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Polymorphism in Cholesteryl Esters: Cholesteryl Palmitate*

Abstract: The thermal transitions in cholesteryl palmitate, an ester having liquid-crystalline states, have been evaluated and compared to previously reported data. A sample synthesized from carefully purified cholesterol and palmitic acid using *p*-toluene sulfonic acid compared directly with previously reported data. A sample of recrystallized ester from a commercial source showed a slightly depressed solid-to-mesophase transition temperature. However, the mesophase proved to be smectic, commonly reported as monotropic with respect to the solid phase. Both smectic-to-cholesteric and cholesteric-to-isotropic-liquid transitions were sharp, indicating a pure material. It is postulated that a specific impurity, possibly an isomeric cholesterol ester, is responsible for this previously unreported effect. This effect of related compound interaction has been reported for nematic-mesophase-forming compounds. Thin layer chromatography, microscopy, depolarized light intensity analysis, differential scanning calorimetry and NMR spectroscopy data are offered in support of this postulate.

Introduction

The formation of mesophases, or liquid-crystalline states, of the smectic and cholesteric types has long been recognized as a property of cholesteryl esters [1]. The mesophases formed are frequently determined by the direction of the process, i.e., heating or cooling. For example, cholesteryl myristate exhibits solid, smectic, cholesteric and isotropic-liquid phases on both heating and cooling. Cholesteryl nonanoate exhibits solid, cholesteric and isotropic-liquid phases on heating, but on cooling a smectic mesophase appears between the cholesteric and the solid phases. The smectic mesophase is said to be monotropic with respect to the solid phase. Cholesteryl laurate exhibits no mesophase (see exception below) on heating, but on cooling a cholesteric and a smectic mesophase appear between the isotropic-liquid and the solid phases. The smectic and cholesteric mesophases are monotropic with respect to the solid phase. In the monotropic cases the phase rule and laws of thermodynamics are not violated, since each phase is always in equilibrium with the next higher temperature phase that is stable at the transition temperature. Since 1967 the thermodynamic properties of these interesting materials have been studied in this series of papers. Valuable insights and contributions have been made by other workers during this same time period [2–5].

In part VIII [6] of this series it was noted that significant variations occurred in the transition heats and temperatures of the solid phase, depending on the way in which the solid phase had been formed—from solution, from the isotropic melt or from the mesophase. Solid-phase polymorphy was further explored for cholesteryl propionate, as reported in part XX [7]. This compound was found to form two solid phases with melting points of 95.2 and 98.0°C, depending on the solvent used for recrystallization. In addition, the high melting point form was found to form predominantly on very slow cooling ($<1°$/min) of the mesophase. Interconversion between solid forms without the presence of the mesophase was not noted after repeated efforts. The crystal morphology of the two solid phases is probably fundamentally different in the propionate case, since the higher melting point form is predominantly prismatic and the lower melting point form, spherulitic [7].

In part XXII [8] of this series cholesteryl heptadecanoate was reported to exhibit a high and a low melting point form. It was not possible to separate the two forms in very pure material. However, the introduction of impurities produced predominantly the lower melting point form. The impurity content need be only 1.5 percent to produce *all* low melting point form [8]. The very pure heptadecanoate ester gave only the high melting point form when recrystallized from the mesophase.

* Part XXVII of a series on Order and Flow in Liquid Crystals.

Table 1 Comparison of cholesteryl palmitate transition data; T_m in °C is the temperature at endothermal minimum for DTA or DSC measurements, ΔH is the transition entropy in kcal/mole, and ΔS is the transition entropy in cal/mole-°K; [ ] indicates a monotropic transition.

	Transition											
	Solid → smectic			*Solid → cholesteric*			*Smectic → cholesteric*			*Cholesteric → isotropic liquid*		
Sample	T_m	ΔH	ΔS	T_m	ΔH	ΔS	T_m	ΔH	ΔS	T_m	ΔH	ΔS
Van Schuppen[a]	75.47	13.27	38.0				76.74	0.463	1.32	81.42	0.317	0.894
Synthetic[a]				77.51	13.69	39.1	[76.4	0.41	1.21]	82.00	0.317	0.892
Sell and Neumann[b]				77			[75.5		]	80.0		
Gray[c]				79			[78.5		]	83.0		
Ennulat[d]	77.5	14.4	41.0				[78.1	0.39	1.09]?	82.6	0.29	0.82
Porter, Barrall and Johnson[e]				79.6	14.2	40.3	[64.0	0.36	1.07]	70.0	0.28	0.82
Davis and Porter[f]				77.3	14.0	40.0	[76.5	0.36	1.04]	81.6	0.28	0.78

