and asclepic acid. The fact that the shorter omega-chain gives rise to lower T_t values as well as smaller ΔH_t and ΔS_t values means that the "interfacial melting" is primarily determined by the length of the omega-chain. In this regard, it is worth noting that petroselinic acid, a positional isomer of oleic and asclepic acids having the omega-chain length of 12, does not reveal "interfacial melting".9) In the longer omega-chain, disordered molecular motions would be hindered as in the delta-chain.

It was worth noting here again that despite of the existence of gamma-alpha forms in oleic acid and asclepic acid, they form a eutectic system in a binary mixture as fully described in **Chapter 3.** This implies critical importance of the position of the cis double bond. Difference of the two CH_2 segments between the two acids gives rise to remarkable resistance against the co-crystallization behavior.

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Chapter 3

Phase Behavior of Binary Mixture Systems

1. Introduction

In Chapter 2, we described the polymorphism and crystal growth in a series of cis-monounsaturated fatty acids, oleic acid (OA), erucic acid (EA), petroselinic acid (PSA) and asclepic acid (APA).

In these studies, it is worth noting that there are similarity and dissimilarity in the polymorphic behavior of the above three positionally isomeric fatty acids of OA, PSA and APA. The similarity was seen in the polymorphic transformation from gamma-form to alpha-form, which occurred in OA and APA. The most typical dissimilarity was observed in PSA with respect to the molecular structure of its two forms, as compared to other two acids.

To briefly summarize the polymorphic behavior of the three fatty acids (Table 3-1) in terms of the molecular structural properties.[1-9] Polymorphism of PSA is quite different from others. OA and APA have common polymorphs of gamma-form and alpha-form. Yet, the most stable beta-form of OA does not occur in APA, and a transition from alpha-form to alpha'-form in APA is not observed in OA. From these data, it is manifest that the position of the cis-double bond drastically influences major aspects of the polymorphic behavior. This conclusion then

Table 3-1: Polymorphism of Oleic Acid, Petroselinic Acid and Asclepic Acid

	OA	PSA	APA
polymorph	γ ↔ α ↔ melt β ⤢	LM → HM ⤡ ⤴ melt	γ ↔ α ↔ α' ↔ melt
melting points(℃)	α:13.3 β:16.2	LM:28.5 HM:30.5	α':13.8
order-disorder transition	γ(order) α(disorder)	no	γ(order) α(disorder)
Transition temperature(°C)	-2.2C		-15.0C

specific subcell	γ ($O'_{//}$)
	α ($O'_{//}$)
	β (//-like)
		LM ($O_{\perp}$)
		HM ($O_{\perp}$ and $M_{//}$)
			γ ($O'_{//}$)
			α ($O'_{//}$)
			α'($O'_{//}$)

arises a problem as to what happens in binary mixture systems consisting of the above three isomers.

Studies on the binary mixtures of cis-unsaturated long-chain compounds, involving alkene molecules, have been very scarce. Cis-ω-9-octadecene/octadecane binary system forms a simple monotectic system.10) Similarly, the oleic acid/palmitic acid mixture system forms a simple eutectic system.11) Mod et al. reported the simple eutectic diagrams for six binary systems of the unsaturated fatty acids, involving the PSA/OA system.12) They measured freezing points of the binary mixtures, claiming the formation of the eutectic mixture with very little mixing, neither compound formation nor solid-solution. However, no data on polymorphic transformations were reported and the freezing phenomena would be influenced by polymorphism. The purity of the samples employed may be questioned. No mixture system involving asclepic acid has been studied. This Chapter deals with the behavior of melting and order-disorder transformations in the two binary systems of PSA/OA and APA/OA, using ultra-pure samples.

2. Methods

The crystallization examined with DSC was done by melting the mixture (ca.10-20mg) at 80 C, so that no structure in the melt phase was retained, and, by cooling with a DSC computer program for temperature control. The rate of heating was 2 C/min. We changed the rate of cooling from about 50 C/min to 0.2 C/min, in order to examine the effect of cooling rate on the phase behavior. The crystallization procedure using the X-ray diffraction apparatus was done in the following manner. The molten sample (100 mg, >80 C) was mounted on a sample holder made of copper, which was attached to a thermostat in a chilled room whose temperature was below 0 C. The sample was solidified at different rates of cooling by using temperature-regulated liquid N_2 gas. The rapid-cooling was about 50 C/min and slow-cooling was about 2 C/min. The thermostat was then set up on the X-ray goniometer, rapidly sealed and evacuated by a vacuum pump. The temperature of the sample was regulated with liquid N_2 reservoir, for cooling, placed at the top of thermostat, and with a metal resistance for heating which was placed close to the sample. The accuracy of the temperature was ±1 C and the rate of temperature change was ranged from 1 C/min to 10 C/min. Since the sample holder was of vertical type, the experiment on the melt crystallization process itself was not possible.

3. Results

a. Petroselinic Acid / Oleic Acid

Figure 3-1 shows the typical DSC heating thermograms of the OA/PSA mixtures at different concentrations. All of the samples were crystallized at the cooling rate of about 50 C/min, which we called rapid crystallization. High temperature peaks correspond to the melting of high-temperature stable polymorphs. At the concentrations of PSA/OA=35-18/65-82, only one melting peak appeared, indicating an eutectic point in this range of the concentration ratios. The DSC endothermic peaks appeared in low-temperature regions, being due to the order-disorder transformation of the oleic acid crystals (solid arrows). It is interesting to note that exothermic peaks were detectable just

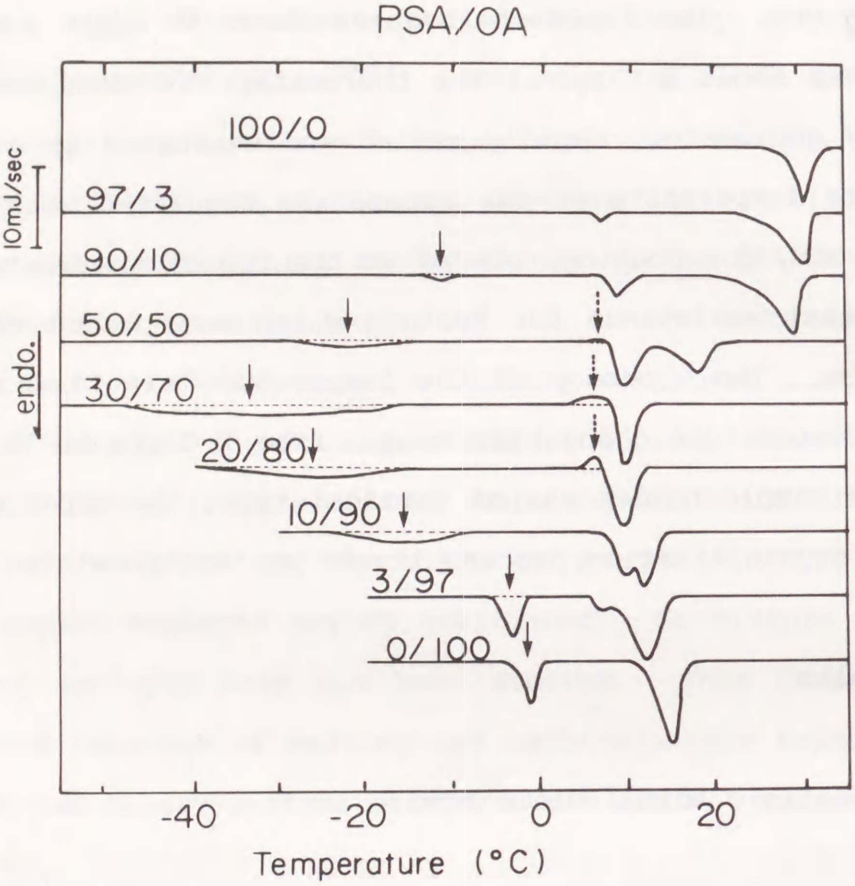

Figure 3-1 DSC heating thermograms of the mixture system of petroselinic acid (PSA) and oleic acid (OA). All of the mixtures were crystallized from melt at a cooling rate of 50 C/min, and heated at 2 C/min.

below the melting points either of OA or of PSA crystals in the PSA/OA concentration ranges from 40/60 to 20/80 (broken arrows). No exothermic peak was detectable in other ranges of the concentration ratios. By decreasing the rate of cooling for crystallization, the DSC heating thermogram differed in the above exothermic peaks, and in the transformation peaks as well. However, the melting behavior did not change due to lowering of the rate of crystallization.

