Polymorphism of POP and SOS[1] III. Solvent Crystallization of β_2 and β_1 Polymorphs

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Single crystals of β_1 and polycrystals of β_2 of POP and SOS were obtained from acetonitrile solution. The crystallization behavior of the two polymorphs was almost the same in POP and SOS; rapid cooling of the solution preferentially crystallized the metastable β_2 form, and the most stable β_1 form crystallized in a very low solute concentration at the expense of β_2, via solution-mediated transformation. The single crystal of β_2 revealed needle-like irregular shape, whereas well-defined slender rhombic shape was observed in β_1. The solubilities of the β_1 forms of POP and SOS in tetradecane solution were precisely measured in a temperature range of $10 \sim 20°C$. This study showed that the solvent crystallization was the single way to grow the β_1 crystal, since melt-cooling and melt-mediated transformation did not crystallize β_1 both in POP and SOS.

The polymorphism of triacylglycerol (TG) depends on the chemical structure of three acyl chains. Up to now, symmetrical monosaturated triacylglycerols have been well studied (1). Many naturally occurring and biologically active triacylglycerols involve one or two unsaturated acyl chains. The polymorphism of TG involving the unsaturated acyl chains has been poorly elucidated, and the literature involves a lot of contradiction (2-8).

Recently, we have carried out systematic studies on the mixed saturated-unsaturated acyl TGs, particularly on TGs having oleoyl chains. Thermal and structural behavior of the polymorphism of symmetric Sat-O-Sat (Sat: saturated acyl, O: oleoyl) (9-11), O-Sat-O (12), and non-symmetric sn-triacylglycerols (O-O-Sat) (13) have been studied. As for the Sat-O-Sat TGs, the authors observed five independent polymorphs of α, γ, pseudo-β', β_2 and β_1 in SOS, AOA and BOB, and six polymorphs of α, γ, pseudo-β_2', pseudo-β_1', β_2 and β_1 in POP, (S: stearoyl, A: arachidoyl, B: behenoyl, P: palmitoyl). The occurrence and transformation behavior of polymorphs of POP and SOS may be illustrated in Figure 1. The physical properties of the five forms in SOS and six forms in POP were fully explained (10) in relation to the previous reports; α was the same as that reported in the literature (3-8), γ and pseudo-β' have the same physical properties as those reported in other literature (2-8), although the nomenclature was different. Two β forms, β_2 and β_1, were discovered in our study. As to the nomenclature, we basically obeyed the Larsson's principle (14), which took into account the subcell packing structure of the independent polymorph. But additional naming was used in order to characterize very complicated polymorphic structures (10).

In this report, we describe the solvent crystalliza-

tion of β_2 and β_1 from acetonitrile, and solubility values of β_1 in tetradecane.

MATERIALS AND METHODS

We employed POP and SOS with purity of 99.9%, and acetonitrile and tetradecane of 99% purity (Nakarai Chemicals, Tokyo, Japan). The samples of high purity POP and SOS were provided by Fuji Oil Co., Izumi Sano, Japan and they were prepared by the following processes. Initial materials were prepared by solvent crystallization of cocoa butter using n-hexane and synthesized through enzymatic esterification using triolein and ethyl palmitate. The 99% sample was first obtained by a preparative HPLC (Killoprep 250, Millipore Co., Ltd., MA). The 99.9% samples were produced with the Same HPLC using the initial material of 99% purity. The purity of 99.9% was determined by HPLC (Japan Spectroscopic Co., Ltd., Tokyo, Japan), which showed no detectable peak of the impurity.

