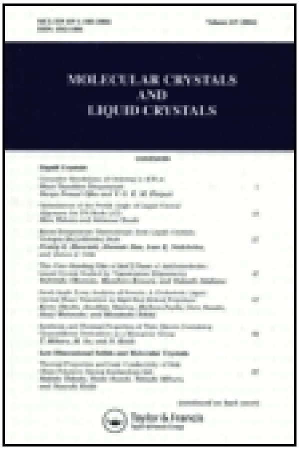

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# Liquid Crystalline Properties of Ergosteryl Fatty Acid Esters

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# Liquid Crystalline Properties of Ergosteryl Fatty Acid Esters‡

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**Abstract**—Thirteen fatty acid esters of ergosterol (24α-methyl-5, 7, 22-cholestatrien-3β-ol) have been prepared and their liquid crystalline properties studied with the polarizing microscope. Ergosteryl acetate is not mesomorphic. Saturated even-chain esters from the butyrate to the laurate form enaniotropic smectic transitions. The myristate and palmitate form monotropic transitions, the smectic mesophases being formed only upon cooling the isotropic liquid. The stearate also forms a monotropic transition, the smectic mesophase being formed below the melting point. The heptanoate, nonanoate and tridecanoate odd-chain esters also form the enaniotropic transitions. An odd-even effect on the liquid crystalline properties is observed in this series of esters.

## 1. Introduction

The cholesteryl fatty acid esters represent a thoroughly studied class of mesomorphic substances. Many of these esters are polymorphic, forming both a smectic and a cholesteric mesophase.[1,2] The cholesteryl $n$-alkyl carbonates also form a cholesteric mesophase.[3] A number of different optical[4–6] and calorimetric methods[7–9] have been used to study the mesomorphic properties of these esters. Interest has been directed at preparing esters of structurally related sterols in an effort to investigate the effect of structural modifications on mesomorphic properties. Sterols which were chosen for such studies include β-sitosterol

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‡ Part V of a series. See Ref. 10 for Part IV.

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(24α-ethylcholest-5-en-3β-ol), stigmasterol (24α-ethyl-5, 22-cholestadien-3β-ol), stigmastanol (24α-ethylcholestan-3β-ol) and ergosterol (24α-methyl-5, 7, 22-cholestatrien-3β-ol). The structures of these sterols are illustrated in Fig. 1. While no mesomorphic behavior has been detected with various stigmasteryl fatty acid esters by two groups,[10,11] Russian workers have found these esters to form monotropic smectic transitions.[12] These workers note, however, that the isotropic melts of these substances must be cooled very rapidly to detect these smectic transitions. The mesomorphic properties of stigmasteryl esters are thus unusually susceptible to the rate at which the thermal events occur. This may be why other workers have not detected these transitions. Various β-sitosteryl fatty acid esters also form smectic mesophases.[13]

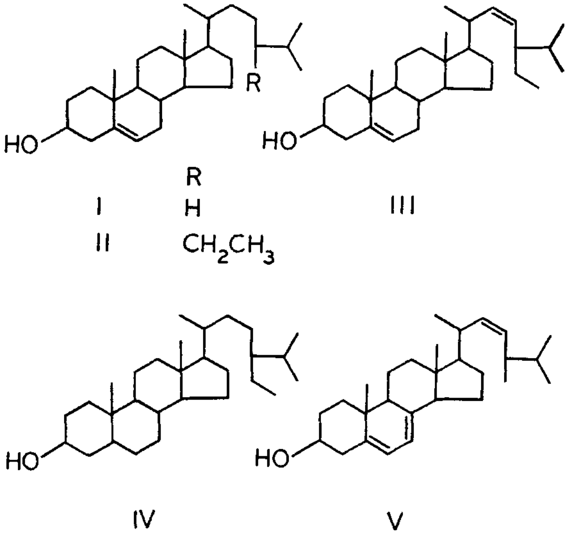

Figure 1. Structural formulas of (I) Cholesterol, (II) β-sitosterol (24α-ethylcholest-5-en-3β-ol), (III) Stigmasterol (24α-ethyl-5,22-cholestadien-3β-ol), (IV) Stigmastanol (24α-ethylcholestan-3β-ol) and (V) Ergosterol (24α-methyl-5, 7, 22-cholestatrien-3β-ol).