Figure 3-2 shows the effect of the crystallization rate on the DSC heating thermogram of the PSA/OA mixture at the concentration ratio of PSA/OA=30/70. One melting peak was detectable, since this concentration was very close to the eutectic point. As to the polymorphic transformation, its endothermic peak, which was a single broad peak at 50 C/min, was split into two (solid arrows). At the same time, the peak shape became narrower, and the transition temperatures were elevated, with decreasing rate of crystallization from 50 C/min to 0.2 C/min. It is worth noting that the higher transition temperature did not change appreciably with decreasing rate of crystallization, being -2 C which is the same as the gamma-alpha transformation temperature of pure oleic acid. Meanwhile, the transition peak at the low-temperature side, for example at -30 C at the 1 C/min cooling, increased in temperature as well as in size upon further decrease of the crystallization rate. Quite similar result was observed in the mixture of PSA/OA=80/20. However, different feature was observed in the polymorphic transformation in the mixtures of PSA/OA=50/50 and PSA/OA=80/20. By decreasing the crystallization rate, the transition temperature was raised, but no splitting was observed. As to the exothermic peak just below the melting points (broken arrows), it vanished only when

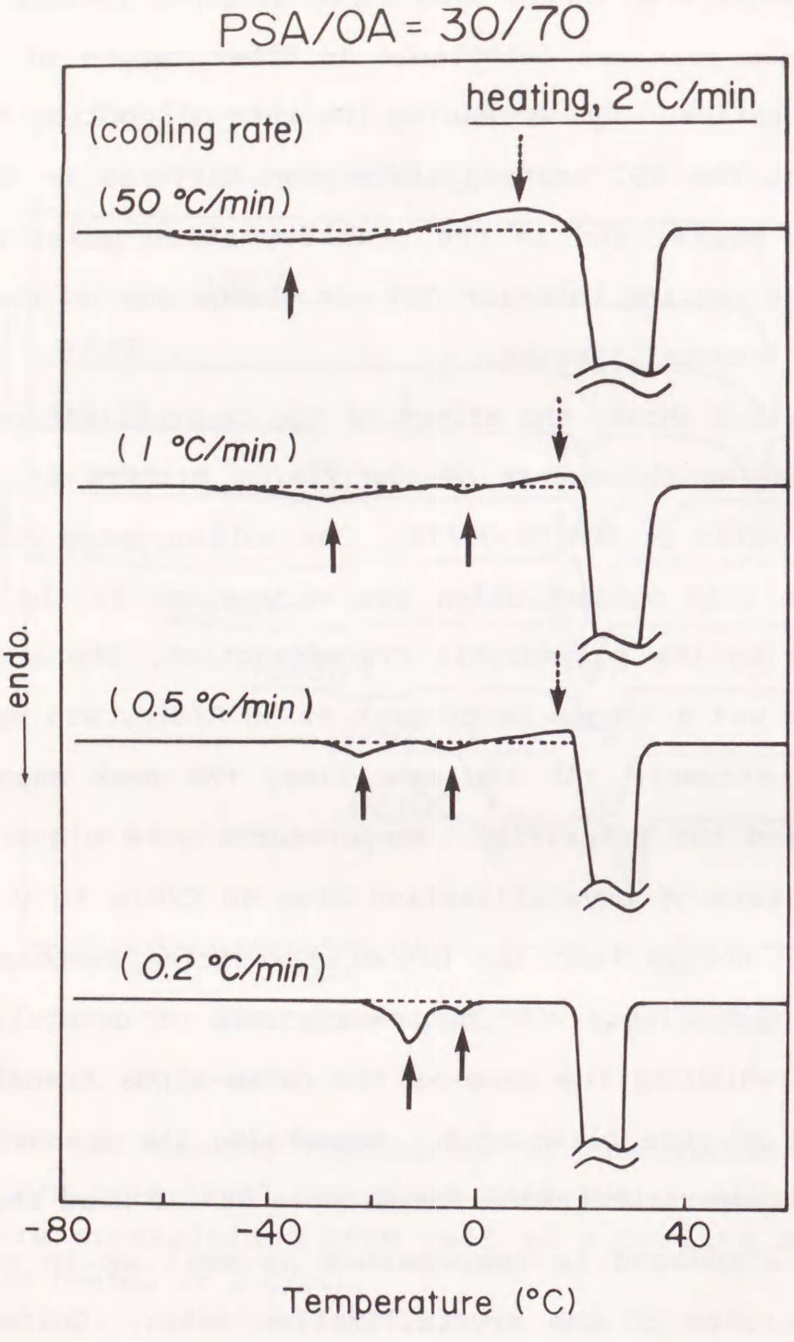

Figure 3-2 DSC heating thermograms of the mixture of PSA/OA=30/70 as a function of cooling rate from the melt. The heating rate was 2 C/min.

the rate of crystallization was lowered to 0.2 C/min. It is inferred that these effects of the crystallization rate may be related to the crystallization processes of the eutectic mixtures as discussed in the next section.

Figure 3-3 shows the X-ray diffraction patterns obtained in the PSA/OA =50/50 mixture at 4 C. There are two long spacings with the values of 4.8 nm and 4.5 nm, which correspond to LM form of PSA and alpha-form of OA, respectively.[7,13] The short spacing pattern is rather complicated, but we found that it is a superposition of the alpha-form of OA and LM form of PSA. The same X-ray diffraction patterns as Figure 3-3 were observed at PSA/OA=10/90, 30/70 and 80/20.

Figure 3-4 shows the temperature change of the X-ray short spacing spectra of the mixture of PSA/OA=50/50, which was solidified by the rapid cooling. At -30 C, the X-ray diffraction spectra of gamma-form of OA and the LM form of PSA are detectable. On heating to 0 C (Figure 3-4B), the spectra corresponding to gamma-form of OA in Figure 3-4A disappeared and alpha-form of OA appeared, whereas the spectra of the PSA LM form unchanged. This proves that the broad DSC peak around -20 C in Figure 3-1 is due to gamma-alpha transition of the oleic acid crystals. At 10 C (Figure 3-4C), the diffraction pattern drastically changed; alpha-form of OA disappeared, a broad peak appeared in a 2θ range of 15 - 25 , and weaker specific diffraction spectra were observed. This specific X-ray diffraction pattern corresponds to LM form of petroselinic acid, and the broad line underlying the spectra of LM form is ascribed to the melting of oleic acid alpha-form. The same results were obtained at PSA/OA=10/90, 30/70 and 80/20, although the transformation temperatures of gamma-alpha and relative intensity of X-ray

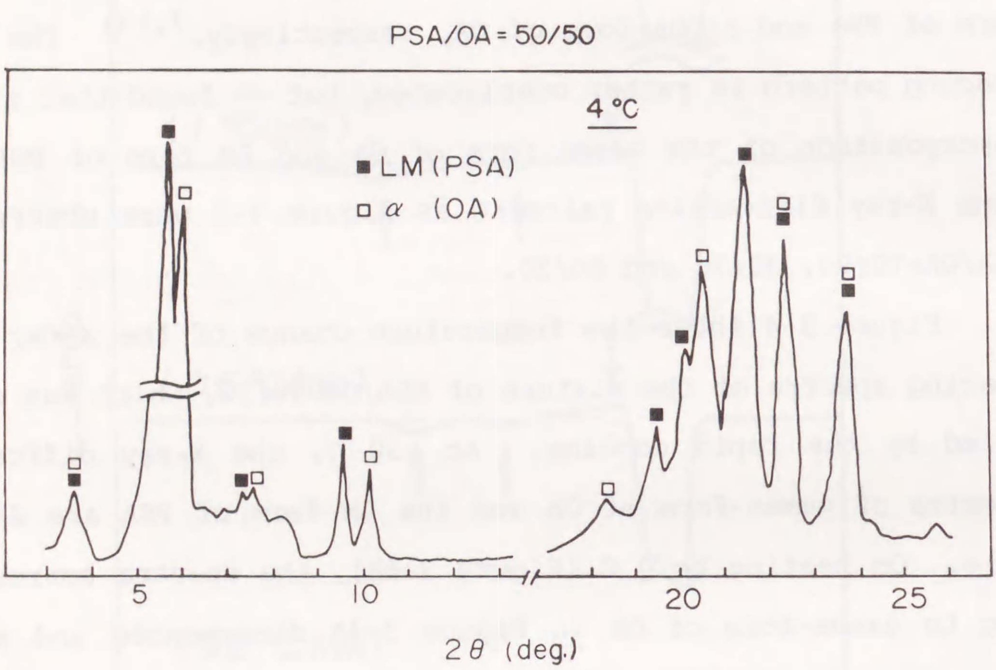

Figure 3-3 X-ray diffraction spectra of the mixture of PSA/OA =50/50 taken at 4 C, exhibiting long and short spacings of LM form of petroselinic acid (■) and α-form of oleic acid (□).