[a] All DSC temperatures from the present study are accurate to ±0.07°C except as specified by an omitted decimal place, in which case the accuracy is ±0.1°C.
[b] P. J. Sell and A. W. Neumann, *Z. Physik. Chem. (N. F.)* **65**, 13 (1969).
[c] G. W. Gray, *J. Chem. Soc.*, 3733 (1956).
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The present study was concerned with the investigation of the possible solid-phase polymorphy or selective impurity depression of cholesteryl palmitate. This ester has been reported by a number of workers [2, 3, 6, 9, 10] to undergo a transition to the cholesteric mesophase on heating, followed by a cholesteric → isotropic liquid transition. On cooling the isotropic liquid, the cholesteric mesophase forms. At lower temperatures the cholesteric mesophase undergoes a transition to the smectic mesophase, followed by formation of the solid phase, somewhat supercooled. These data, collected in Table 1, indicate that the smectic mesophase is monotropic with respect to the solid and cholesteric phases. The cholesteric mesophase has been reported as being monotropic with respect to one solid phase as well [6]. Thus the existing literature strongly indicates that cholesteryl palmitate may have a complex solid-phase polymorphy. Collaterally, the next lower member, cholesteryl myristate, has been reported without exception to be completely reversible in all mesophases; i.e., the smectic and cholesteric mesophases form on both heating and cooling [5, 6, 9, 10]. Cholesteryl laurate would behave as does cholesteryl myristate were it not for the spontaneous conversion of the low melting point solid form near 85°C to a higher melting point (about 95°C) form. This renders monotropic both the cholesteric and the smectic mesophases [11, 12]. Davis and Porter have succeeded in isolating the pure low temperature solid phase of the laurate ester [12]. Their sample, prepared by careful precipitation from *n*-pentanol, apparently lacked nuclei of the high temperature form, thus preventing the usual solid-phase recrystallization.

Indeed, it may be possible to form low melting point crystal forms of all the even *n*-alkyl cholesteryl esters. Preliminary evidence indicates that the solid-phase polymorphy of the cholesteryl esters may be at least as complex as the mesophase polymorphy. In part this apparent polymorphy may be due to selective impurities previously reported for nematic systems [13].

Experiment

• *Preparation of samples*

Cholesteryl palmitate from two sources was studied. A sample of cholesteryl palmitate from van Schuppen Chemicals, Veenendaal, Holland, was recrystallized from warm ethanol three times, washed with cold ethanol and dried for 24 hours at 10^{-6} Torr. This produced a fine needle-like product with no odor of acid or oxidized cholesterol. Thin layer chromatography on silica plates using a 1:1 tetralin-hexane mobile phase produced only a single spot [14]. This material is referred to in the text as the van Schuppen sample.

A sample of the palmitate ester was synthesized from cholesterol and palmitic acid, both recrystallized from ligroin. The palmitic acid was obtained from Eastman Kodak and was twice recrystallized from ligroin. The cholesterol was obtained from van Schuppen Chemicals as a specially prepared high purity material. It is not known if van Schuppen Chemicals used this identical lot in the preparation of their ester. Into a flask equipped with a Stark tube (to monitor water production) was placed 0.064 mole cholesterol, 0.0722 mole palmitic acid, 1 g

