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The Polymorphism of Cholesteryl Esters: Differential Thermal and Microscopic Measurements on Cholesteryl Myristate†

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Abstract—The techniques of differential thermal analysis and photomicroscopy have been combined to eludicate the complex phase relationships and crystallography of cholesteryl myristate. Also, the amount of polarized light rotated by the sample as a function of temperature has been measured at a fixed sample thickness. Cholesteryl myristate exhibits a complex liquid crystal mesophase system on heating. In addition to the phase transformations obtainable on heating, photomicrographs were obtained of the phases which result on subjecting the smectic and cholesteric mesophases to a very low shear. Excellent correlation was obtained between the present work and earlier studies of the myristate ester. The combined instrumental and microscopic method appears to be a valuable tool in the study of subtle phase changes in complex systems.

The study of cholesteryl ester polymorphism has been complicated by the nature of the multiple-phase transformations. In many cases, the liquid crystal phases formed on cooling from the melt are different from those formed on heating from the solid phase.[1,4,11] For example, cholesteryl stearate shows only a single transition, from solid to isotropic liquid, on heating from the solid phase.[1,7] On cooling the melt, the cholesteric and smectic mesophases each form readily in their respective temperature ranges. In addition to this, the solid phase formed from the melt can be different from the solution-grown solid phase.[1] Crystal structure can even vary

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with solvent and temperature of crystallization. These considerations make particularly difficult any intercomparison of data between different esters and between different methods of analysis.

Cholesteryl myristate is one of the few compounds in the ester series of straight chain, saturated acids which does not exhibit irreversible phase behavior.[1,7]

$$\text{Solid} \xleftrightarrow{73.6^\circ\text{C}} \begin{array}{c}\text{Smectic}\\ \text{Mesophase}\end{array} \xleftrightarrow{79.9^\circ\text{C}} \begin{array}{c}\text{Cholesteric}\\ \text{Mesophase}\end{array} \xleftrightarrow{85.5^\circ\text{C}} \begin{array}{c}\text{Isotropic}\\ \text{Liquid}\end{array}$$

The states formed on cooling or heating the melt or solution crystallized materials appear to be identical both with respect to temperatures and heats of transition.[1,3] For these reasons, this material was chosen for a comparative study by optical microscopy, differential thermal analysis (DTA), and measurement of polarized light rotated by the sample as a function of intensity and temperature (LIA). By consolidating these three techniques for a relatively reproducible system, it is possible to gain an insight into the interesting optical and related properties of mesophases formed by cholesteryl esters. The method of purifying the cholesteryl myristate was described previously.[1]

In addition, photomicrographs have also been obtained which show the effects of shear on the smectic and cholesteric phases of cholesteryl myristate. The smectic state at low shear is important from a biomedical viewpoint, since this is a common state for cholesteryl esters in living systems. Several publications have pointed out that elevated serum cholesterol concentrated by dietary saturated fats is due mainly to their myristic acid content.[9]

Experimental

Differential Thermal Analysis

A Du Pont differential thermal analyzer equipped with the micromelting point block was used to obtain the differential thermogram shown in Fig. 1. A 3-mg sample of cholesteryl myristate was heated at 5°/min. from 20°C to 110°C in a nitrogen atmosphere. Glass beads were used as reference.

Polarized Light Rotation Intensity and Microscopy

A Zeiss Ultraphot II photomicroscope equipped with polarizer and analyzer nicole prisms and strain-free polarizing optics was used to obtain all photomicrographs. The microscope was modified for the study of rotated polarized light intensity as a function of temperature by introducing a cadmium telluride photoconductive cell at the movie camera focus. The resistance changes of the photoconductive cell were measured and amplified in the conventional manner and recorded on the y axis of a 1-mV per inch Moseley x-y recorder. The photometer was set so that a 7-mV signal at the recorder corresponded to complete transmission through the nicole prisms oriented in a parallel fashion. The recorder zero was arranged to correspond to the light throughput when the prisms were crossed. The cholesteryl ester was heated on a modified Zeiss