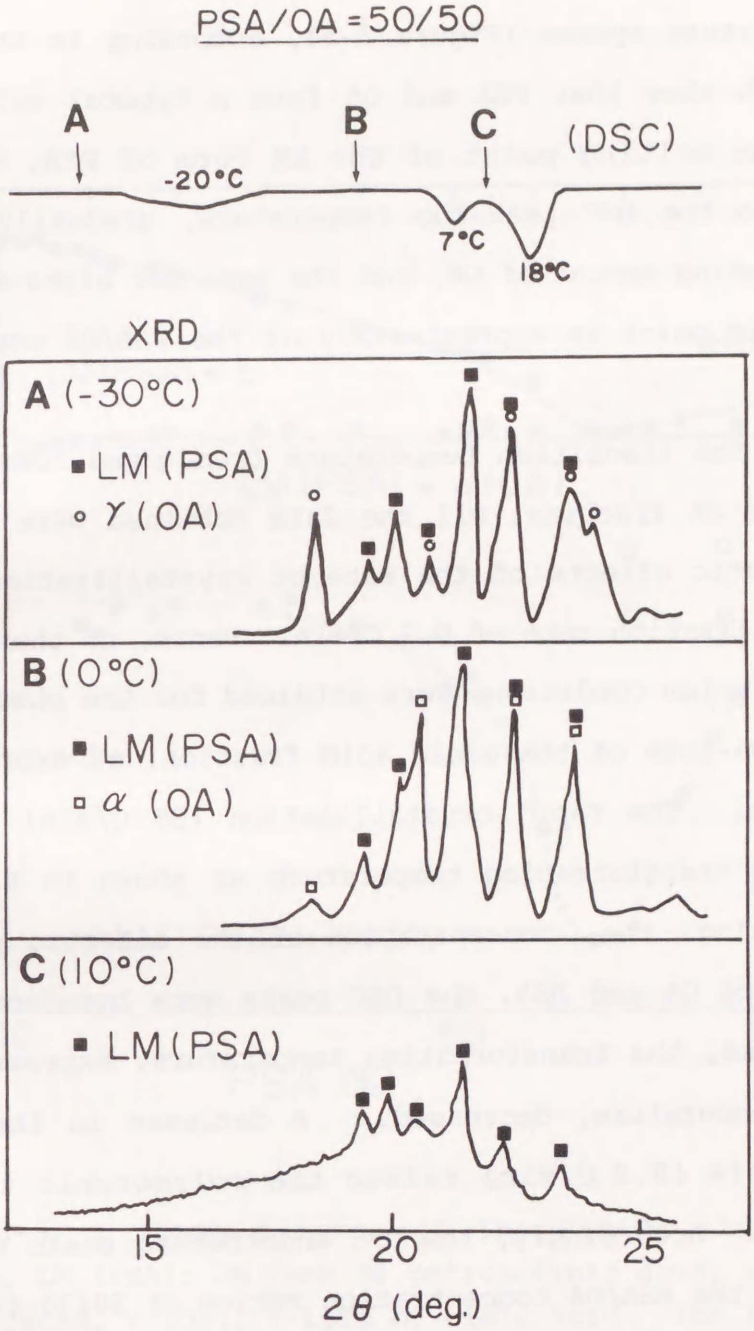

Figure 3-4 X-ray diffraction short spacing spectra of the mixture of PSA/OA=50/50 taken at -30 C (A), 0 C (B) and 10 C (C), which correspond to different phase regions denoted in a DSC heating thermogram. The spectra are of LM form of petroselinic acid (■), γ-form of oleic acid (○) and α-form of oleic acid (□).

diffraction peaks were different.

All of the above data enabled us to depict a phase diagram of this mixture system (Figure 3-5), according to the liquidus lines which show that PSA and OA form a typical eutectic property. The melting point of the LM form of PSA, which corresponds to the DSC peak-top temperature, gradually decreased with increasing amount of OA, and the same for alpha-form of OA. The eutectic point is approximately at the PSA/OA concentration of 35/65.

As to the transition temperature from gamma-form to alpha-form of the OA fraction, all the data obtained were influenced by the kinetic effects of the rate of crystallization, even at the crystallization rate of 0.2 C/min. Hence, no thermodynamically equilibrium conditions were attained for the phase behavior of the gamma-form of the oleic acid fraction, as expressed by a dashed line. The rapid crystallization (50 C/min) remarkably lowered the transformation temperature as shown in Figure 3-1. On approaching the concentration of the eutectic point from both sides of OA and PSA, the DSC peaks were broadened and, at the same time, the transformation temperature, expressed by the peak top temperature, decreased. A decrease in the crystallization rate (0.2 C/min) raised the polymorphic transition temperature. Accordingly, the DSC endothermic peaks were split into two at the PSA/OA concentration ratios of 30/70 (-3.8 C and -14.0), and 20/80 (-3.8 C and -14.0 C). However, the 50/50 and 75/25 mixtures did not split the gamma-alpha transition at the lowered crystallization rate, but only raised the transition temperatures to -7.2 C and -5.4 C, respectively.