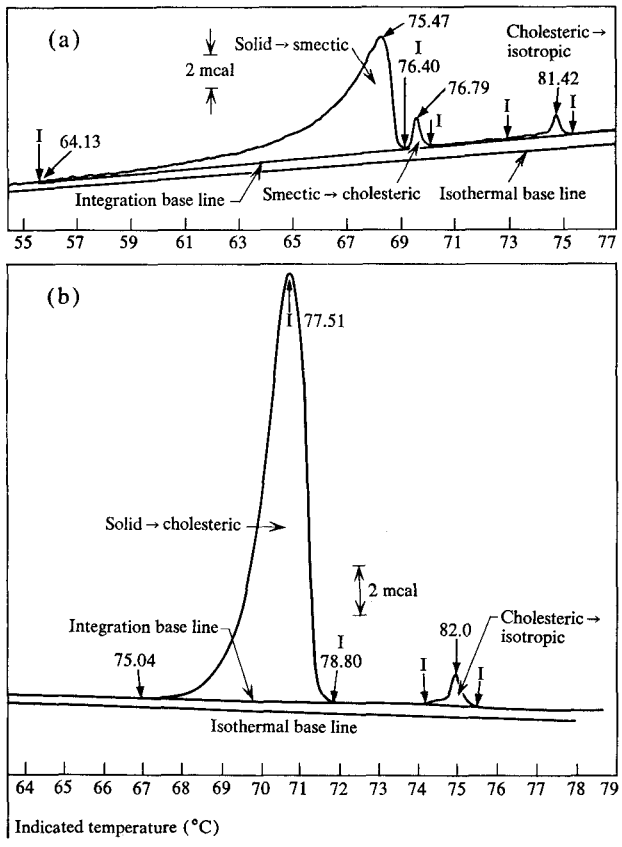

Figure 1 Differential scanning calorimeter traces of (a) van Schuppen cholesteryl palmitate (recrystallized in ethanol), 2.472-mg sample, 2.5°C/min heating rate; and (b) synthetic cholesteryl palmitate, 2.501-mg sample, 2.5°C/min heating rate. Integration limits are denoted by I. Chart temperatures are corrected for slope and error.

p-toluene sulfonic acid and 150 ml ligroin. The mixture was refluxed for 90 minutes until 95.5 percent of the cholesterol had reacted as indicated by the water in the Stark tube. The reaction mixture was transferred to a separatory funnel and washed successively with five 10-ml portions of hot 20% ethanol-water; five 10-ml portions of hot 26% ethanol-water, 2% sodium carbonate; and additional ethanol-water until a blue litmus reaction was obtained. The ligroin phase was dried over anhydrous sodium sulfate. The dry ligroin phase was reduced to 50 ml and allowed to stand at room temperature overnight. This produced a fine suspension which was chilled, filtered, washed with cold ligroin, recrystallized twice from acetone and vacuum sublimed at 10^{-6} Torr. The product was put through the same recrystallizations as the van Schuppen sample to insure as nearly identical products as possible. This material is referred to in the text as the synthetic sample.

Time-averaged NMR spectra of both the van Schuppen and the synthetic esters were identical. This technique is capable of detecting isolated proton resonances present to a minimum extent of 0.05 percent under favorable conditions and should be applicable for noncholesterol-based impurities.

• *Scanning calorimetry*

The technique used for thermal and differential scanning calorimetry (DSC) purity measurements has been described elsewhere [15–17]. The scanning calorimeter traces were quite different for the two samples; see Fig. 1. The van Schuppen sample had an indicated purity of 97.31 mole % and exhibited three transitions on heating (solid → smectic, smectic → cholesteric and cholesteric → isotropic liquid). The synthesized sample had an indicated purity of 99.52 mole % and exhibited in the DSC only two transitions on heating (solid → cholesteric and cholesteric → isotropic liquid). Both samples showed three transitions on cooling. The data are given in Table 1.

• *Microscopy*

The samples were observed and photographed with a Ziess Ultraphot II microscope between crossed polarizers. The samples were heated and cooled on a Mettler FP-2 stage equipped for both heating and cooling. The amount of light rotated (depolarized light intensity, or DLI) was measured on the second melting of the sample by a simple photometer similar to that described previously [18–20].

Results

• *Microscopy and DLI*

The unusual behavior of the van Schuppen and synthesized cholesteryl palmitate esters was first noted on routine microscopic examination of the samples. This examination showed that a well-defined smectic structure was formed on heating the van Schuppen sample; see Figs. 2(a), (b) and (c). In addition, a transient smectic form appeared in the synthetic sample on heating. A smectic front passed across the sample as the solid converted to the cholesteric mesophase. This is illustrated in Fig. 2(d).

The smectic mesophase in the van Schuppen sample is shown in the DLI curve, Fig. 3(a). The smectic form appears as a decrease in the depolarized light intensity from 70.5 to 74.3°C. Above 74.3°C the DLI increases to a level slightly higher than that of the initial sample. At 76.3°C the smectic mesophase streams and rapidly converts to the cholesteric texture. At 76.7°C the cholesteric texture begins to "open" with dark areas (substrate-induced fast vibration direction aligned with the polarizer) growing larger. At 80.9°C the cholesteric texture begins to stream rapidly and flash [see spike at 80.9°C in Fig. 3(a)]. At 80.95° the cholesteric texture transforms to the isotropic liquid and the DLI drops to instrument zero.