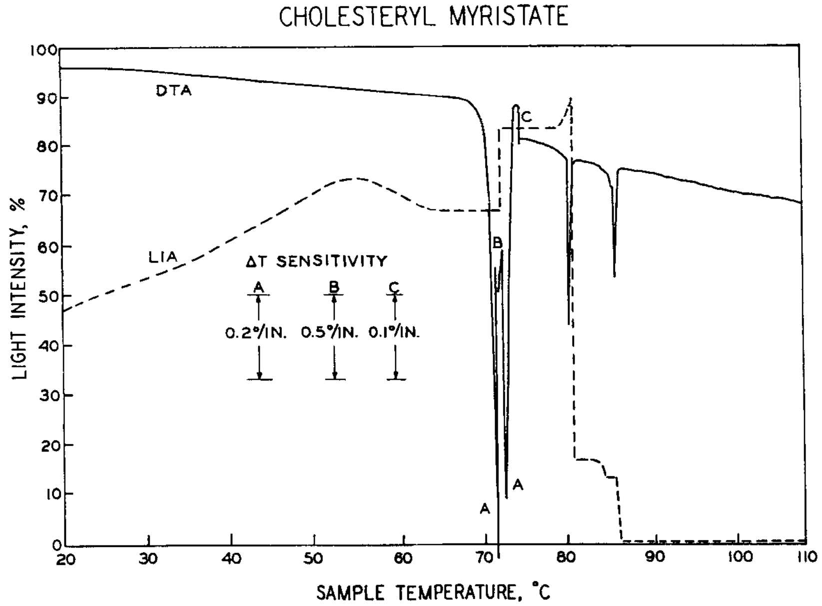

Figure 1. Polarized Light Intensity (LIA) and Differential Thermal Analysis (DTA) Curves of Cholesteryl Myristate. 3-mg sample heated at 5° per min.

hot stage. The modification consisted of changing the thermocouple system to copper-constantan and repositioning the thermocouple for more accurate sample temperature measurement. This thermocouple was located as close as possible beneath the sample cover slip. A second temperature program thermocouple was placed in contact with the ceramic heater frame of the hot stage. The stage was programmed at 5°C per min. with a temperature controller. Temperature was controlled to ± 0·02°C. Ice reference junctions were used on both the sample and program thermocouples. The output of the sample-ice junction thermocouple pair was recorded on the 0.5-mV per inch x axis of the Moseley x-y recorder. The LIA curve shown in Fig. 1 was obtained in this way.

The sample was mounted in a 0.2-mm thick layer between two thin glass cover slips separated by a 0.2-mm spacer. Heating and cooling of the sample on the hot stage was carried out under a stream of flowing dry nitrogen. The ester was premelted and cooled to the solid phase at 2°C per min. The final temperature of the solid phase prior to LIA and photomicroscopy was 10°C. This treatment effected good contact between sample and glass and produced a uniform solid phase. The low shear experiments were carried out by sliding the top cover slip gently over the bottom cover slip *in situ* on the stage. The shear sample was the same as that used in the photomicrographs and the LIA curve.

All experiments were carried out in quadruplicate on fresh samples. The photomicrographs given in this paper are one set of the four which are representative of the group. All temperatures are averages of the four experiments. An average deviation of 0.05°C was determined.

Results

Photomicrographs: The solid phase of cholesteryl myristate at 20°C consists of highly birefringent platelets of irregular shape exhibiting a distinctly wrinkled appearance, see Fig. 2. The birefringence and wrinkling of the platelets are characteristic of the solid phase. In this particular preparation, the predominant birefringent color was a green-blue with bright yellow lines in between the deeper wrinkles. The irregular dark zones in Fig. 2 were due to voids in the solid preparation.

