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THE EFFECT OF PURITY ON THE THERMODYNAMIC PROPERTIES OF CHOLESTERYL HEPTADECANOATE*

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ABSTRACT

The thermodynamic properties of two samples of cholesteryl heptadecanoate have been measured and contrasted. As little as 2% impurity (presumably oxidized cholesterol and free acid) profoundly effects all of the thermodynamic properties. The mesophases are the most sensitive to alteration and distortion by impurities. A transition diagram has been developed for this ester.

INTRODUCTION

The effect of purity on smectic and cholesteric mesophase transitions has not been studied in quantitative detail in the past. Gray[1] reported differences in transition temperatures between materials he had synthesized and materials previously reported by Jaeger[2], Friedel[3], and Lehmann[4]. It was presumed that these differences were due to purity variations. However, no attempt was made to quantitate the purity of the compounds in question or to establish a relationship between purity and transition heats and temperatures. Ennulat[5] has shown using the van't Hoff equation to determine purity, that transition temperature depression in the mesophase is an order of magnitude less sensitive to impurities than the solid → mesophase transition. It was suggested that this is due to the lower heat of transition of the mesophase as opposed to the solid, which cannot be correct.

If the following case is considered, the flaw becomes obvious. The van't Hoff equation is as follows:

$$X = \frac{\Delta H}{RT_m^2} \Delta T$$

where X = mole fraction of impurity
R = gas constant in kcal/mole/°K
T_m = melting point of 100% pure material in °K
ΔT = melting point depression

It is obvious that ΔT is inversely proportional to ΔH, and as ΔH becomes smaller ΔT must become larger. In a given sample, the mole fraction of impurity must be constant,

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*Part XXII of a Series on "Order and flow of liquid crystals".

barring vaporization on heating. Therefore, with a ΔH smaller by a factor of ten in the mesophase transitions as opposed to the solid → mesophase transition, the ΔT should be ten times larger. Although the reverse case was observed by Ennulat[5] the data are subject to several interpretations.

If the van't Hoff equation does apply to the mesophases as well as the solid transition, solution conditions must be very complicated. Fundamentally, the van't Hoff equation describes the entropy of mixing of a material which is insoluble in one phase (solid) but soluble in another phase (mesophase or isotropic liquid). The question arises as to how a material can be "soluble" in the mesophase to give a characteristic melt broadening to the solid → mesophase transition and then "soluble" in the isotropic liquid to give a second characteristic broadening to the mesophase → isotropic liquid transition. The situation becomes doubly complicated when more than one mesophase exists. Indeed, previous gas chromatographic studies have indicated selective solubilities (heats of mixing) for various materials in different mesophases[6-8].

The purpose of this study was to examine in detail the mesomorphic and solid transitions of a liquid crystal-forming material, cholesteryl heptadecanoate, as a function of compound purity and use the data to examine the above contentions.

EXPERIMENTAL

A sample of cholesteryl heptadecanoate was obtained from Applied Science Laboratory, P.O. Box 440, State College, Pa., U.S.A. The material as received had a rancid odor. Thin layer chromatography showed a single small spot other than the ester. (This same result has been obtained with alcohol recrystallized product which had been allowed to stand three months under atmospheric conditions). A portion of the Applied Science cholesterol heptadecanoate was recrystallized once from 90% ethanol, dried for 48 h under vacuum at 50°C and vacuum sublimed from the mesophase. This treatment gave a yield of approximately 90% of recrystallized ester.

A Perkin–Elmer DSC-1B scanning calorimeter was used for the calorimetric study. Samples were examined at a heating rate of 5°/min at sensitivities of 4 and 1 mcal/sec. The average sample size was 2–3 mg. Purity analysis was carried out on 6-mg samples heated at 2.5°/min. The temperature axis was calibrated with the melting points of benzene, heptane, benzoic acid, naphthalene and indium. All temperatures used for purity calculation as well as those quoted in the tables were corrected for thermal resistance of the sample pan holder using the slope of indium[8a] (99.999%). The areas of various curves were converted to calories using the heat of fusion of 99.99% naphthalene. The value 4536 cal/mole was accepted as the heat of fusion[9]. All samples and standards were encapsulated under dry nitrogen in volatile sample holders. Weighings were done on a Cahn two-pan electrobalance using an empty volatile sample pan and lid as tare. Areas were integrated with a polar planimeter. All calculations including the purity analysis were carried out with a computer program developed at IBM. The DSC purity analysis method was essentially the same as that described by Gray[10] and used by Ennulat[5] and Plato and Glasgow[11]. Although there are objections to the method, the technique appears satisfactory for relative purity determination in most cases.