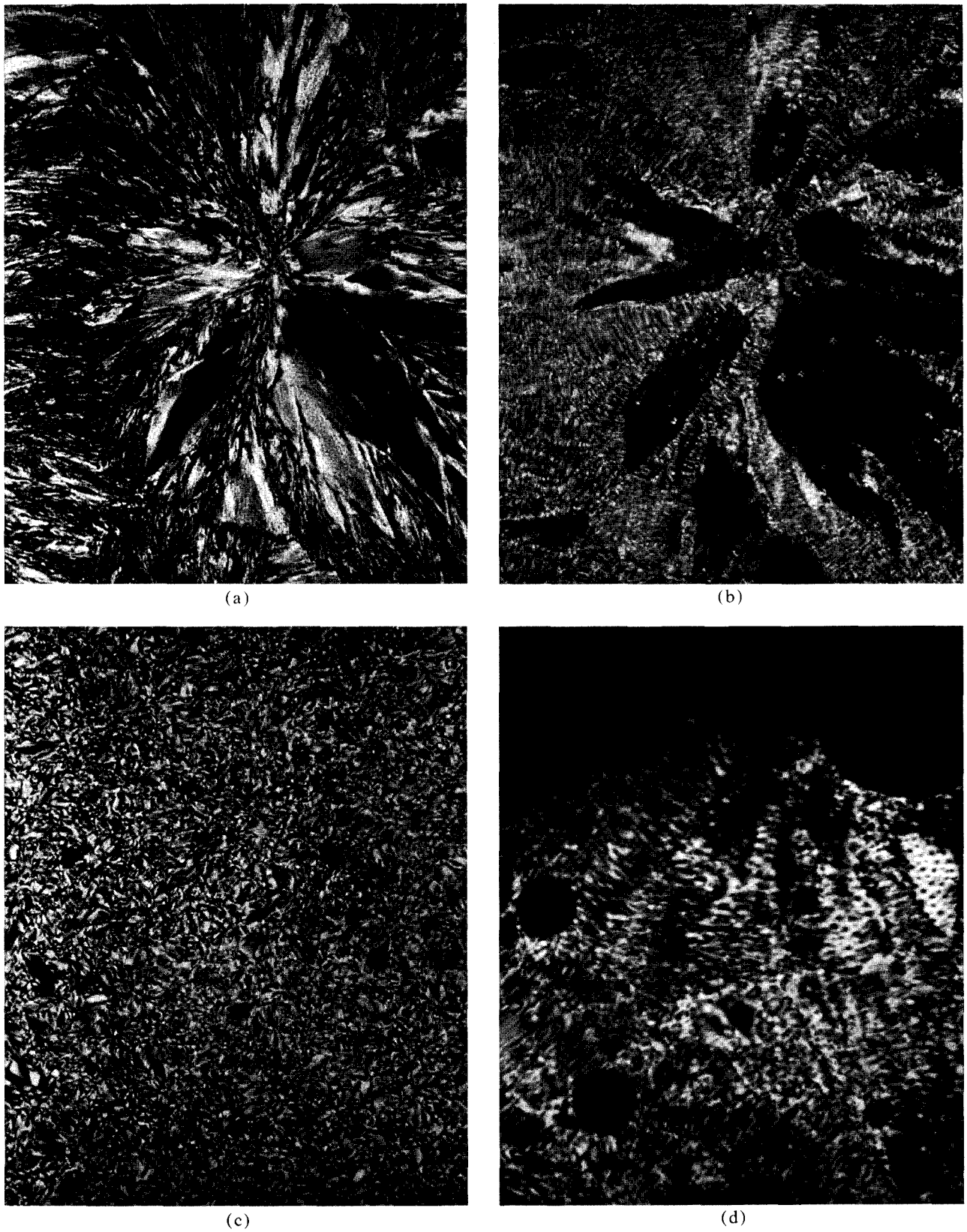

Figure 2 Photomicrographs (90×) of cholesteryl palmitate using crossed polarizers, 1-mm-thick samples between coverslip and slide. Van Schuppen sample: (a) solid phase at 50°C; (b) smectic mesophase forming at 75.8°C on heating; and (c) fully perfected and thermally stable smectic mesophase formed on heating at 76.6°C. Synthetic sample: (d) Smectic front (middle area) shown passing through the solid phase (bottom) as the cholesteric mesophase (top) is formed at 78.0°C.