In the solvent crystallization, acetonitrile was chosen, since the solubility was low enough for better crystallization (e.g., in POP, 2 g solute/100 g solvent at 14.5°C). The solution was put in a thermostated glass cell (30 cc), and cooled to the crystallization temperature. The rapid cooling of the solution of high solute concentration often crystallized metastable polymorphs such as γ, pseudo-β' or β_2, not the stable β_1. The crystallization, however, of γ and pseudo-β' yielded quite irregular-shaped crystals which rapidly converted to more stable forms. Hence, we could not easily control the growth conditions of the crystals of the two forms. By contrast, the growth of β_2 was controlled more easily; the most typical conditions were: the saturated solution at 40°C was cooled to 22°C at the rate of about 0.5°C/hr. The β_1 crystal was obtained via solution-mediated transformation from β_2; new β_1 crystals started to grow at the expense of β_2 crystals in the solution at a constant temperature. β_1 appeared approximately two hours after the solution-mediated transformation was initiated. It was difficult to singly grow the β_1
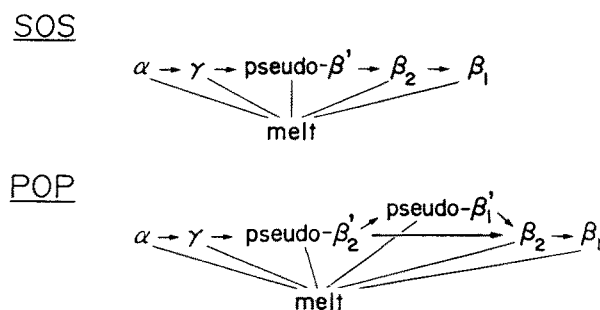

FIG. 1. Transformation cycles of independent polymorphs of POP and SOS. The solid-state undergoes irreversible transformation from α to β_1 (10). All polymorphs except β_1 are formed by melt crystallization (11).

crystals, instead of through $\beta_2 \rightarrow \beta_1$ solution-mediated transformation. There were two reasons for this difficulty: (a) the difference in solubility between β_2 and β_1 was rather small, and (b) the growth rate of β_1 was extremely low in comparison to β_2. Hence the growth conditions of β_1 were very limited, and not actually attained. The crystallized sample was examined by X-ray diffraction (XRD) (Rigaku, CuK$_\alpha$) and Differential Scanning Calorimetry (DSC) (Rigaku DSC-8230). The melting point was defined, by computerized date-processing, as a crossing point between the base line and the tangential line of a maximal slope of the initial endothermic peak. The data were compared with those of the crystal obtained by melt crystallization (11) and polymorphic transformation (10).

Solubility for β_1 of POP and SOS was determined by measuring the temperature at which the β_1 crystal reached equilibrium with the tetradecane solution whose concentration was precisely measured.

RESULTS AND DISCUSSION

Solubility. Solubility curves of the β_1 form of POP and SOS in tetradecane are shown in Figure 2, and numerical data are displayed in Table 1. Solubilities of POP and SOS increased with increasing temperature, the difference being almost the same at all temperatures. The difference in the saturation temperature between the POP and SOS at a fixed concentration was 5.4 ~ 5.5 °C.

The solubility expressed by molar fraction, and 1/T (K^{-1}) did not follow Van't Hoff equation in both POP and SOS. It was impossible to measure the solubility of β_2 since the solution did not reach the equilib-
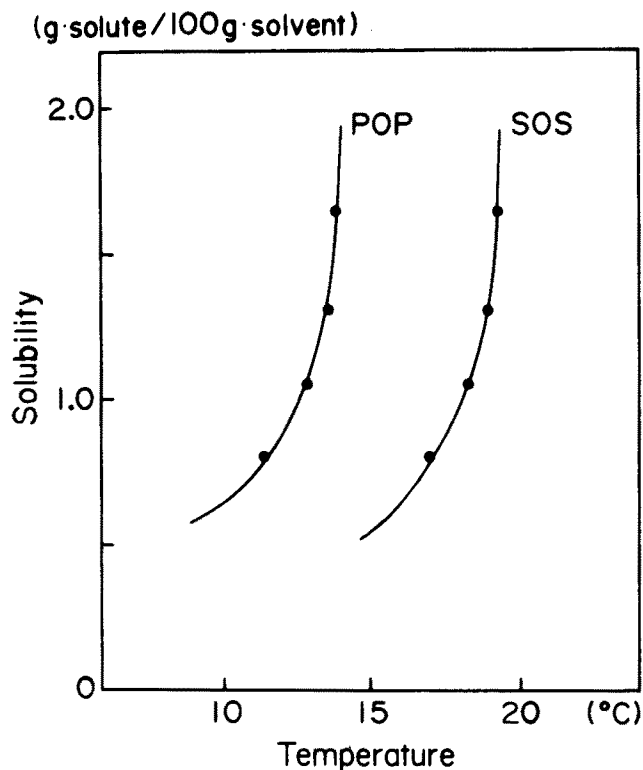
rium with β_2 due to the solution-mediated transformation to β_1.