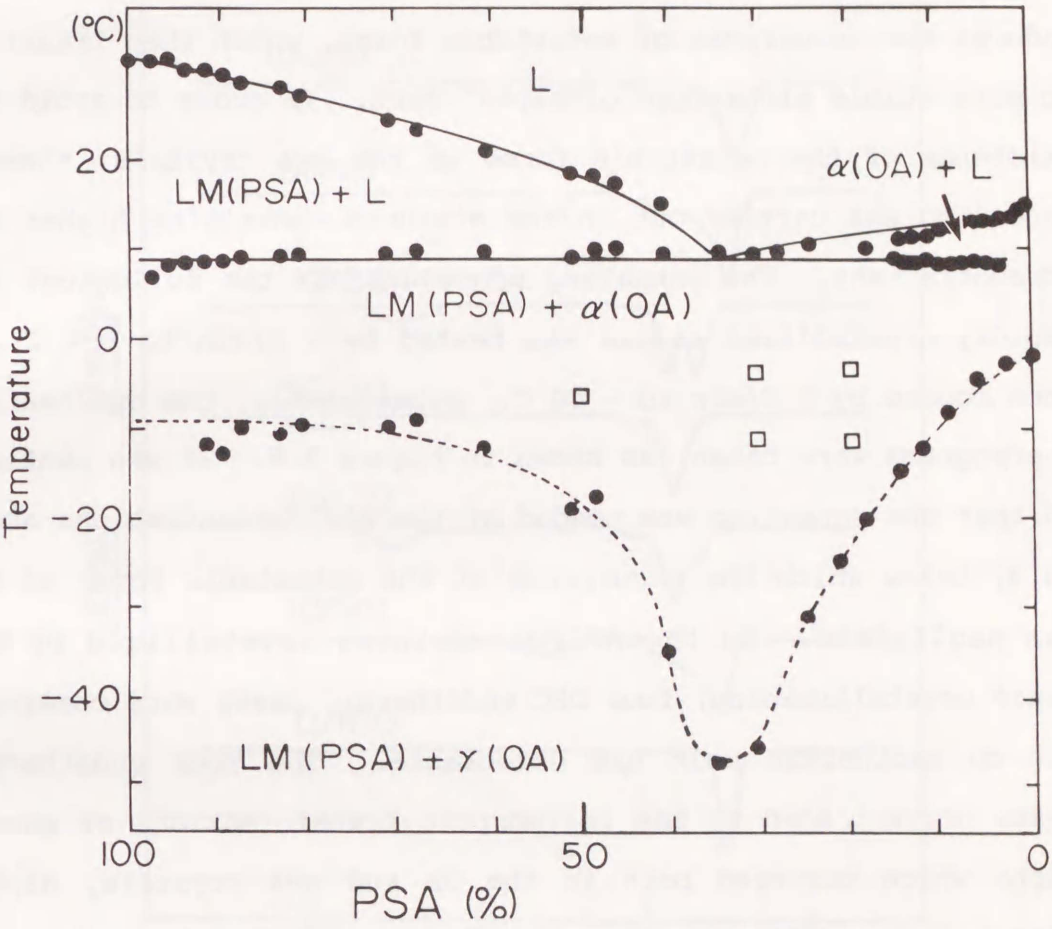

Figure 3-5 A phase diagram of the PSA/OA mixture system: L;liquid, LM (PSA); LM form of petroselinic acid, α (OA); α-form of oleic acid, γ (OA); γ-form of oleic acid. The liquidus lines and γ→α transition temperatures (dashed line) at the rapid cooling (●) and slow cooling (□) are all due to peak-top temperatures of corresponding DSC heating thermograms.

b. Asclepic Acid / Oleic Acid

Figure 3-6 shows the DSC heating thermograms of the APA/OA mixtures, which were rapidly crystallized. We already observed[6)] that the rapid crystallization of pure asclepic acid induces the occurrence of metastable forms, which then transform to more stable alpha-form or alpha'-form. In order to avoid the existence of the metastable forms in the APA crystals, thermal annealing was carried out in the mixtures containing higher APA concentrations. The annealing procedure was the following: the rapidly crystallized sample was heated by 1 c/min to - 4 C and then cooled by 1 C/min to - 60 C. Subsequently, the DSC heating thermograms were taken, as shown in Figure 3-6. It was confirmed that the annealing was needed at the APA concentrations above 80 %, below which the occurrence of the metastable forms of APA was negligible. In the APA/OA mixtures crystallized by the rapid crystallization, four DSC endothermic peaks were observed, but no exothermic peak was detectable. The four endothermic peaks corresponded to the polymorphic transformations of gamma-alpha which occurred both in the OA and APA crystals, alpha-alpha' in the APA crystals, the melting of alpha'-form of APA and the melting of alpha-form of OA. The effect of the crystallization rate was manifest only in the polymorphic transformation from gamma-form to alpha-form. The eutectic point is approximately at the concentration of APA/OA=50/50.

Figure 3-7 shows the effect of the crystallization rate on the DSC endothermograms of the APA/OA mixture at the concentration ratio of 50/50. There is one melting peak at the peak-top temperature of 4.5 C, since this concentration corresponds to the eutectic point. This peak did not change appreciably by

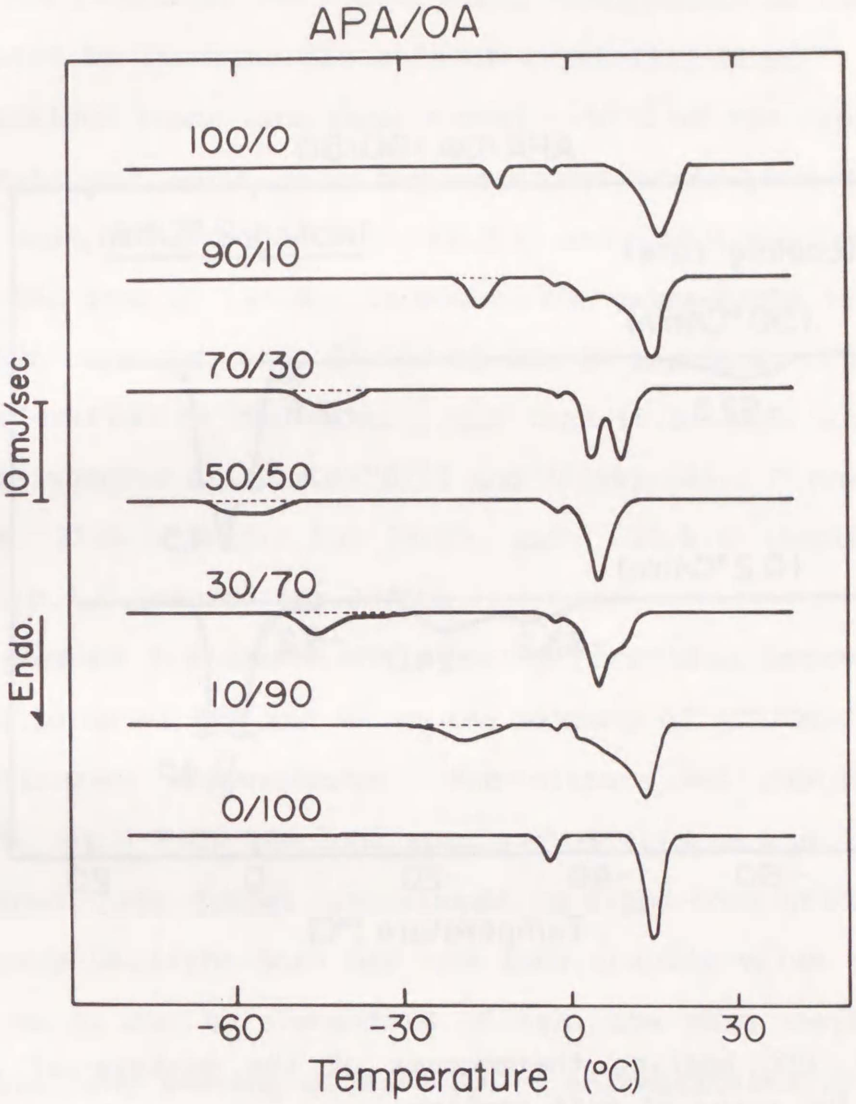

Figure 3-6 DSC heating thermograms of the mixture system of asclepic acid (APA) and oleic acid (OA).

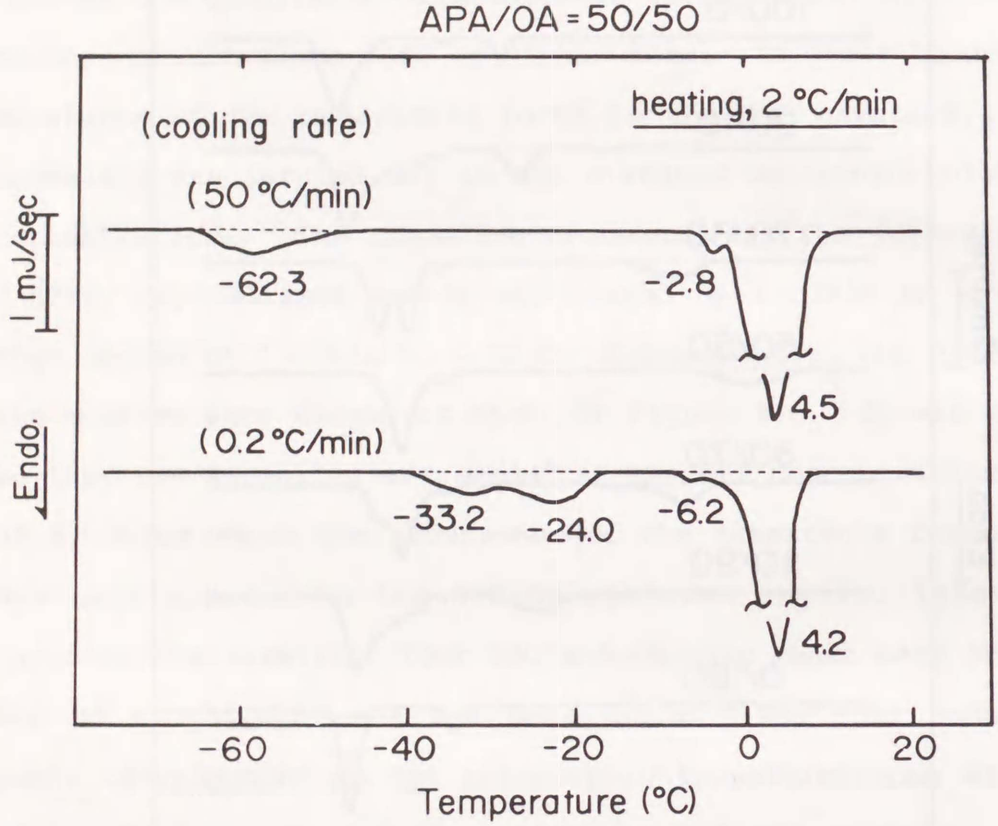

Figure 3-7 DSC heating thermograms of the mixture of APA/OA =50/50 at two rates of melt cooling.