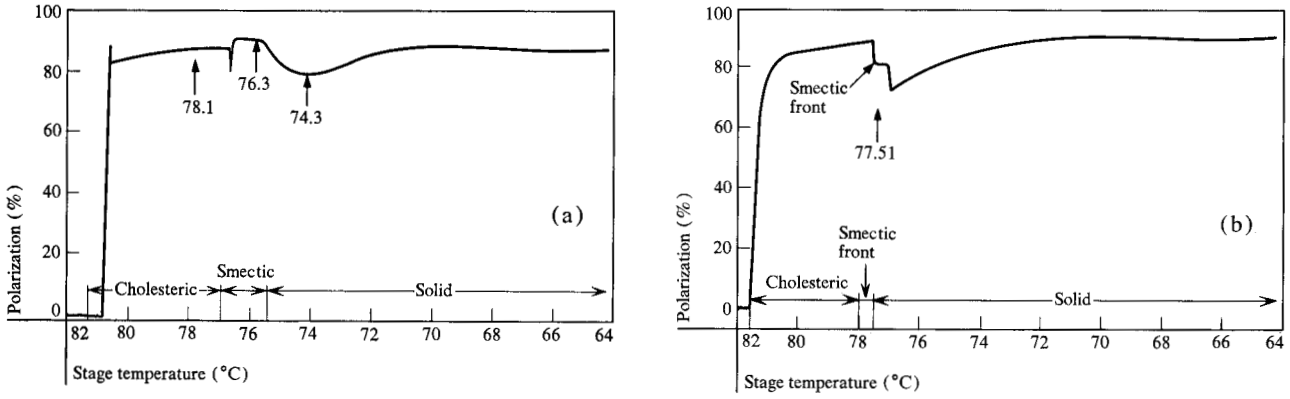

Figure 3 Depolarized-light transmission intensity curves for (a) van Schuppen and (b) synthetic cholesteryl palmitate, 1-mm-thick samples, 2°C/min heating rates.

The DLI curve of the synthetic sample, Fig. 3(b), shows a step in the DLI curve between the solid and the cholesteric forms. During this step the transient smectic phase appears to the observer. No alteration was noted on either the van Schuppen or the synthetic sample on repeated reheating.

• *Scanning calorimetry*

The DSC curve on the synthetic sample, shown in Fig. 1(b), resembles work reported previously, as illustrated by the data in Table 1, on the number of transitions and the transition temperature on heating. The solid → cholesteric mesophase transition agrees well with the observations by Sell [10], Ennulat [2], and Davis [3]. The cholesteric → isotropic-liquid transition temperature of the synthetic sample is above those reported by Sell and Neumann [10] and Porter, Barrall and Johnson [6], but agrees well with those of Davis and Porter [3], Ennulat [2] and Gray [9]. The palmitate sample originally studied by Barrall, Porter and Johnson [6] was probably about 96 percent pure, which is low by present standards. The cooling thermogram of the synthetic sample agrees in number and temperatures of transitions with previous work. It is also interesting to note that there is generally excellent agreement among all previous workers who measured transition entropy—even mesophase entropies. This is particularly noteworthy in view of the small size of these transitions and the well-recognized effects of certain impurities on transition range and temperature [2, 7, 8].

The van Schuppen sample reproducibly gives a unique heating thermogram, Fig. 1(a). The smectic phase appears without ambiguity, thermally well-separated from the large thermal event. Separate portions of impure sample were recrystallized from ethanol, acetone and hexane and gave identical results. The smectic → cholesteric transition temperature and entropy compare closely to the synthetic sample and the data reported by Davis and Porter [3]. In addition, the transition occurs at a lower temperature than those observed by Gray [9] and by Ennulat [2]. The cholesteric → isotropic-liquid transition temperature is in agreement with that observed by Davis and Porter [3]. The purity of the sample calculated from the solid → smectic transition was found to be 97.31 mole %. Similar calculations on the smectic → cholesteric and cholesteric → isotropic-liquid transitions gave purities of 99.53 mole % and 99.99 mole %, respectively. This unusual variation suggests that an impurity is present that is insoluble in the solid phase (excluded from the lattice), but is about equally soluble in the smectic, cholesteric and isotropic-liquid phases. In a previous study of cholesterol heptadecanoate [8], the impurity present in that sample (the unreacted acid) was found to be soluble only in the isotropic liquid. Ennulat [4] has obtained similar data on cholesteryl nonanoate.