The samples were heated from solid to mesophase to isotropic liquid at 5°/min and 4-mcal/sec sensitivity. The isotropic liquid was then cooled at 5°/min at 1-mcal/sec sensitivity until both mesophases had formed — but the solid phase had not formed. The mesophases were reheated at 5°/min and 1-mcal/sec sensitivity back to the isotropic liquid. This program was repeated twice. The sample was then cooled at 5°/min to room temperature where the solid phase reformed. The solid was melted a second time as above and the mesophase treatment repeated. Three separate samples of each material purity were run. The reason for this rather elaborate treatment was to obtain heating as well as cooling data on monotropic mesophases.

RESULTS

The cholesteryl heptadecanoate sample obtained from Applied Science laboratory had a purity of 97.94% from an analysis of the solid → mesophase (cholesteric) transition peak shape. The data on three separate samples were: 97.90%, 97.98% and 97.94%. An area correction of 4% was required for the linearization of the melting temperature *vs.* reciprocal fraction melted plot. The extrapolated melting point of 100% pure material was 77.95°C. The thermal data are given in Table I. Typical DSC heating scans are shown in Fig. 1 for the solid → mesophase → isotropic

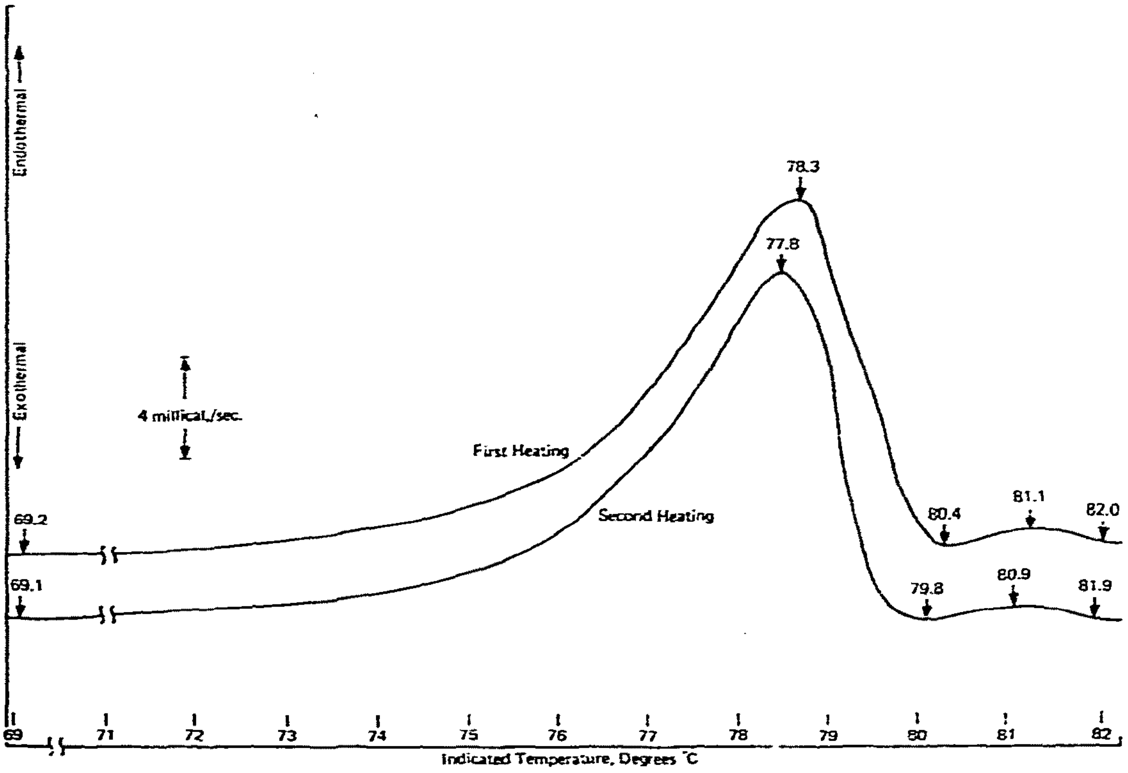


Fig. 1. Heating of 97.94% cholesteryl heptadecanoate.

liquid transitions. Detailed thermograms of the mesophase heating and cooling curves are given in Figs. 3 and 4.