decreasing the rate of crystallization. Small peaks at - 2.8 C (rapid crystallization) or - 6.2 C (slow crystallization) correspond to the transformation from alpha-form to alpha'-form in the APA crystals. The gamma-alpha transformation was most influenced by lowering the rate of crystallization. The single gamma-alpha transition peak around - 60 C at the rapid crystallization was split into two, and the transition temperatures were both elevated, e.g., -33.2 C and -24.0 C. We confirmed that the peak at -33.2 C is due to the gamma-alpha transition in the APA crystals, and -24.0 C in the OA crystals. The splitting and elevation in the gamma-alpha transition were also observed in the samples of APA/OA=70/30 and 20/80; -45.2 C (rapid), -32.0 C and -21.5 C (slow) for 70/30, and -30.0 C (rapid), -20.9 C and -10.4 C (rapid) for 20/80.

Figure 3-8 shows the X-ray diffraction patterns of the alpha forms of APA and OA in the mixture of APA/OA=70/30, taken at different temperatures. The mixture was rapidly crystallized. At - 7 C, two long spacings of 4.15 nm and 4.10 nm were obtained. The former corresponds to alpha-form of OA, although the pure OA alpha-form has the long spacing value of 4.33 nm. 4.10 nm is due to alpha-form of APA, the pure sample of which has the long spacing of 4.10 nm. A complicated short spacing pattern was found to be superposition of two alpha-forms of APA and OA as denoted in Figure 3-8. It is reasonable to observe weaker diffraction peaks of OA compared to APA, since the concentration of OA is smaller. On heating to 7 C, the long spacing of alpha-form of OA disappeared and, correspondingly, the short spacing pattern of alpha-form of OA also disappeared. Accordingly, the X-ray diffraction spectra of this mixture proved that the mixture of APA/OA =70/30 is of eutectic nature.

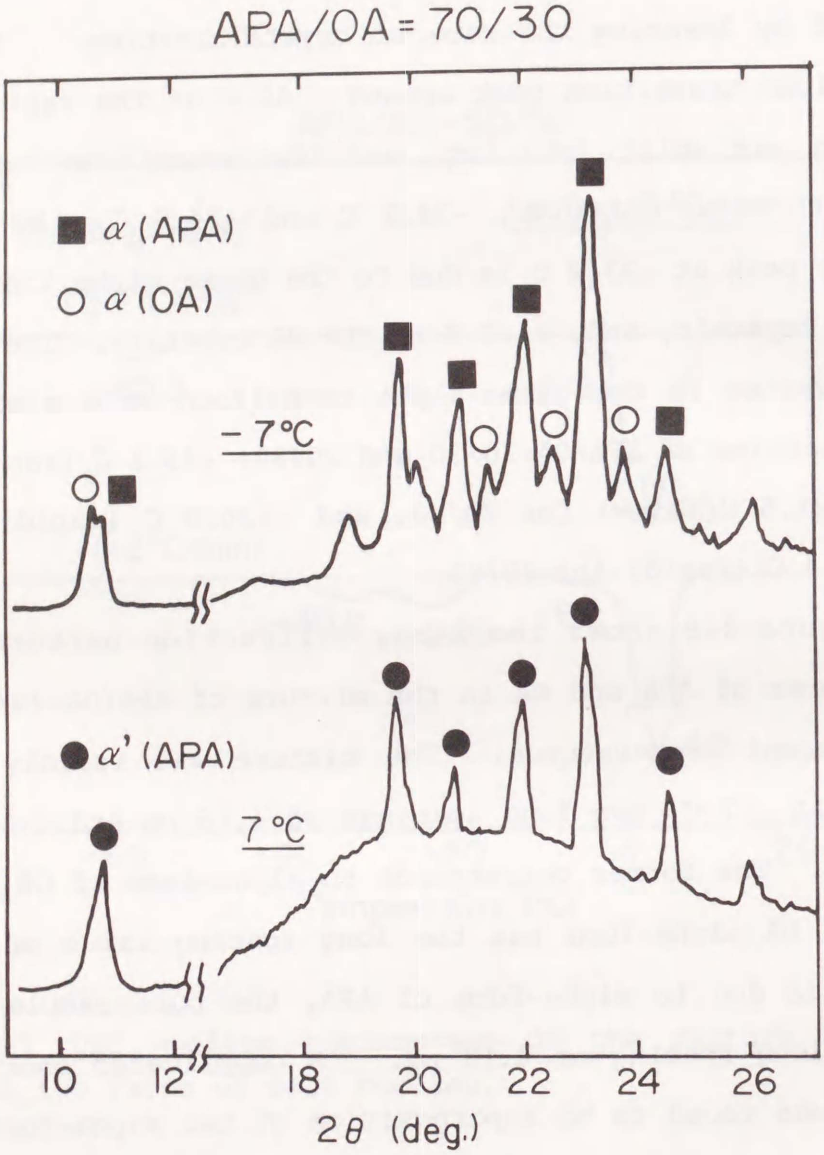

Figure 3-8 X-ray diffraction short spacing spectra of the mixture of APA/OA=70/30 taken at -7 C and 7 C. α (APA); α-form of asclepic acid, α (OA); α-form of oleic acid and α' (APA); α'-form of asclepic acid.

The similar X-ray diffraction data were obtained in wider range of the APA/OA concentration ratios, except for the gamma-forms, since there is very small difference between long spacing values of gamma-form of oleic acid and asclepic acid.[8)]

Summarizing all of the above data, we depicted the phase diagram of asclepic acid and oleic acid mixtures in Figure 3-9. The gamma-alpha transformation temperatures at the slow and rapid crystallizations are shown, being dependent on the rate of crystallization. Due to the same reason as the PSA/OA system, the phase behavior of the gamma-form of OA and APA involves certain kinetic effects, as depicted in a dashed line. The liquidus line indicates the eutectic property of this mixture system, showing the eutectic point around APA/OA=50/50. The transformation temperature from alpha-form to alpha'-form in the APA crystals did not change appreciably with changing the relative concentration of APA. However, the temperatures of the gamma-alpha transformations which occurred both in the APA and OA crystals, were lowered on approaching the eutectic point, when the mixture was crystallized rapidly. These phenomena were quite similar to those of the PSA/OA mixture system. In the case of the slow crystallization, the gamma-alpha transformation temperatures were raised and split into two for each as precisely described above.