Conclusions

A sample of cholesteryl palmitate prepared in our laboratory from carefully purified cholesterol and palmitic acid compares closely in all ways with material described by at least three other groups [2, 3, 9]. However, a sample of the palmitate ester obtained from van Schuppen Chemicals was somewhat different. That material shows a clearly defined, sharp smectic mesophase on heating, as well as the previously reported cholesteric mesophase. Differential scanning calorimetry indicates that the solid-to-smectic transition is relatively broad and characteristic of material with purity no higher than 97.31 mole %. However, the mesophase transitions are very sharp and characteristic of material of at least 99 mole % purity. In addition, thin layer chromatographic techniques, which have been demonstrated to be sufficiently sensitive and efficient to separate as little as 0.05 percent unreacted acid, alcohol and cholesterol, do not find a trace of impurity. Contamination of the sample with other esters is also ruled

out by the same method. A previously completed study of adsorbed solvent phenomena indicates that the van Schuppen sample is solvent free [7]. An earlier study of cholesteryl heptadecanoate has shown that as little as 0.1 percent unreacted starting material or solvent greatly broadens and shifts the mesophase transitions to lower temperatures by several degrees [7, 8, 12]. Such is not the case with the van Schuppen sample. For these reasons, it is necessary to postulate an impurity that is insoluble in the solid phase but is equally soluble in the smectic, cholesteric and isotropic-liquid phases. Since it is the entropy of mixing that results in peak broadening and transition-point depression, this entropy would be absent in true solutions. The effects of impurity solubility have been described in detail previously [7, 12]. The identity of this postulated impurity, about two percent of the sample, remains in doubt as all efforts to separate it have been without success. It is possible to speculate on the basis of known contaminants, but this would be guesswork in consideration of the large number of possible contaminants.

We know that the impurity must have an R_f value under the separation conditions equal to that of cholesteryl palmitate or its presence would be revealed as a separate chromatographic spot. The impurity must have a structure almost identical to that of the palmitate ester or careful NMR would locate an unusual resonance. The NMR method is normally sensitive to as little as one percent impurity of the acid or cholesterol starting material. A readily available material which could satisfy all of the above criteria is the ester of *cis*-Δ^5-cholestene-3β-ol [1]. The *cis* isomers that have been explored simply melt. All cholesterol that is commercially available is of biological origin. As such there is a chance of some variation in isomeric content. Other isomers are possible, but an isomeric palmitate ester accounts for all of the chromatographic, purity and microscopic effects. If equal efficiency in purification is assumed, isomeric variation could also account for the small variations in results among workers reporting calorimetric data derived from differential thermal analysis and differential scanning calorimetry.

The van Schuppen sample was submitted to the following fractional solutions and recrystallizations:

1. Wash with 100 cm^3 acetone in an ultrasonic bath about 4 to 5 minutes
2. Vacuum dry
3. Wash with 100 cm^3 ethanol in an ultrasonic bath about 4 to 5 minutes
4. Vacuum dry
5. Dissolve in 100 cm^3 acetone/ethanol (50/50) solution by boiling on hot plate; cap and bring to room temperature under fume hood
6. Vacuum dry
7. Dissolve in 100 cm^3 acetone/ethanol (50/50) solution by boiling on hot plate; cap and put in refrigerator to precipitate crystals rapidly.

This treatment produced a material in every way *identical* with the synthetic sample. The success of partial solution of the crystal *surface* in the ultrasonic bath indicates that the impurities are sorbed on the crystal after growth is complete, i.e., during the digestion period. Partial solution probably takes advantage of the change in solubility with temperature between the desired ester and the impurity. Vacuum sublimation was not successful in removing the impurity.

Therefore it appears that by introduction of the proper impurity it is possible to move the solid-mesophase transition to lower temperature. This shift reveals the presence of a normally monotropic mesophase on heating. Similar work with impurities and two-component systems has been reported in detail by Dave et al. [13, 21, 22] for nematic-mesophase-forming materials. The van Schuppen palmitate ester behavior can best be explained by a selective impurity rather than by separate crystal forms of the solid.

Indeed, monotropic mesophases would not appear on cooling were it not for the tendency of the transition to the solid to supercool. Earlier work has shown that the monotropic propionate ester does not form a mesophase at all on cooling if the cooling rate is less than 0.5°/minute. The solid nucleates at a temperature below the melting point but above the cholesteric mesophase formation temperature [23].

This study has implicated the role of a very selective impurity in depressing the transition temperature of a single phase. The mesophases, normally regarded as very sensitive to impurities, were left relatively unaffected. The demonstrated possibility of such an impurity further complicates the solid-phase properties of the cholesteryl-ester-mesophase-forming materials.

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