TABLE I

THERMAL DATA FOR CHOLESTERYL HEPTADECANOATE 97.94 MOLE % BY DSC

T_b	T_m	T_e	ΔH (cal/g)	ΔH (kcal/m)	ΔS (cal/m/°K)	Transition
First heating solid						
69.2	78.3	80.4	20.0	12.8	36.4	Solid → cholesteric
80.4	81.1	82.0	0.283	0.169	0.477	Cholesteric → isotropic liquid
First cooling						
81.0	79.3	77.4	0.457	0.292	0.829	Isotropic liquid → cholesteric
74.6	73.7	—	0.558	0.356	1.027	Cholesteric → smectic
—	72.6	71.5				
First heating smectic mesophase						
72.6	74.9	—	0.665	0.425	1.22	Smectic → cholesteric
—	75.7	76.8				
79.2	80.6	—	0.449	0.287	0.812	Cholesteric → isotropic liquid
—	81.2	82.0				
Second cooling						
79.9	79.1	77.5	0.463	0.296	0.841	Isotropic liquid → cholesteric
74.6	73.7	—	0.491	0.314	0.906	Cholesteric → smectic
—	72.6	71.5				
Second heating solid						
69.1	77.8	79.8	17.3	11.0	31.4	Solid → cholesteric
79.8	80.9	81.9	0.401	0.256	0.723	Cholesteric → isotropic liquid
Second hecting smectic mesophase						
72.5	74.4	—	0.633	0.404	1.16	Smectic → cholesteric
—	75.7	—				
—	77.3	78.3				
78.3	80.4	—	0.513	0.328	0.928	Cholesteric → isotropic liquid
—	81.0	82.1				

The recrystallized heptadecanoate ester showed a calculated purity of 99.70% from an analysis of the solid → cholesteric endotherm obtained on second heating. In this case as in the previous sample, the results on three separate samples are very close together. The extrapolated temperature of fusion for the 100% pure material was found to be 77.98 °C, in good agreement with the 97.94% material. The thermal data for the 99.70% ester is shown in Table II. Typical thermograms for the solid → mesophase → isotropic liquid heating are shown in Fig. 2. Detailed thermograms of the mesophase transitions are shown in Figs. 3 and 4.

DISCUSSION

The more pure heptadecanoate ester exhibited solid phase polymorphy in material which had been freshly sublimed. This was evident on microscopic examination between crossed polarizers and from the thermogram in Fig. 2.