The smectic properties of stigmasteryl *n*-alkyl carbonates[14] and *n*-alkyl thiocarbonates[15] have been reported. These workers have more recently made a very thorough study of the mesomorphic properties of a number of other carbonates and thiocarbonates of cholesterol and cholestanol.[17] These include the 5α-cholestanyl *n*-alkyl carbonates, 5α-cholestanyl *S*-alkyl thiocarbonates, *S*-cholesteryl *O*-alkyl thiocarbonates, cholesteryl *S*-alkyl thiocarbonates, *S*-cholesteryl *S*-alkyl thiocarbonates, cholesteryl xanthates and cholesteryl trithiocarbonates. The mesomorphic properties of ergosteryl palmitate have recently been reported.[10] The liquid crystalline behavior of the homologous series of fatty acid esters of this sterol has now been studied.

## 2. Experimental

All reagents and solvents were analytical grade. The solvents were distilled before use. The fatty acyl chlorides were purchased from the Eastman Kodak Company and were of Eastman Grade. Ergosterol, obtained from the Sigma Chemical Company, was crystallized three times from acetone, m.p. 165° (Literature, m.p. 165°).[18] The ergosterol was homogeneous as was demonstrated by both thin-layer and gas–liquid partition chromatographic analyses. The esters were prepared, purified and stored in the dark to minimize degradation due to irradiation. Esters were prepared by esterification of 200 mg of ergosterol with a 1.5 molar excess of the acyl chloride in 100 ml of anhydrous benzene containing 3 ml of pyridine. The solution was refluxed under anhydrous conditions for one half hour. After allowing it to cool to room temperature, 100 ml of ether was added and the solution was washed three times with equal volumes of 10% hydrochloric acid, three times with 5% potassium hydroxide, followed by three washes with water. The organic layer was dried with anhydrous sodium sulfate and the solvent evaporated on a steam bath under a stream of nitrogen. The residue was dissolved in benzene and then added to an alumina column (30 × 1.5 cm) containing the Merck acid-washed adsorbant (15 gm). The ester was eluted with 300 ml of benzene and crystallized four times from methanol-ether. Thin-layer and gas–liquid partition chromatography were employed to further establish the purity of the crystalline esters. The purified esters were chromatographed on 250 $\mu$ thick layers of silica gel G. The solvent system was ether-hexane, 7:93 (v/v). Spots were visualized by spraying the plates with anisaldehyde reagent and then heating them at 80°. The esters could be separated in this system according to fatty acyl chain length. Representative $R_f$'s are as follows: ergosteryl acetate, $R_f$ 0.38; hexanoate, $R_f$ 0.58; palmitate, $R_f$ 0.67. Gas–liquid chromatography was carried out using a Barber–Colman Model 5000 gas chromatograph equipped with a hydrogen flame detector. A 1% SE-30 column was prepared by the method of Kuksis *et al.*[19] A 60 cm long glass column (4 mm i.d.) was packed with Gas Chrom *Q* (100/120 mesh, Applied Science Laboratories) and coated with 1% SE-30. The column was conditioned for 72 hrs. at 320° with the carrier gas ($N_2$) flowing at 150 cc/min (thermal stripping). The column was operated at 290° with an $N_2$ flow rate of cc/min.

Melting points and phase transition temperatures were determined using a Nalge–Axelrod hot-stage polarizing microscope. Samples were sandwiched between glass cover-slips and were heated at a rate of 3°/min and cooled at a rate of 2°/min. The melting point is defined as the temperature at which the crystalline structure forms either the isotropic liquid or a birefringent liquid (mesophase). The smectic → isotropic transition temperature is the transition from the mesophase to the isotropic liquid. In all cases this temperature differed by no more than one degree from the isotropic → smectic transition temperature. This is another index of the purity of the preparations. Photographs were obtained with an Exakta 35 mm single lens reflex camera. With the 10X objective (50 diameters magnification) a 10 sec exposure gave a sufficiently sharp photograph.