4. Discussion

In the present study, we have mostly paid attention to the chain-chain interactions between the positionally isomeric cis-mono-unsaturated fatty acids by observing the phase behavior of the binary mixture. In general, three extreme cases are pos-

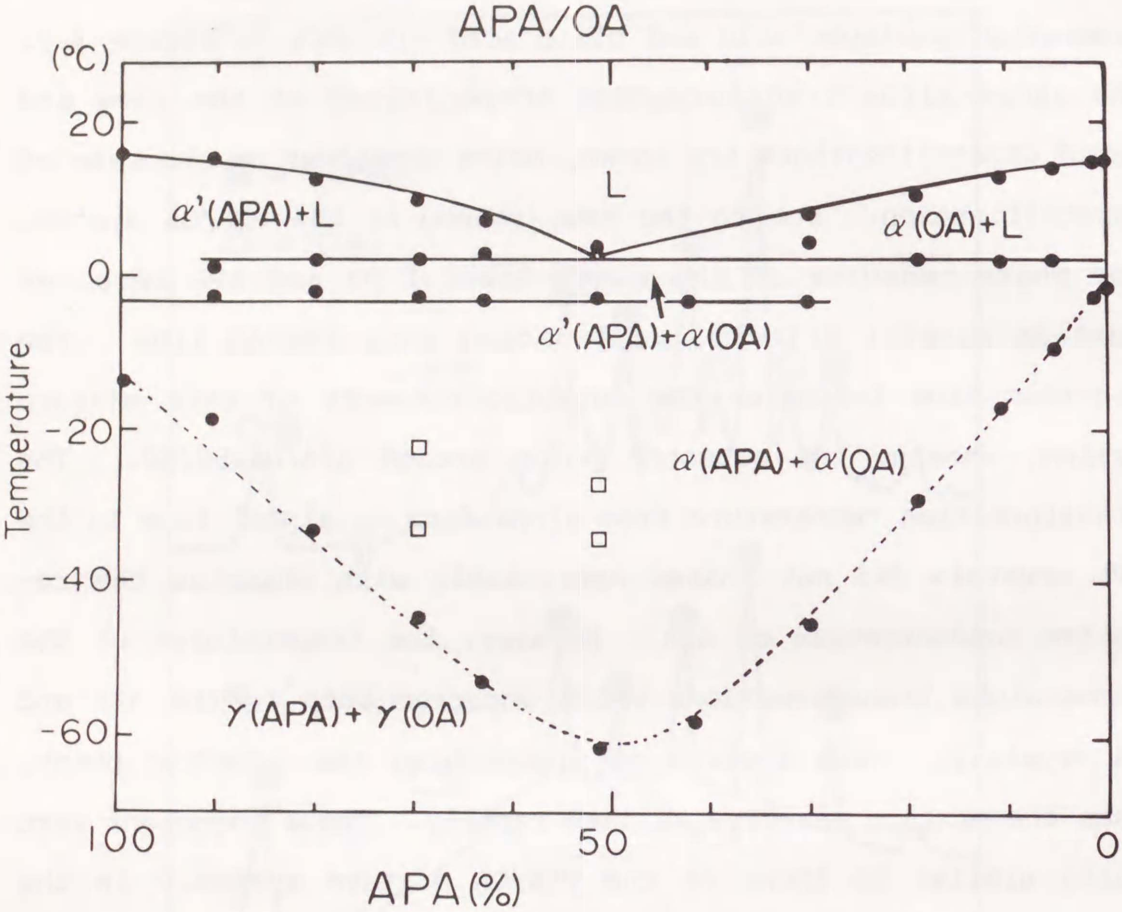

Figure 3-9 A phase diagram of the APA/OA mixture system. L;liquid, α' (APA); α'-form of asclepic acid, α (OA); α-form of oleic acid, α (APA); α-form of asclepic acid, γ (OA); γ-form of oleic acid and γ (APA); γ-form of asclepic acid. Liquidus lines and polymorphic transition temperatures (dashed line) are due to peak-top temperatures of corresponding DSC heating thermograms. (●) rapid crystallization and (□) slow crystallization.

sible to occur in the binary mixture, assuming that two components are completely miscible in the liquid state (melt); solid-solution, compound formation and eutectic mixture. In the solid-solution (mixed crystal), the two component molecules share the same crystalline lattice, in which the molecules are distributed through the structure at random. As to the compound formation system, it often occurs in specific ranges of the concentration ratio, due to certain geometrical matching or chemical reactions between the component molecules which otherwise often reveal the eutectic system. The eutectic system is ascribed to scarce molecular interactions. Actually, however, there are modified mixture systems of each extreme case, which are caused by partial mixing of the host molecules into the guest crystalline lattices.

The problem as to which case among the above three systems actually occurs, may primarily depend on the molecular interactions between the two components in the crystalline state. This is quite true for fatty acids and related compounds. For example, the molecules forming the solid-solution are chemically similar and not too different in size, as revealed in a mixture system of lauric acid (n_c=12)/myristic acid (n_c=14) in limited ranges of concentration ratios.10,11) No solid-solution has been observed in the mixtures involving the cis-unsaturated fatty acids. The occurrence of the compound formation was indicated in the binary mixtures of even-numbered saturated fatty acids with the difference in the chain lengths by two carbon atoms.10,11) Very recently, the compound formation was observed in the triacylglycerol mixture of SOS (1,3-distearoyl-2-oleoyl-glycerol) and SSO (1,2-distearoyl-3-oleoyl-glycerol) near the concentration ratio of 1:1.14)

It seems that the factors discriminating the compounds employed in the present study are simple; the position of the double bond with the same total carbon atoms of the chains. However, there is another peculiarity, polymorphism. Polymorphism may largely modify the phase behavior whatever the nature of the mixture phase, compared to the cases in which the pure components and the mixture reveal single crystalline phases. In this regard, Iwahashi et al. reported that, in the mixtures of optically active R-1-2-distearoylglycerol and S-1,2-distearoylglycerol, an eutectic mixture was formed in the stable beta polymorph, but the solid solution of a displacement type was formed in the metastable alpha-form.16) The similar results were obtained in the mixture of SOS/SSO,17) where a metastable alpha-form shows the complete solid-solution, whereas the stable beta-form showed either eutectic or compound formation system. In binary mixtures of odd n-alkanes that differ in length by two carbons, a conformationally disordered phase reveal the solid-solution, but a phase separation occurs in an ordered phase below critical temperatures that change as a function of concentration.16) Obviously, many of fats and lipids reveal polymorphism. Hence, their binary mixture systems must be examined in relation to the kinetic and thermodynamic aspects in the polymorphic behavior.

Accordingly, we first discuss the kinetic properties of polymorphism of each component, which are prerequisite for interpreting the kinetic properties of the binary mixture systems. Thereafter, the experimental data of the mixture systems of PSA/OA and APA/OA will be discussed.

a. Kinetic Aspects of Polymorphism of Pure Components

The thermodynamic properties of the polymorphism of the three fatty acids were displayed in Table 3-1. Hence, some kinetic properties are explained here.

Among the three forms of oleic acid, beta-form is most stable, yet gamma-form as well as alpha-form are metastable at all temperatures. The crystallization from melt at cooling rates faster than 0.1 C/min yields only alpha-form, which transforms to gamma-form on cooling at about -3.6 C. Upon heating, gamma-form transform to alpha-form at - 2.2 C. Although beta-form is most stable, the polymorphic transformations either from gamma-form to beta-form below -2.2 C, or from alpha-form to beta-form between -2.2 C and 13.3 C, are hindered in the crystals grown from the melt. This is a kinetic effect due to certain steric hindrance. Therefore, the occurrence of alpha-form or gamma-form must be important in the present mixture systems.

As to petroselinic acid, two forms, LM and HM, occur during the melt crystallization. LM form is more stable than the HM form below 18.7 C. The rate of nucleation of LM form became predominant with increasing rate of crystallization, and, with decreasing temperature of crystallization, particularly below 20 C. Therefore, the occurrence of LM form is relevant in the present mixture system.

Asclepic acid has three forms; gamma-form which is stable below -15.0 C where gamma-form transforms to alpha-form on heating. The alpha-alpha' transition occurs at -1.4 C on heating, being only detectable with DSC, not with X-ray diffraction. In addition, it appears that there are two metastable forms, which crystallizes faster than any of the above three forms upon rapid crystallization. These metastable forms, however, transforms rapidly to the more stable forms, by annealing the crystallized sample (see the previous section). Therefore, the occurrence of the three forms of asclepic acid displayed in Table 3-1 is relevant in the present experiment. We note again here that the molecular property with respect to the ordering (gamma-form)-or-disordering (alpha-form) in the omega-chain is almost identical in asclepic acid and oleic acid.

b. Phase Behavior of Binary Mixture

b.1 Eutectic Property

According to the liquidus lines and the X-ray diffraction spectra of the crystals present in the phase regions differentiated by the DSC endothermograms, we conclude that PSA/OA and APA/OA are of eutectic nature. No solid solutions were detected down to a concentration of 3 % of the minor component in the two systems. As to the PSA/OA system, Mod et al., reported the same conclusion, although they dealt with the freezing experiment.12)

The eutectic points lie at the concentration ratios of 35/65 PSA/OA, and 50/50 for APA/OA. A single liquid phase is present above the liquidus lines between the eutectic points and the melting points of every single components in the two mixture systems.