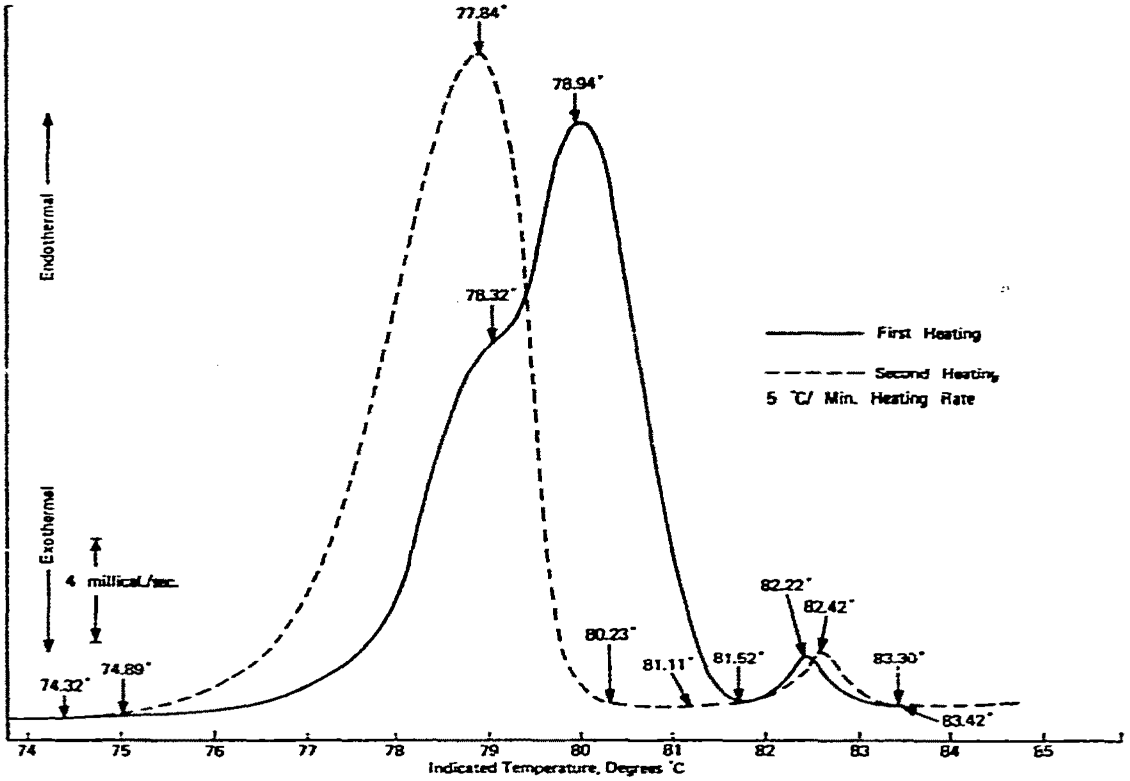


Fig. 2. Differential scanning calorimetric heating curves of 99.70% cholesteryl heptadecanoate.

Two overlapping endotherms are evident at 78.32 and 78.94°C in the first heating. The higher temperature form is not present on reheating the same sample recrystallized from the melt. The heat of fusion of the low temperature solid, 14.10 kcal/mole, is 0.75 kcal/mole lower than the mixed crystal form seen on first heating. This is a significant amount of lowering, especially when viewed as an entropy change (see Table II). The low temperature solid is 2 cal/mole/°K less ordered than the high temperature form. This is the correct order of magnitude for a rotational disorder which could be present in the lower melting solid. On simple recrystallization of the sample (dissolution of the solid in ethanol followed by *total evaporation* of the solvent) the high temperature form reappears in the first heating curve.

The less pure sample of the ester, 97.94%, exhibited a single low-temperature endotherm on both first and second heating for the conversion of the solid to the mesophase. However, the high temperature side of the endotherm on first heating has a slightly distorted shape. This may be due to the melting of a small amount of high-temperature solid. The most prominent effect of the impurity is a large reduction in the heat and the entropy of fusion. This reduction is unusually large, far greater than the presence of 2.06% of impurity would indicate. The second melting endotherm of the 99.70% esters is 21.7% larger with respect to transition heat than the 97.94% ester. The same holds true of the entropy of the transition. However, even with these relatively large thermodynamic effects the vertices of the solid → mesophases second