The following abbreviations and terms are used in this paper: *sm* = smectic mesophase; *iso* = isotropic liquid; enaniotropic = reversible transition, formed on both heating and cooling; monotropic = transition formed only on cooling the isotropic liquid.

## 3. Results

The melting points obtained by us for ergosteryl fatty acid esters are compared in Table 1 with the values that have been reported by other workers. In most instances there is good agreement. Although a series of fatty acid esters of ergosterol has been prepared by other workers,[11] no mesomorphic behavior of these esters was reported. The discrepancies that do exist

TABLE 1 Melting Points and Phase Transition Temperatures of Ergosteryl Fatty Acid Esters

| Esters[a] | Melting point, °C Observed[b] | Melting point, °C Literature | Observed transition temperature, °C |
|---|---|---|---|
| Acetate | 179–180 | 177[c]<br>181[d] | ... |
| Butyrate | 127 | 134[c]<br>129.5[e] | iso → sm 142 |
| Isobutyrate | 153 | 162[f] | iso → sm 157 |
| Hexanoate | 119 | 125.5[c] | iso → sm 141.5 |
| Heptanoate | 94 | ... | iso → sm 130 |
| Octanoate | 110 | 121[c] | iso → sm 137.5 |
| Nonanoate | 89–90 | ... | iso → sm 131 |
| Decanoate | 109–110 | 117.5[c] | iso → sm 132 |
| Dodecanoate (Laurate) | 114 | 116[c] | iso → sm 127.5 |
| Tridecanoate | 103 | ... | iso → sm 118.5 |
| Tetradecanoate (Myristate) | 115 | 115[c] | iso → sm 118 |
| Hexadecanoate (Palmitate) | 109 | 110[c]<br>107[g]<br>106—108[h] | iso → sm 110 |
| Octadecanoate (Stearate) | 113 | 113[c]<br>110.5[g] | iso → sm 104 |

[a] Crystallized from methanol-ether.

[b] Defined as the solid → mesophase or the solid → isotropic liquid transition.

[c] A. Kuksis and J. M. R. Beveridge, *J. Org. Chem.* **25**, 1209 (1960).

[d] W. Bergmann, *Ann. Rev. Plant Phys.* **4**, 383 (1953).

[e] C. Tanret, *Compt. Rend.* **147**, 75 (1889).

[f] C. E. Bills and E. M. Honeywell, *J. Biol. Chem.* **80**, 15 (1928).

[g] A. Angeletti, G. Tappi and G. Biglino, *Ann. Chim.* (*Rome*) **42**, 502 (1952).

[h] A. E. Oxford and H. Raistrick, *Biochem. J.* **27**, 1176 (1933).

can be explained by the methods used to determine the melting points and by other factors such as the means of synthesis and degree of purification of the esters, the solvent systems employed for crystallization and the method of drying the crystalline samples. A standard melting point apparatus was used by other workers for the determinations. The melting point reported by them presumably was the temperature at which the crystalline structure began to disappear to a liquid. The majority of these esters, however, have a waxy texture near the melting point and melt to hazy, turbid liquids. This is especially true with ergosteryl octanoate. In such situations it is difficult to detect the true melting point. We define the melting point as the temperature at which the crystalline structure disappears. With a polarizing microscope we observed either the darkening of the field of view (isotropic liquid), or the change to a distinctly different birefringent pattern (mesophase).