In the case of PSA/OA, LM form of petroselinic acid are present together with liquid, between the liquidus and solidus lines at the PSA concentrations above 35 %. By contrast, the region of the coexistence of liquid and alpha-form of oleic acid is present between the liquidus and solidus lines at the OA concentrations above 65 %. Below the solidus line, two phase regions are present;"LM form of petroselinic acid + alpha-form of oleic acid" above the transformation temperatures, which were changeable by changing the concentration ratio, and " LM form of petroselinic acid + gamma-form of oleic acid" at the low-temperature region. Since no transformation occurred in LM form of petroselinic acid below 18.7 C and the X-ray diffraction spectra clearly evidenced the gamma-alpha transformation of oleic acid, the existence of the gamma-alpha transformation detectable at the OA concentration down to 10 % is another proof of formation of the eutectic mixture system. The eutectic nature of the mixture of petroselinic acid and oleic acid, meaning that the two chains do not interact strongly, seems reasonable. Obvious reasons for that are far different physical properties of the crystals of the two acids, in particular with respect to their molecular structures. The subcell packing of alpha-form and gamma-form are $O'_{//}$, whereas the $O_{\perp}$ packing is revealed in LM form of petroselinic acid. Correspondingly, lesser interactions between the omega-12 double bonded molecules (PSA) and omega-9 double bonded molecules (OA) might cause the separation of the crystal phases of the two acids. As to more subtle molecular interactions, a problem may arise as to how the two molecules can be mixed each other, revealing an overall eutectic property of the phase diagram. This may be related to thermal behavior of the gamma-alpha transition of oleic acid fractions, as will be discussed below.

In the case of APA/OA, there are two phase regions in which the solid and liquid coexist; alpha'-form of asclepic acid + liquid and alpha-form of oleic acid + liquid, which are present at the APA concentrations above and below 50 %, respectively. Below the solidus line, three phase regions are present, depending on the gamma-alpha transitions in asclepic and oleic acid, and on the alpha-alpha' transition in asclepic acid. The occurrence of the alpha-alpha' transition, which only occurs in asclepic acid,$^{6)}$, is another proof of the formation of the eutectic mixture. It seems that the eutectic nature in the APA/OA mixture may be unexpected, since the difference in the position of the double bond at the carbon chains is only two, and since two common polymorphic forms are present in the two acids. However, the thermodynamic stability and kinetic aspect of the polymorphism of the two acids reveal some contrast. gamma-form and alpha-form of oleic acid are metastable, whereas gamma-form, alpha-form and alpha'-form of asclepic acid are most stable. Due to kinetic effects, rapid cooling crystallizes the metastable forms of asclepic acid, and, by contrast, alpha-form of oleic acid was formed by rapid cooling. In this regard, the interaction between the two molecules in the liquid phase would be diminished, as expressed in the different crystallization behavior of the polymorphs of each component. This situation would be present even in the mixtures with the APA concentrations lower than 80 % , where the occurrence of the metastable forms of asclepic acid was depressed.

Although not displayed in the present article, we confirmed that the mixture of asclepic acid and petroselinic acid also forms a simple eutectic system like the binary systems of PSA/OA and APA/OA. This is quite reasonable, since the difference in the position of the double bond between APA and PSA is maximum, 5 carbon atoms, among the three binary mixture systems.

The lesser interactions between the three positional isomers may partly be explained by geometrical properties of molecules in melt. According to FT-NMR analysis of oleic acid, Iwahashi et al., recently claimed that the fragmental movements of carbon atoms of a dimerized molecule in melt are limited both at the positions of $(COOH)_2$ and the double bond, the extent of restriction at the two positions being the same.[17)] The values of $1/\tau_C$, an inverse of the effective correlation time for the rotational reorientation which was calculated from $^{16)}$C-NMR spin lattice relaxation time T_1, are drastically lowered on approaching the double bond. The specific restriction of the fragmental movement at the double bond position may diminish the interactions between the omega-7 (asclepic acid), omega-9 (oleic acid) and omega-12 (petroselinic acid) double-bonded molecules.

b.2 Partial Mixing

The thermal behavior of the polymorphic transformations in the two binary mixture systems is a measure of partial mixing of molecules in the eutectic crystal fractions.

The gamma-alpha transition temperature in the oleic acid crystal fraction of the PSA/OA mixture was decreased on approaching the eutectic point, when the mixture was rapidly crystallized (>50 C/min). At the same time, the corresponding DSC endothermic peaks were broadened. However, by decreasing the rate of crystallization (0.2 C/min), the transition temperature was raised, and the endothermic peaks became much narrower (Figures 3-2 and 3-5). In particular, two endothermic peaks appeared in the mixtures of the PSA concentrations of 35% and 20 %. The similar reduction in the gamma-alpha transition temperatures due to the rapid crystallization was observed in the

APA/OA mixtures (Figures 3-7 and 3-9). The slow crystallization raised the transition temperatures and split each of the DSC peak in the mixtures of APA/OA=70/30 and 50/50.

The rapid crystallization may cause two effects; formation of small crystal domains, and partial mixing which occurs through the incorporation of guest molecules as impurities into host crystals.

The formation of the small crystal domains is reflected in subtle exothermic peaks in the PSA/OA mixtures, whose concentration ratios were close to the eutectic point (Figures 3-1 and 3-2). This anomaly may be ascribed to latent heat released by coagulation of the small crystal domains just below the melting point. However, the size effect of this kind may not lower the transition temperature appreciably, since very rapid crystallization (>100 C/min) did not lower the gamma-alpha transition of oleic acid so much as observed in Figures 3-1,3-2,3-6 and 3-7; -2.2 C at slow crystallization and -3.6 C at very rapid crystallization. Hence, we assume that the reduction in the transformation temperature at the rapid crystallization is ascribed to the partial mixing. To prove it, Figure 3-10 shows the decrease in the gamma-alpha transition temperature of oleic acid caused by an addition of petroselinic acid or asclepic acid to oleic acid. The mixture was rapidly crystallized by cooling at 50 C/min. In this range of concentration of guest molecules, the decrease in the gamma-alpha transition temperature was a linear function of the concentration of the added molecules, being more enhanced for asclepic acid than for petroselinic acid. In accordance with the decrease in the transformation temperature, enthalpy values of the gamma-alpha transition decreased by the addition of the guest molecules, although no

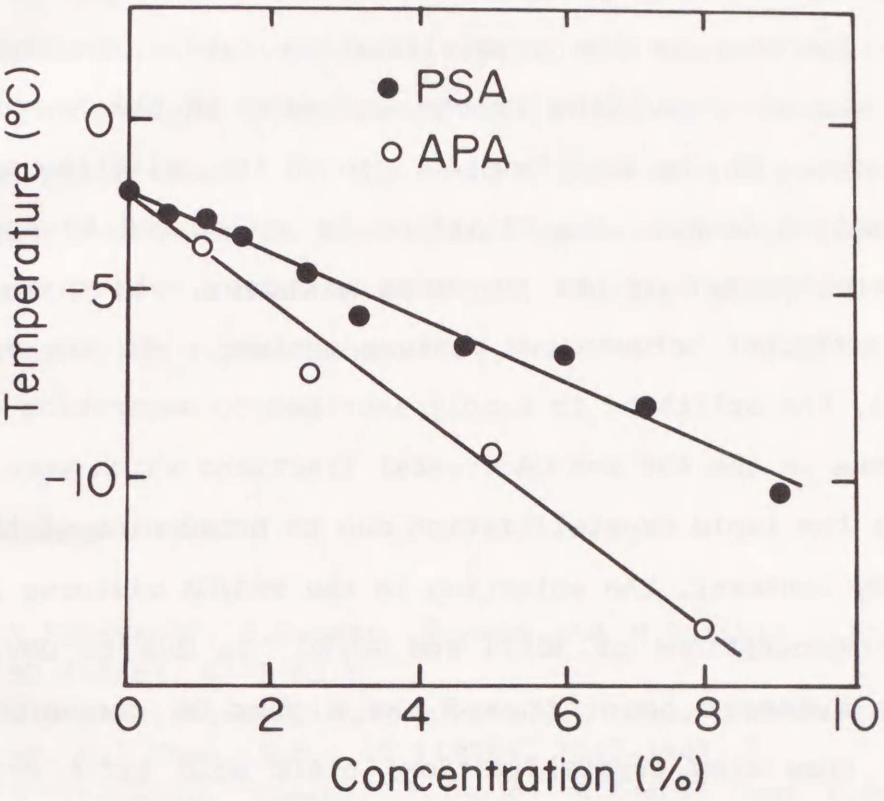

Figure 3-10 Effects of addition of asclepic acid (APA) and petroselinic acid (PSA) on γ→α transition temperature of oleic acid. The sample was cooled by 50 C/min.

appreciable difference between APA and PSA was detected. Form this we conclude that the degree of partial mixing is higher in the APA/OA mixture system than in the PSA/OA mixture system. This conclusion may be rationalized by the fact that the difference in the position of the double bond is smaller in the APA/OA mixture than in the PSA/OA mixture.