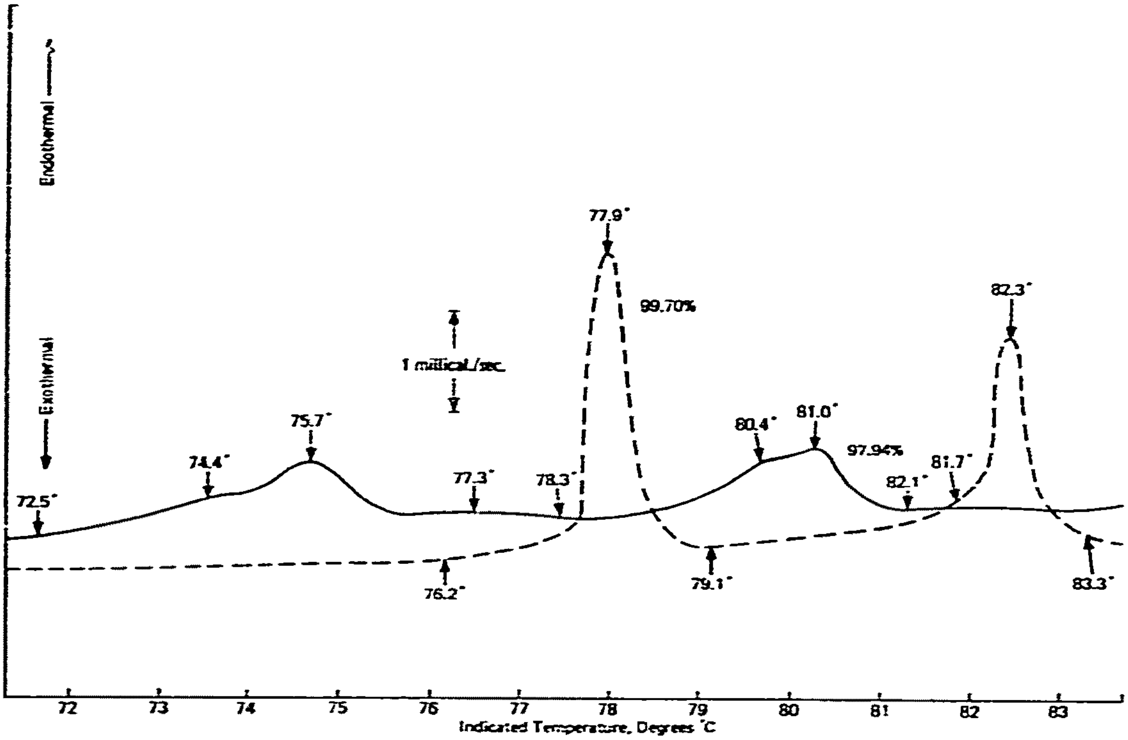


Fig. 3. Differential scanning calorimetric curves of mesophase heating.

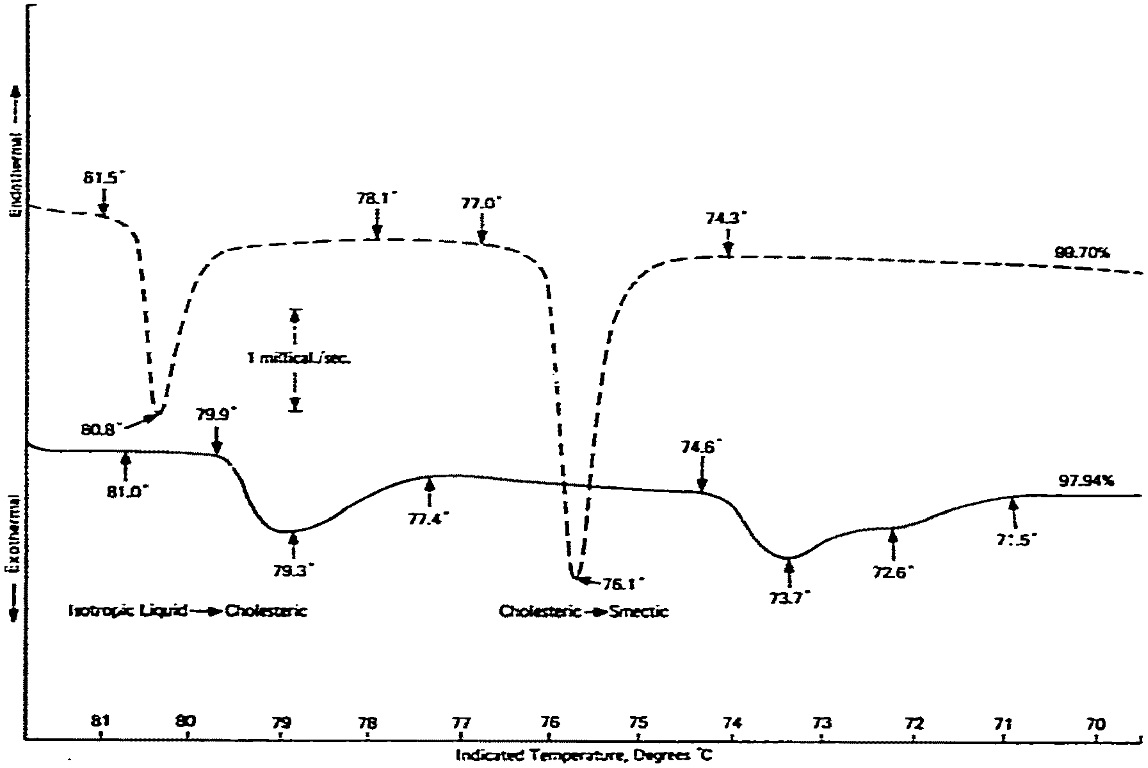


Fig. 4. Differential scanning calorimetric curves of mesophase cooling.