The esters used for our determinations were of high purity as was demonstrated by gas–liquid partition chromatographic analyses. A representative chromatogram is illustrated in Fig. 2. All of the esters exhibited such a homogeneous peak with a steady baseline, indicating the absence of homologues. The logarithm of the retention time in millimeters is plotted as a function of the fatty acyl chain length for the series of esters in Fig. 3. A linear relationship is obtained, indicating a well-defined homologous series of substances. Gas–liquid chromatographic analyses using cholesteryl esters as the stationary phase have demonstrated the unusually high heats of solution of various organic solvents in these substances.[20] These retained organic solvents which are not completely removed can dramatically affect the thermodynamic properties of mesomorphic substances.[21] Although in such instances the melting point is usually not changed, the mesophase transition temperature can be drastically altered. An earlier preparation of ergosteryl palmitate had a *sm* → *iso* transition temperature 8° lower than that found in the present study, although the melting points were identical.[10] For the present investigation, the samples were crystallized from

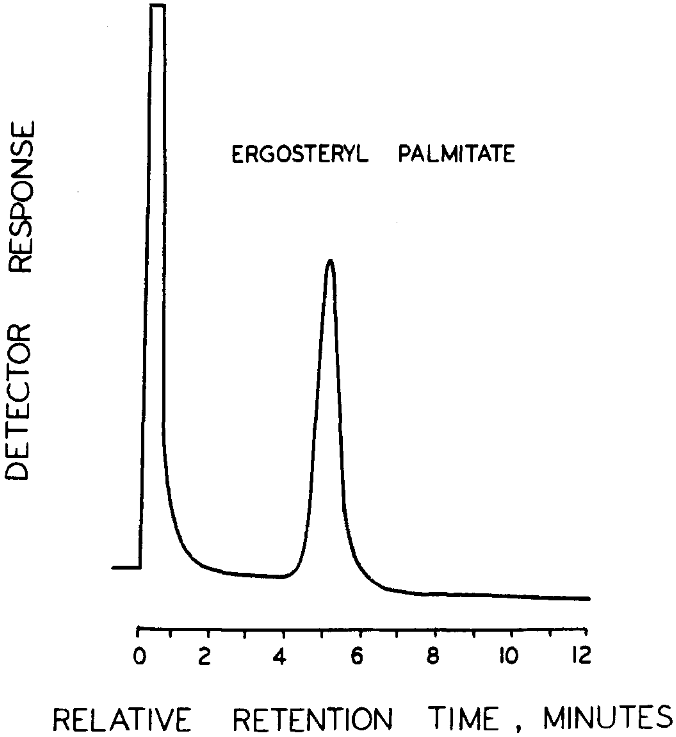

Figure 2. Reproduction of the gas–liquid chromatographic tracing of ergosteryl palmitate.

methanol–ether and carefully dried *in vacuo.* Such a procedure hopefully eliminated these difficulties. A detailed description of the mesomorphic properties of these ergosteryl esters listed in Table I follows.

*Ergosteryl Acetate*—The crystalline structure melted directly to the isotropic liquid at 179–180°. Upon cooling, no mesophase was detected, the melt crystallizing at 76°.

*Ergosteryl Butyrate*—Many focal–conic groups were observed upon heating the solid to 132°. At 142° the isotropic liquid formed, the fan-texture appearing upon cooling to this same temperature. The crystalline structure formed at 110°.

*Ergosteryl Isobutyrate*—This ester melted at 153° to a birefringent liquid. The mesophase consisted of crosses, many of