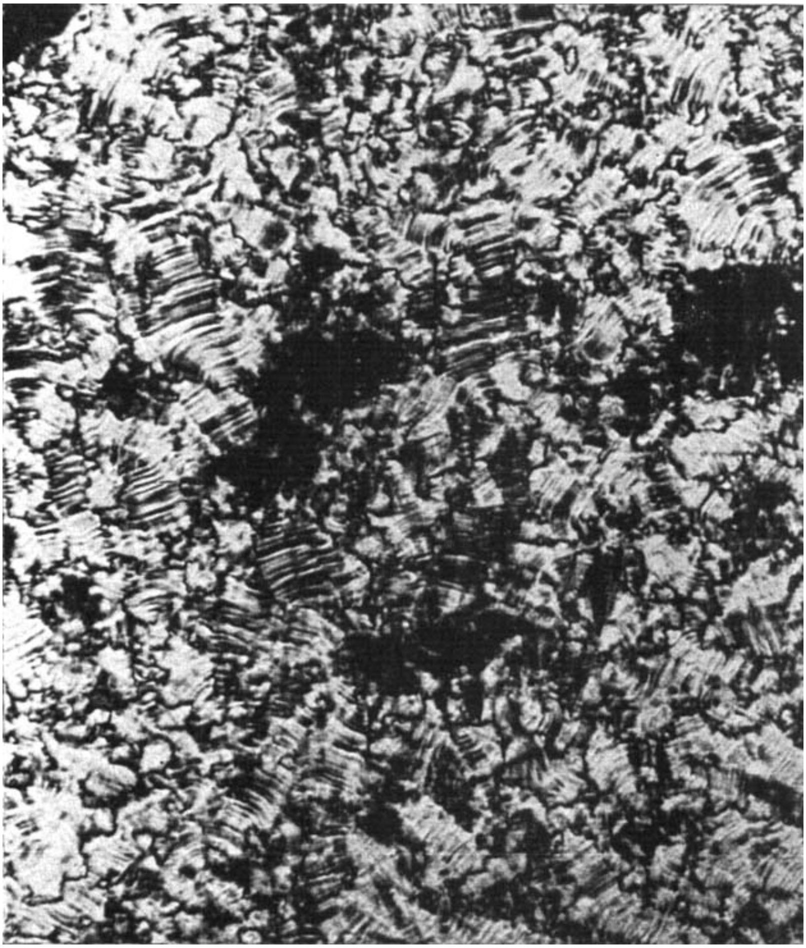

Figure 2. Cholesteryl Myristate Solid Phase at 20°C Crossed Nicoles, Zeiss Ultraphot II Microscope at 272 Diameters Magnification.

On heating the solid phase the field brightens, and the birefringent colors become more intense. Near 55°C, the field begins to darken slowly and the width of the wrinkled bands slowly increases. On heating further, at 73°C, the field begins to flow, and the bire-

Figure 3. Cholesteryl Myristate Smectic Phase at 77°C. See Fig. 2 for conditions.

fringent colors disappear sharply giving rise to a white field in which the general outlines of the solid phase plates are preserved, but the wrinkles are absent. The wrinkles appear to flatten out suddenly. The phase formed at 73°C is the smectic mesophase according to