TABLE II

THERMAL DATA FOR CHOLESTERYL HEPTADECANOATE 99.70 MOLE % BY DSC

T_b	T_m	T_e	ΔH *(cal/g)*	ΔH *(kcal/m)*	ΔS *(cal/m/°K)*	*Transition*
First heating solid						
74.9	78.3	—	23.24	14.85	42.2	Solid → cholesteric
—	78.9	81.5				
81.5	82.2	83.3	0.576	0.368	1.04	Cholesteric → isotropic liquid
First cooling						
78.1	80.8	81.5	0.574	0.368	1.04	Isotropic liquid → cholesteric
74.3	76.9	77.0	0.780	0.498	1.42	Cholesteric → smectic
Heating smectic mesophase						
76.2	77.9	79.1	0.758	0.484	1.39	Smectic → cholesteric
79.1	81.7	—	0.680	0.435	1.19	Cholesteric → isotropic liquid
—	82.3	83.3				
Second cooling						
77.6	80.7	80.9	0.529	0.338	0.969	Isotropic liquid → cholesteric
74.9	76.1	77.6	0.728	0.465	1.33	Cholesteric → smectic
Second heating solid						
74.3	77.8	80.2	22.09	14.10 (14.38)	40.2	Solid → cholesteric
81.1	82.4	83.4	0.628	0.401	1.13	Cholesteric → isotropic liquid
Heating smectic mesophase						
76.8	77.7	78.9	0.701	0.448	1.28	Smectic → cholesteric
79.7	80.4	—	0.63	0.403	1.13	Cholesteric → isotropic liquid
—	81.8	—				
—	82.2	83.2				

heating endotherms for the two samples are identical within the limits of DSC sensitivity. On the basis of a simple visual or microscopic melting point these two obviously different samples appear to be identical. This serves to reinforce the idea that thermodynamic data are required for an adequate understanding of mesophase transitions.

The second transition which occurs on heating the heptadecanoate ester is the cholesteric → isotropic liquid transition. On first heating, the 99.70% sample obscures a portion of this transition with the tail of the endotherm due to the high temperature form. In Table II the data given for this transition are probably low for first heating due to this overlap. The two heats of transition for the first and second melt are 0.368 and 0.401 kcal/mole. The value of 0.401 kcal/mole is probably more correct. Irrespective of which value is ultimately correct, the 97.94% sample exhibits a much smaller, ~54%, transition heat for the same cholesteric → isotropic liquid transition. In addition, the location of the vertices of the endotherms are measurably different, *i.e.*, 1.1 °C for the first heating and 1.5 °C for the second heating. The endotherm for the 97.94% sample being lower in all cases as would be expected.

Therefore, this transition is almost twice as sensitive on a transition-heat basis and over ten times as sensitive on a transition-temperature basis to impurities as the solid → cholesteric transition. Although this supports the assumptions made in using the van't Hoff equation (the smaller the transition heat, the greater the effect of impurities) some interesting questions concerning solubility of impurities remain. On cooling, the isotropic liquid cholesteryl heptadecanoate exhibits two mesophase transitions, isotropic liquid → cholesteric and cholesteric → smectic. The smectic phase is monotropic since the formation of the solid phase must be postponed by supercooling for the smectic phase to form.

Due to the supercooling of the solid phase it is possible to study the mesophases in both heating and cooling modes. Since the microscopic textures of the mesophases are different depending on the mode of formation, it is reasonable that small differences in transition heats should exist. An examination of the data for the 99.70% material in Table II indicates that the cholesteric → isotropic liquid transition is 12.6% larger than the cooling curve isotropic liquid → cholesteric transition for the 99.70% ester. The smectic → cholesteric transition is only 3.11% smaller than the cholesteric → smectic transition. This 3.11% difference is *not* significant. From these data the dissolution of the open net-like cholesteric mesophase formed from either the smectic or solid phase requires ~12.6% more energy than the formation of the moss-like cholesteric mesophase from the isotropic liquid. The smectic mesophase transition to or from the cholesteric appears to be independent of the direction of the phase change.