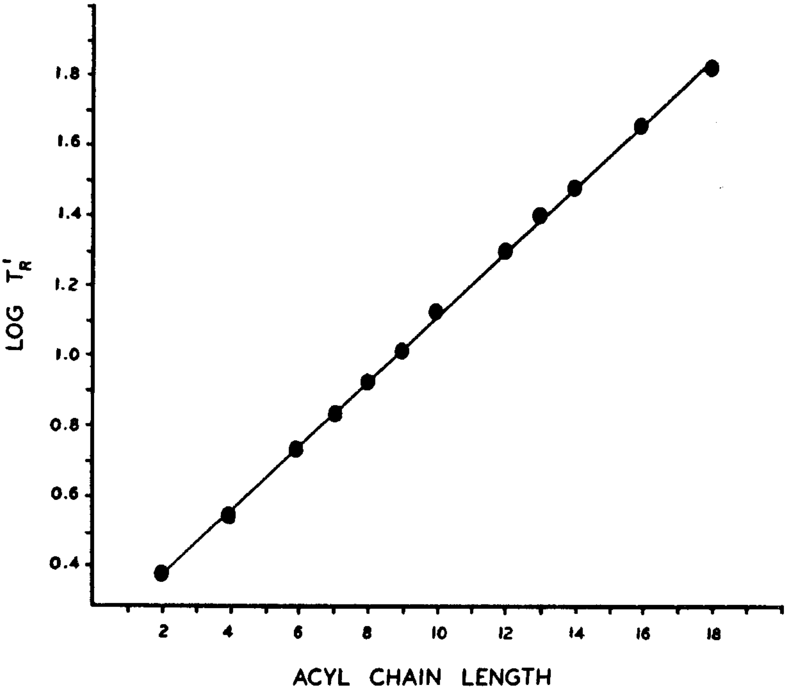

Figure 3. Gas–liquid partition chromatographic analysis of ergosteryl fatty acid esters on 1% SE-30.

which were surrounded by three concentric rings. The isotropic liquid appeared at 157°. Upon cooling, the fan-texture structure formed at 157° and the crystalline phase at 117°.

*Ergosteryl Hexanoate*—The crystals melted at 119° to a birefringent liquid containing many small focal–conic groups. At 127° larger crosses appeared in the mesophase, the isotropic liquid being formed at 141°. Upon cooling, a beautiful fan-texture (smectic type A morphology) (22) formed at 142°. The crystalline solid appeared at 78°.

*Ergosteryl Heptanoate*—This ester melted at 94° to a highly birefringent liquid. The mesophase contained many small focal–conic groups which re-arranged to the fan-texture at 115°. At 128° some spherulites appeared at the periphery of the melt, the isotropic liquid forming at 130°. Upon cooling, the fan-texture appeared at 129°, persisting until crystallization occurred at 48°.

*Ergosteryl Octanoate*—Upon heating the crystals, a birefringent liquid appeared at 110°, consisting of small focal–conic groups. The isotropic liquid was formed at 138°. Large focal–conic groups invaded the melt upon cooling down to 137°. At 56° a crystalline front moved across the melt.

*Ergosteryl Nonanoate*—At 89–90° the crystals melted to form many small focal-conics. Larger focal–conic groups formed at 118°, with spherulites at the periphery of the melt. At 130° the isotropic liquid appeared and at 131° on the cooling cycle the fan-texture reformed. The crystalline phase was formed upon cooling to 47°.

*Ergosteryl Decanoate*—The crystals melted to the small focal–conic texture at 109–110°. The texture consisted of small focal–conic groups and many crosses surrounded by three concentric rings, similar to the mesophase formed by the heptanoate. At 131° the isotropic liquid appeared. Upon cooling, the fan-texture was formed at 132° and the crystalline phase at 54°.

*Ergosteryl Dodecanoate* (*Laurate*)—This ester melted directly to the small focal–conic texture at 114°. At 120° the large focal–conic groups appeared and also many crosses with the three concentric rings. The isotropic liquid formed at 127° upon cooling, the fan-texture at 128°. The crystalline phase appeared at 59°.

*Ergosteryl Tridecanoate*—The small focal–conic texture formed at 102–103°, re-arranging to the large groups at 113° which disappeared to the isotropic liquid at 119°. Upon cooling, the fan-texture was formed at 118° and the crystalline structure at 52°.

*Ergosteryl Tetradecanoate* (*Myristate*)—This ester melted directly to the isotropic liquid at 115°. A monotropic fan-texture appeared upon cooling to 118° and the crystalline phase at 82°.

*Ergosteryl Hexadecanoate* (*Palmitate*)—The isotropic liquid appeared upon heating the crystalline structure to 109°. When cooled to 110° the fan-texture appeared. The crystalline phase formed at 102°.

*Ergosteryl Octadecanoate* (*Stearate*)—At 113° the crystals melted to an isotropic liquid. Upon cooling the melt to 104°, a fan-texture which contained many crosses was formed. A beautiful multi-colored crystalline solid was formed at 81°.