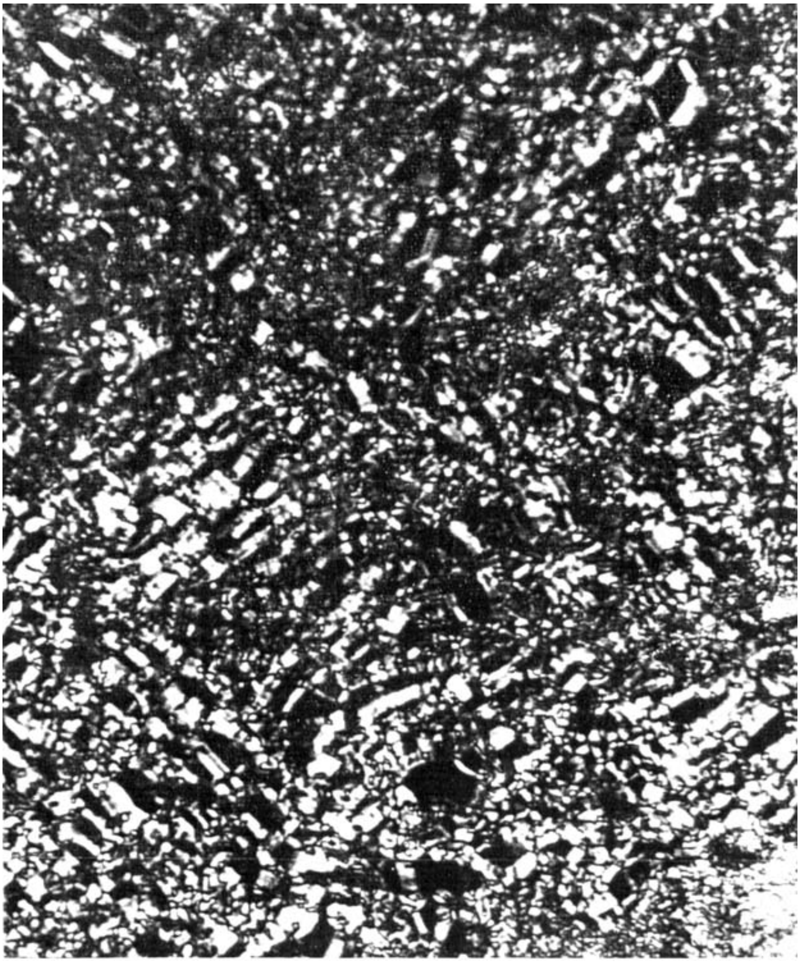

Figure 4. Cholesteryl Myristate Cholesteric Phase at 83°C. See Fig. 2 for conditions.

Gray.[7] Figure 3 shows this mesophase at 77°C. The phase in characterized by a high degree polarized light rotation, lack of birefringence colors, and the presence of light and dark lines across the irregular leaflets.

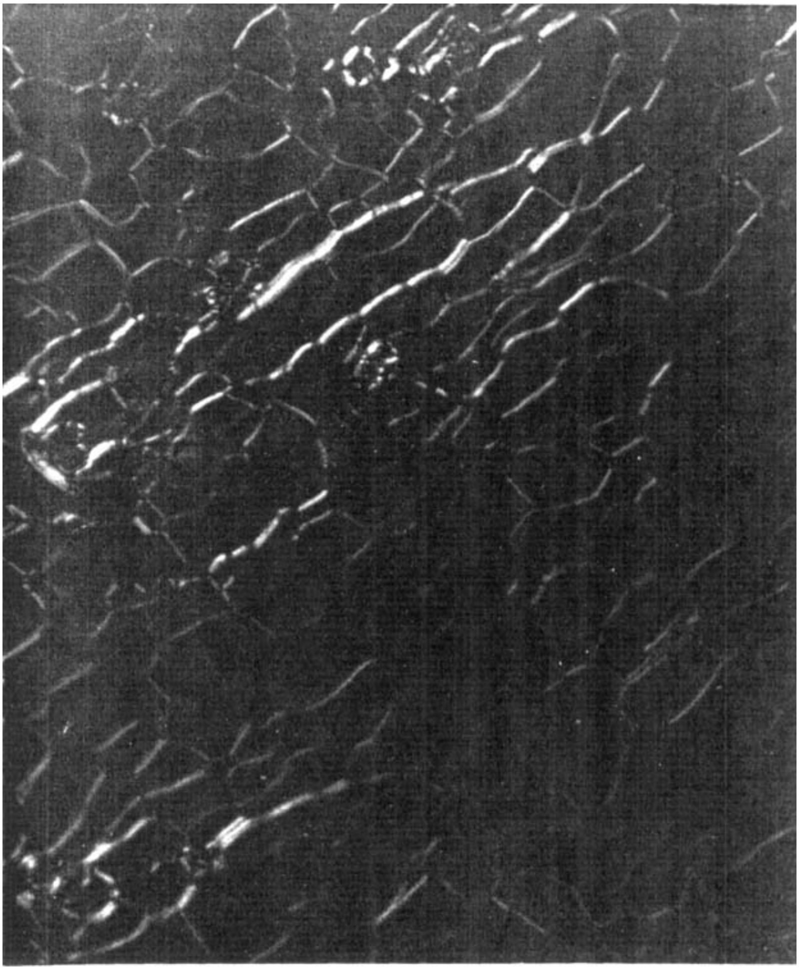

Figure 5. Cholesteryl Myristate Cholesteric Phase Sheared at 83°C. See Fig. 2 for conditions. Shear was at 45° to the long axis of the photograph.