The decrease in the crystallization rate diminished the partial mixing, resulting in the increase in the transition temperatures. At the same time, it caused the splitting of some DSC transition peaks. The splitting is influenced by crystallization processes of the eutectic mixtures, which would be somehow different between two mixture systems. In the mixture of APA/OA, the splitting is simply ascribed to separation of the transitions in the APA and OA crystal fractions which were overlapped at the rapid crystallization due to broadening of the DSC peaks. By contrast, the splitting in the PSA/OA mixtures at two PSA/OA concentrations of 30/70 and 20/80 is due to deviation from the eutectic point toward the higher OA concentration region. Upon slow crystallization, oleic acid first crystallized from the mixture melt along the liquidus line. At the eutectic point, both petroselinic acid and oleic acid crystallized at the same time. In this case, two kinds of oleic acid crystals would be present; the first-solidified crystals were grown from the mixed liquid, and the second-solidified ones were grown at the eutectic point. The former crystals of oleic acid contain lower amounts of petroselinic acid molecules as the impurity, compared to the latter crystals, giving rise to higher gamma-alpha transition temperatures. At the PSA/OA concentration of 50/50, no splitting was observed, since the crystallization of oleic acid occurred only when the mixture melt, coexist-ing with the petroselinic acid crystals, reached the eutectic point.

By decreasing the rate of crystallization, the melting points of the eutectic crystal fractions did not change so much as the gamma-alpha transformation temperature both for the PSA/OA and APA/OA mixtures. This means that the gamma-alpha transformation is very sensitive to the partial mixing. Since the gamma-alpha transformation is due to disordering of the omega-chain segment in the alpha-form, the incorporation of the foreign molecules in the ordered gamma-form may accelerate the formation of the disordered phase. Further study will be needed to clarify this phenomenon.

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Chapter 4

Summary

Crystal growth and polymorphic transformations in cis-monounsaturated fatty acids were studied with X-ray diffraction, differential scanning calorimetry and polarized optical microscopy.

The goal of this study is to clarify, on a molecular-level, the physical chemical properties of unsaturated lipids which play critical roles in functional activities of biological organisms and lipids containing foods. Varying the position of a cis double bond in the aliphatic chain, we have dealt with four cis-unsaturated fatty acids, oleic acid (OA; $C_{18:1}\omega9$), erucic acid (EA; $C_{22:1}\omega9$), petroselinic acid (PSA; $C_{18:1}\omega12$) and asclepic acid (APA; $C_{18:1}\omega7$). In these compounds, the difference in the position of a cis double bond is rather small; 2 (OA/APA) or 3 (OA/PSA) carbon atoms, but we found that it gave rise to remarkable influences on the structural, thermodynamical and kinetics aspects of the polymorphic behavior.

This study clarified the occurrence and transformation of polymorphs, thermodynamic stability, crystallization kinetics and crystal structures, in crystalline states of each substance. In addition, the phase behavior of binary mixtures of oleic acid and its two positional isomers are also clarified to investigate molecular interactions between them.

In this study, new crystal structures were discovered, quite differing from those reported for saturated fatty acids. They are alpha, alpha_1, gamma and gamma_1 forms in erucic acid, LM(low-melting) and HM(high-melting) forms in petroselinic acid, and alpha, alpha' and gamma forms in asclepic acid. Being based on the analysis of thermodynamic stability and molecular structures, it was found that a total of the polymorphic behavior revealed quite diversified phenomena depending on the position of a cis double bond in the aliphatic chain. An order-disorder transformation of a new type, due to conformational disordering of the aliphatic chain segment between the double bond and CH_3 end group, which was first discovered in oleic acid by Suzuki and co-workers in 1985, was also discovered in erucic and asclepic acids. From this, we concluded that this order-disorder transformation is characteristic in a certain group of cis-monounsaturated fatty acids.

As to the crystallization kinetics, we found that the crystal growth phenomena from melt and solution phases were significantly influenced by the polymorphic modifications themselves, and by a specific structure existing in the melt in the case of oleic acid. The principal results are summarized as follows;

(1) Concerning the polymorphic effects, alpha and alpha_1 forms of erucic acid showed almost the same properties both in the rates of crystal growth and nucleation. However, beta-form of oleic acid showed significantly smaller values (10^{-5}-10^{-3} times) in the growth rate than that of alpha-form. This suggests that the crystallization kinetics is remarkably dependent on the

polymorphic structures. This difference may originate from the specific effects of the cis double bond.

(2) The effects of the melt structure of oleic acid have been observed in that melt preheating prior to the crystallization influences the rate of nucleation of alpha-form. This result supports Iwahashi's findings (1990) which maintain a possibility of existence of three structures in the melt phase, from the view point of crystal growth kinetics.

(3) The effect of melt structures affects the nucleation of alpha and beta form, in a different manner. The nucleation of alpha-form was more sensitive to the melt structure than that of beta-form. This indicates that the difference in the melt structures is related to the nucleation of specific crystalline structures.

(4) The effect of melt preheating on the growth rate of alpha-form was not significant. This indicates a modification in the melt structure at the solid-liquid interface of oleic acid crystals which has theoretically been predicted as a universal phenomenon in organic and inorganic substances.

In the phase diagrams of binary mixtures of PSA/OA and APA/OA systems, this study discovered that the mixtures showed eutectic property. It proved that there are sparing interactions to form a common crystal structure between the positional isomers. Regarding the order-disorder transformations which occur in oleic acid and asclepic acid, the transformation temperatures changed in accordance with the composition and the rate of crystallization. The change is more remarkable in the

APA/OA system than in the PSA/OA system. This behavior is explained in terms of partial mixing of the molecules in the solid state.

To further extend the present research, let us display the following problems which have arisen during the present work;

(a) Detailed physical analyses of crystal and molecular structures of important polymorphic forms such as alpha forms of oleic acid, erucic acid, asclepic acid, beta form of oleic acid, all of which have only been examined in rather thermodynamic or vibrational spectroscopic methods. Particularly, the alpha form may contain most typical molecular properties of cis-monounsaturated fatty acids in its disordered aliphatic omega-chains. X-ray or neutron diffractions using single crystals may be most powerful.

(b) Diffraction analysis of the melt structure of oleic acid or other unsaturated fatty acids which was first examined by Iwashashi's group. In addition, the relationships between the possibly structured-melt and polymorphic crystallization would be relevant to clarify the specific roles of cis-unsaturated fatty acids and their esters, which are observed in the molecular interactions in crystalline, liquid crystalline and membrane states.

(c) Further analysis of the mechanisms of the order-disorder transformation termed "interfacial melting" of the aliphatic chain segments. The effect of length of the omega-chain was confirmed, but it seems that additional factors would be present such as parity of the omega-chain length, odd or even, and minimal chain length of the omega-chain. Systematic study using different kinds of <u>cis</u>-monounsaturated fatty acids will be needed.

(d) Structures and kinetic behavior of the binary mixtures of positional isomers have been more interesting, and another combinations of the mixtures systems, which contain the acids with different omega-chain lengths but the same omega-chain length or vice versa. This may shed new light on the molecular interactions of diversified unsaturated fatty acids.

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