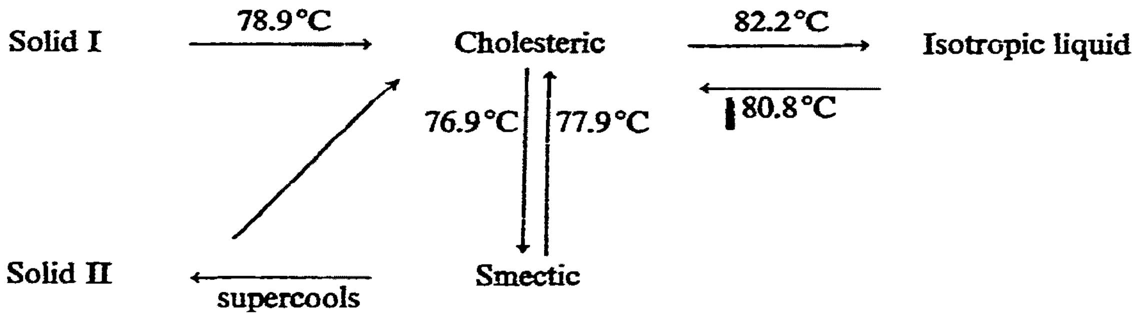
The mesophase transitions exhibited by the 97.94% ester under the same conditions are somewhat different from the transitions in the 99.70% ester. The transitions in the less pure material are characterized by the appearance of a shoulder on each endotherm or exotherm, Figs. 3 and 4, a depression of the transition point and a reduction in the total heat of transition.

The smectic mesophase transition appears to be less sensitive to the impurity with respect to heat of transition than the cholesteric transition. However, transition-temperature depression and additional transition features are comparable between the smectic and cholesteric transitions.

The additional shoulders on the mesophase transitions in the less pure ester suggest that the impurity is inducing several stable molecular arrangements in the mesophases not found in pure material. These new "phases" may be due to alterations in the pitch of the cholesteric helix and variable layer separation in the smectic mesophase (see Ref. 12 for illustrations of these phases). These alterations would create disorder in the mesophases which would result in depressed transition temperature, reduced entropy of transition, and reduced heat of transition.

CONCLUSIONS

From the more pure sample of cholesteryl heptadecanoate examined in this study, the ester has the following transition diagram:



Impurities have a great effect on the temperature, heat and phase distribution of the mesophases. The transition temperature depression is in agreement with the predictions of the van't Hoff equation. It was found that the solid phase of the ester exhibits at least two forms depending on impurity concentration and mode of formation (from ethanolic solution, vacuum sublimation or the smectic mesophase). The temperature of the solid → cholesteric mesophase transition is relatively insensitive to impurity concentration. However, the heat of transition is reduced very sharply by a small amount of impurity. For convenience, the thermodynamic data for the two samples examined on second heating in this study are summarized in Table III.

TABLE III

EFFECT OF PURITY ON CHOLESTERYL HEPTADECANOATE TRANSITIONS

Transition Type	*DSC Purity*									
	99.70 *mole %*					97.94 *mole %*				
	T_b	T_m	T_e	ΔH (*kcal/m*)	ΔS (*cal/m/°K*)	T_b	T_m	T_e	ΔH	ΔS
Solid → cholesteric	74.3	77.8	80.2	14.1	40.2	69.1	77.8	79.8	11.0	31.4
Cholesteric → isotropic liquid	81.1	82.4	83.4	0.401	1.13	79.8	80.9	81.9	0.256	0.723
Cholesteric → isotropic liquid (heating SM)	79.1 —	81.8 82.2	— 83.2	0.403	1.13	78.3 —	80.4 81.0	— 82.1	0.328	0.928
Smectic → cholesteric (heating SM)	76.2	77.7	78.9	0.448	1.28	72.5 —	74.4 75.7	— 78.3	0.404	1.16
Isotropic liquid → cholesteric (cooling IL)	78.1	80.8	80.9	0.368	1.04	81.0	79.3	77.4	0.294	0.829
Cholesteric → smectic (cooling IL)	74.3	76.9	77.0	0.498	1.42	74.6 —	73.7 72.6	— 71.5	0.356	1.027

This study has demonstrated that extreme care must be exercised in all phases of sample purification if accurate and meaningful thermodynamic data are to be obtained on these very sensitive cholesteryl esters.

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