On further heating, the smectic phase maintains a stable intensity and shape until near 79°C where the brightness suddenly increases.

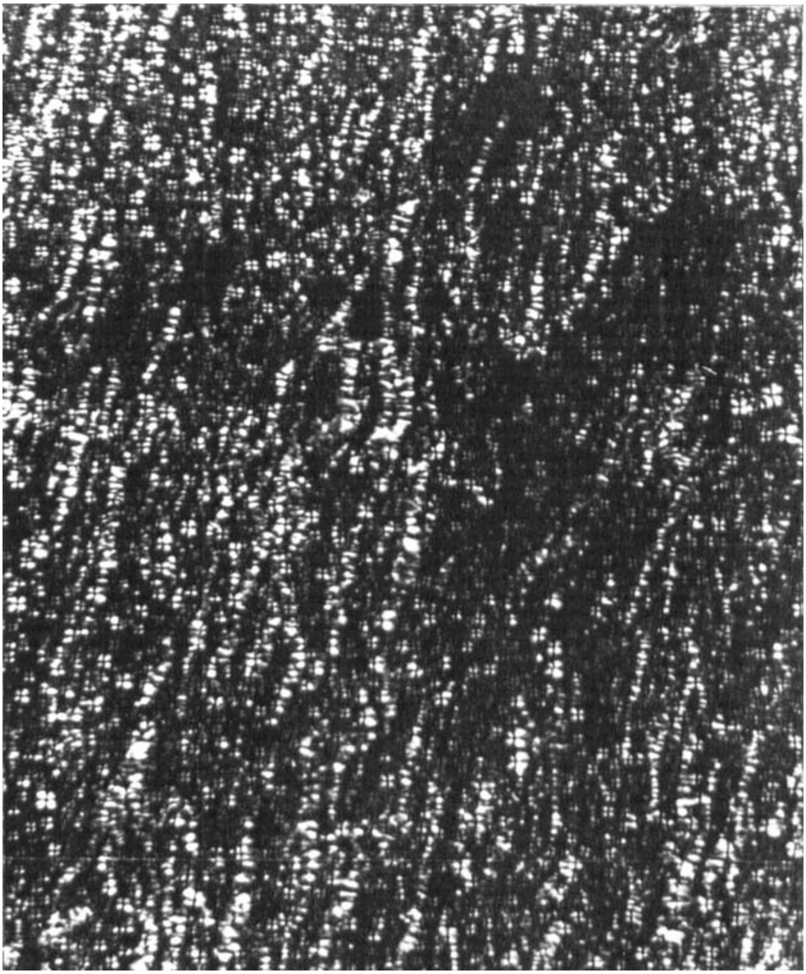

Figure 6. Cholesteryl Myristate Smectic Phase Sheared 77°C. See Fig. 2 for conditions. Shear was parallel to the long axis of the photograph.

Then, *with violent flowing*, the brightness rapidly decreases giving at 83°C the cholesteric mesophase shown in Fig. 4. Near 85°C, the granular structure shown between the brighter rectangular forms in Fig. 4 increases in size. At 86°C, the field begins to stream rapidly with final extinction of light and loss of birefringence occurring at 87.6°C. At this temperature and above, cholesteryl myristate is in the isotropic liquid phase.

The cholesteric mesophase reforms on cooling the isotropic liquid at 2°C per min to give a field almost identical to Fig. 4. When the top cover slip is displaced a few millimeters causing a weak shearing action, the cholesteric mesophase [sometimes called texture[7, 8, 10]] streams and reforms giving rise to an open netlike structure of bright rods (Fig. 5). The brighter portions of the net are parallel to the direction of shear. The smectic phase formed on heating the room temperature solid to 78°C also shows unusual shear phenomena (Fig. 6). The bright smectic plates disintegrate under shear to give a granular structure. Each grain exhibits a distinct optically positive maltese cross. Orientation of granules (sometimes called spherulites) occurs in the direction of shear. When this preparation is cooled, the solid phase reforms, growing from each granule in a circular fashion. The maltese cross grows larger and is highly rayed. This spherulite solid phase has the same LIA curve as the solid grown from an unsheared melt.