## 4. Discussion

The melting points and isotropic → smectic transition temperatures of these ergosteryl esters are plotted as a function of fatty acyl chain length in Fig. 4. There is a steady decrease in the *sm* → *iso* transition temperatures with increasing fatty acyl chain length. For the even-chain esters these values fall on a

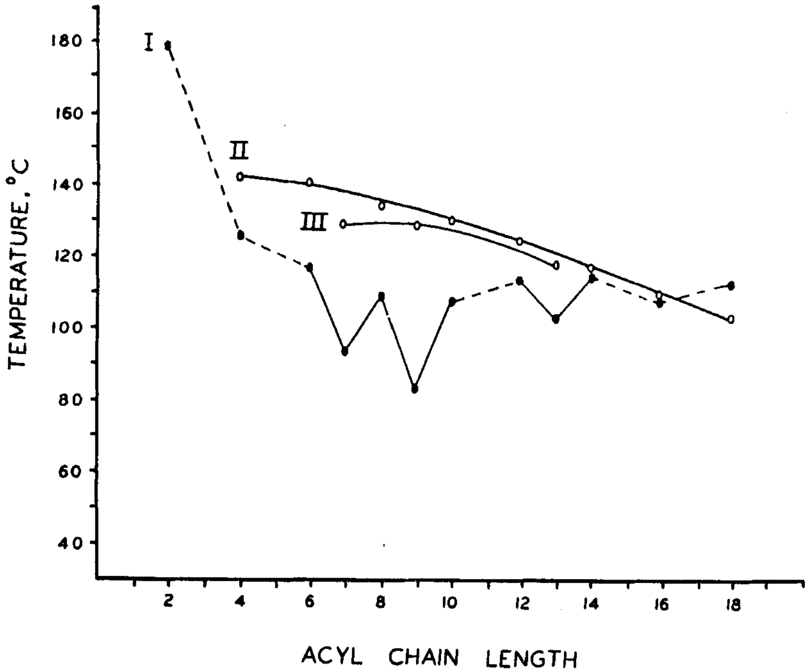

Figure 4. Phase transition temperatures of ergosteryl esters as a function of the fatty acyl chain length. Curve I represents the solid → mesophase or solid → isotropic liquid transition temperatures (melting points) for both the odd- and even-chain esters. Curve II represents the isotropic → smectic transition temperatures for the even-chain esters and curve III is a plot of these temperatures for the odd-chain esters.

smooth curve as is the case with most homologous series of liquid crystalline compounds that have been studied.[1,2,10] The values for the three odd-chain esters are somewhat displaced and therefore have been drawn as a separate curve. Most of the ergosteryl esters described in this communication form enantiotropic smectic transitions. Upon heating these esters, a birefringent liquid containing many small focal conic groups is formed. At a higher temperature this mesophase re-arranges to form a texture containing many large crosses. These changes are not accompanied by a front moving across the melt, as is usually the case with mesophase → mesophase transitions.[1] Instead, the mesophase re-arranges within the melt. Thus the assignment of polymorphic transitions to these microscopically observed changes should await calorimetric measurements. Upon cooling the isotropic melt of such esters, a very well-defined fan-texture (smectic type *A* morphology)[22] forms within the melt. This mesophase then persists until crystallization. The mesophase formed by ergosteryl hexanoate (Fig. 4) illustrates the type of texture formed by these esters. Such enaniotropic transitions are depicted as:

$$\text{Crystalline} \rightleftarrows \text{Smectic} \rightleftarrows \text{Isotropic Liquid}$$

In some instances the texture formed upon heating consisted of small crosses each surrounded by three concentric rings. These rings disappeared rapidly to form the large cross structure. Such an optical pattern may result from nucleation due to dust particles. For esters which formed a smectic mesophase only upon cooling from the isotropic liquid (tetradecanoate, hexadecanoate and octadecanoate) the monotropic transition is assigned.

$$\begin{tikzcd} & \text{Isotropic Liquid} \arrow[dd, shift right] \\ \text{Crystalline} \arrow[ur] & \\ & \text{Smectic} \arrow[uu, shift right] \arrow[ul] \end{tikzcd}$$