Solvent crystallization. Figure 3 shows optical photographs and corresponding XRD spectra and DSC melting peaks of β_2 and β_1 of POP crystallized from acetonitrile solution. Table 2 summarizes XRD short spacing spectra expressed in relative intensity. β_2 revealed an irregular needle shape as shown in Figure 3(a). The XRD long spacing (61.0 A) and short spacing and DSC melting point (35 °C) of the solvent crystallized β_2 are the same as those of melt-crystallized β_2 (10); e.g., melting point of 35.1 °C, XRD long spacing of 61.0 A and XRD short spacing exactly the same as in Figure 3(a). Figure 3(b) shows the concurrent crystallization of β_1 and β_2 during the solution-mediated transformation. Well-developed rhombic β_1 crystals appeared near the needle-like β_2 crystals. The XRD short spacing pattern of the sample shown in Figure 3(b) is somewhat changed from Figure 3(a). DSC melting peaks became double, indicating the existence of the two different polymorphs. With increasing time of incubation, the concentration of β_1 crystals increased at the expense of β_2, because the solubility of β_1 is lower than that of β_2. The long spacing value did not change, because β_2 and β_1 have the same interlamellar distance (10).

Figure 3(c) shows the morphology, XRD and DSC melting peak of the single crystal of β_1. The interplanar angles of the rhombic crystal are 123° and 57°, which are nearly the same as those of β of trilaurin (15). The XRD and DSC data of β_1 are quite identical to those of the β_1 which was transformed in a crystalline state from the melt-crystallized metastable forms (10). The intensity of XRD long spacing spectra of β_1 became higher, because its crystallinity was raised. We confirmed that a total behavior of solvent crystallization of β_2 and β_1 of SOS was the same as that of POP, with respect to the crystal morphology, XRD and DSC data.

Table 3 shows enthalpy values of fusion of β_2 and β_1 of POP and SOS obtained by the solvent crystallization. All values increased by 6 or 10% in comparison to those of the melt-crystallized and transformed samples (10). These increases may be ascribed to better crystallinity, of the type that was observed in the XRD peaks, of the solvent-crystallized sample.

It may be worthy to note that the XRD short spacing patterns of β_2 and β_1 both of POP and SOS are quite similar to those of Form V and Form VI of cocoa butter, respectively (10, 16, 17). The polymorphic behavior of a mixture of POP/POS/SOS using the 99.9% purity of each component showed the same XRD pat-

(g·solute/100g·solvent)

FIG. 2. Solubilities of β_1 of POP and SOS in tetradecane.