DTA and LIA Curves

Excellent correlation exists between the transition temperatures noted by direct optical observation of morphological forms and the DTA and LIA curves shown in Fig. 1. The DTA curve has been discussed in detail elsewhere.[1, 3] The temperatures of transition as noted by the endothermal minimum of the DTA curve are 73.2°C, 80.0°C, and 85.5°C for the solid→smectic, smectic→cholesteric, and cholesteric→isotropic liquid transitions, respectively. This compares closely with 73.6°C, 79.7°C, and 85.8°C reported previously by differential thermal analysis.[1] The small shoulder on the low temperature side of the cholesteric→isotropic liquid endotherm at 85.6°C was not noted in earlier work.[1, 3] At the time of publication of the previous study, the small shoulder was thought to be a possible artifact caused by displacement of grains of sample from the main mass. However, since the shoulder corresponds to features noted on both the direct visual and LIA observation, it is probably a real thermodynamic feature. The cholesteric mesophase apparently has two forms differing slightly in transition temperature to the isotropic liquid phase.

The LIA curve shows a gradual increase in percent depolarization up to 55°C. This could be due either to perfection of the solid phase by freezing of supercooled liquid or by a second-order phase transition. Heat of fusion studies have indicated that crystallization of supercooled liquid crystal phase is a potential operating mechanism.[3] It was found that the heat of transition of solid to smectic mesophase could be 1 to 2 cal/g lower if a sample were quickly reheated directly after freezing. The transition from solid to smectic mesophase at 72°C is sharp on the LIA curve, with no indication of pretransition phenomena in the solid phase. The intensity of the polarized light rotated by the smectic mesophase sharply increases at 79°C. This increase is probably due to a rotational modification of the smectic configuration. The effect has been noted with n-dotriacontane at the premelting chain rotation transition.[2] The transition from smectic to cholesteric mesophase is very sharp at 81°C. The cholesteric mesophase shows two steps in the LIA curve at 85°C and 86.6°C. This is evidence of two cholesteryl modifications. A cooling LIA curve (not shown in Fig. 1) indicated very little supercooling for the isotropic liquid→cholesteric and cholesteric→smectic transitions. However, the smectic→solid transition was depressed by 30°C.

Discussion

Using previously published discussions of cholesteryl ester transitions, it is possible to explain the foregoing data.[8, 10] The wrinkled appearance of the solid phase of cholesteryl myristate is macro-evidence of the intercrystal forces existing in the solid phase. The transition to the smectic phase permits the molecules to move in two directions (in plane) and potentially rotate about one axis freely. Thus, the general outline of the solid phase (plate) is preserved, but the strains of intermolecular ordering decrease. Shearing of the smectic phase destroys the skeletal solid phase arrangement and reduces the structure to the often-reported spherulitic appearance. Thus, the heating of the undisturbed smectic phase produces rotational changes in the smectic array. Prior to conversion to the cholesteric phase, the smectic phase increases in anisotropy probably due to rotation of the long ester side chains. Frenkel's theory predicts a pretransitional effect between similar liquid phases.[6] Conversion to the cholesteric mesophase involves removal of all ordering with the exception of the stacking of the cholesterol plates. The essentially flat structure of the cholesterol molecule favors stacking. The projecting side groups of the ester and the out-of-plane 4-hydrogen cause a helical array. DTA and LIA data indicate two forms of the cholesteryl mesophase. We suggest that these are modifications of the helix. Spatially, several are possible. In any case, the DTA shoulder, the step in the LIA data, and the visual observation cannot be ignored. Some transformation in the cholesteryl phase occurs from 83.9°C to 85°C.

Shearing on the cholesteryl phase causes a netlike structure to appear. The net is probably due to orientational shear effects on the cholesteryl helix. The sheared phase resembles a photomicrograph which was published by Chistyakov[5] of cholesteryl cinnamate in the cholesteryl mesophase. The observation of orientation in cholesteryl esters is important to the evaluation of the mechanism of non-Newtonian flow in the shear rheology of mesophases. It is thus hoped that these investigations of cholesteryl myristate, by three independent and sensitive methods, will be of material aid in untangling some of the confusion which surrounds these interesting compounds.

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