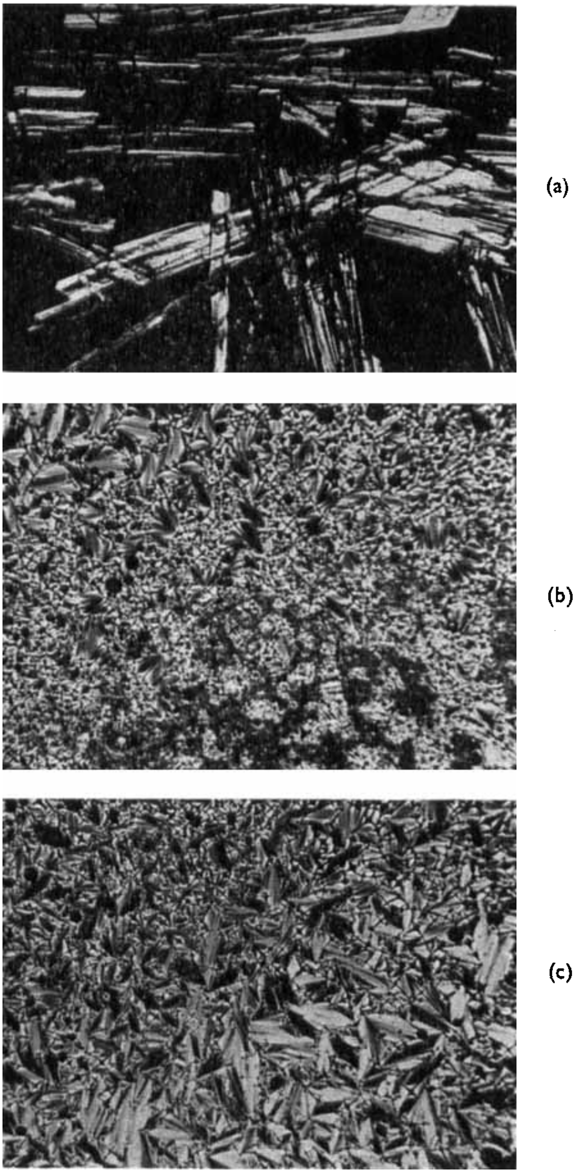

Figure 5. Photographs (50 diameters magnification) of (a) crystals of ergosteryl hexanoate which melt to form the (b) focal–conic texture. Upon cooling the isotropic liquid the (c) fan-texture is reformed.

For these ergosteryl esters there is observed an odd–even effect on the liquid crystalline properties. For the cholesteryl ester series an odd–even effect is also found[1,2] but the isotropic → smectic transition temperature of the odd-chain esters fall on a curve above that for the even-chain esters. In the case of the ergosteryl esters this effect is reversed, the odd-chain curve falling below that for the even-chain esters (Fig. 4). Evidently the relative position of these curves with respect to one another may vary from case to case and is a reflection of the inherent thermodynamic properties of the particular series under study.

Various triterpene fatty acid esters are known to form a cholesteric mesophase.[23] Some of these esters have been isolated from plant sources[24] and cholesteryl fatty acid esters occur commonly in mammalian tissues.[25,26] In addition, ergosteryl palmitate has been isolated from *Penicillium brevi compactum*,[27] and the palmitate and stearate have been identified in cultures of *Saccharomyces cerivisiae*.[28] There is thus an increasing amount of evidence that steryl esters which are known to form either a smectic or cholesteric mesophase occur in living tissues. The fact that these esters exhibit such liquid crystalline properties could be much more than merely coincidental. Their existence in biological systems might suggest some involvement in biochemical processes and should be further investigated.

## 5. Summary

Ergosteryl fatty acid esters from the butyrate through the laurate form enaniotropic smectic transitions. The acetate is not mesomorphic while the myristate, palmitate and stearate form monotropic smectic transitions. There is observed with the liquid crystalline properties of these esters an odd–even effect which is a function of fatty acyl chain length. Some of these esters have been isolated from natural sources.

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