TABLE 1

Solubility Data of β_1 of POP and SOS in Tetradecane

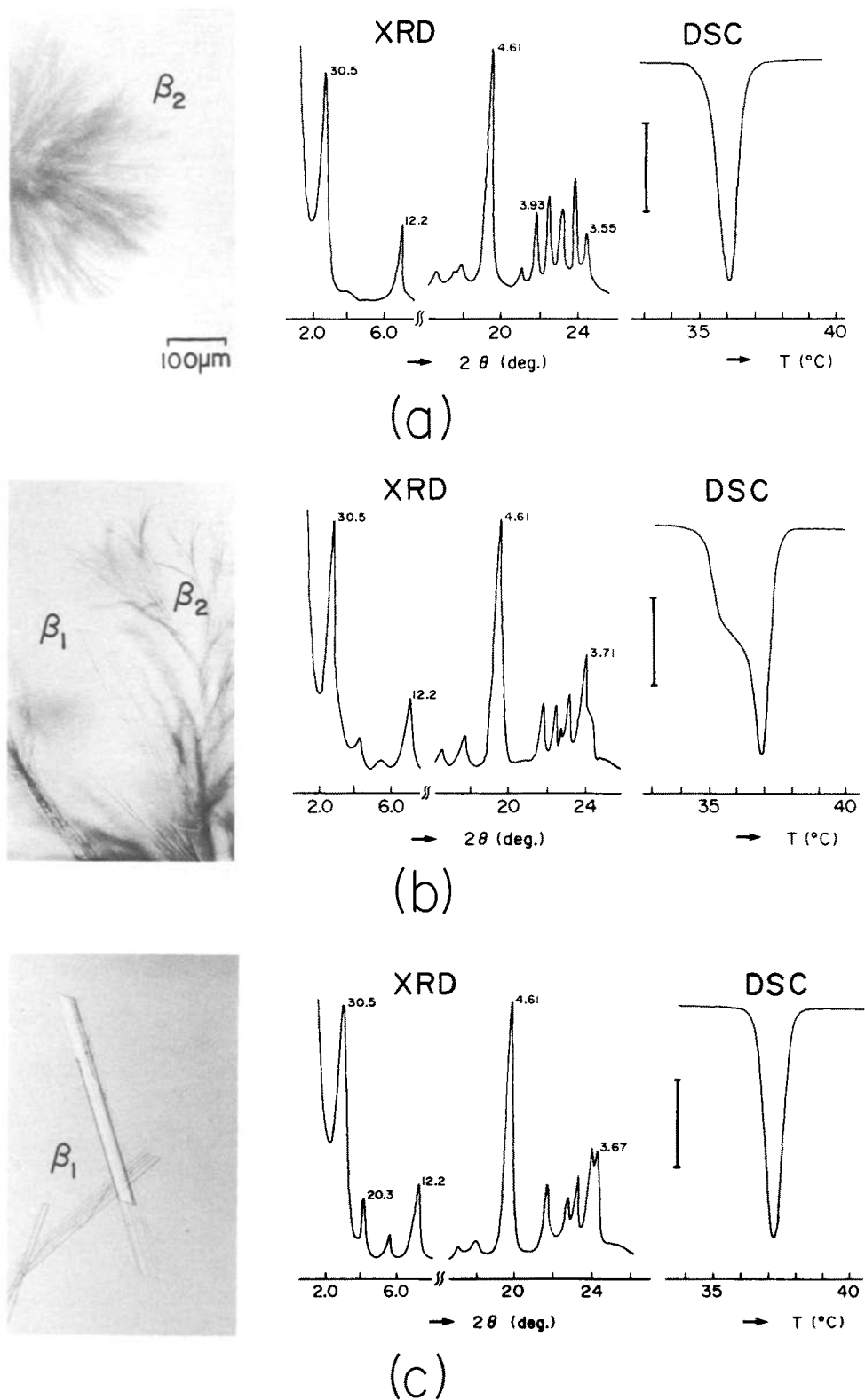| g solute 100 g solvent	Tetradecane solution	
	T$_s$* (POP)	T$_s$ (SOS)
1.65	13.9	19.3
1.30	13.6	19.0
1.05	12.9	18.3
0.80	11.5	17.0

*T$_s$, saturation temperature (°C).

FIG. 3. Optical photographs, X-ray diffraction spectra (XRD) and differential scanning calorimetry (DSC) melting curves of β_2 and β_1 of POP crystallized from acetonitrile solution. (a) β_2 obtained by rapid crystallization, (b) β_2 and β_1 which concurrently crystallized by solution-mediated transformation, (c) β_1. The micrograph of (c) was taken for the crystals out of solution. DSC scale corresponds to 0.5 mcal/sec.

TABLE 2

X-ray Diffraction Short Spacing of β_2 and β_1 of POP Grown from Acetonitrile Solution

Polymorph	Short spacing (Unit, A)
β_2	4.61(vs[a]) 4.07(m), 3.93(m), 3.82(m), 3.72(m) 3.62(w), 3.55(w)
β_1	4.61(vs), 4.07(m), 3.88(w), 3.82(m), 3.72(m) 3.67(m)

[a]vs = very strong, m = medium, w = weak.

TABLE 3

Enthalpy Values (kJ/mol) of Fusion of β_2 and β_1 of POP and SOS

	Solid-state transformation[a]	Solvent crystallization
POP		
β_2	124.4	136.7
β_2	130.2	144.7
SOS		
β_2	143.0	152.3
β_1	151.0	163.3

[a]Reference (10).

terns (18). From these data, we concluded that Form V and Form VI are independent polymorphs of the mixture TG, whose physical behaviors are very similar to β_2 and β_1 of POP and SOS. In this regard, the morphological difference between β_2 and β_1 is indicative for blooming phenomena of the fat containing cocoa butter; i.e., β_1, which corresponds to Form VI revealed more bulky and larger crystal morphology than β_2, which corresponds to Form V.

The present study enabled us to grow large single crystals of β_1 which are useful for structural determination by polarized infrared and Raman spectroscopy, as well as X-ray analyses.

ACKNOWLEDGMENT

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