The Polymorphic and Mesomorphic Behavior of Four Esters of Cholesterol

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The Polymorphic and Mesomorphic Behavior of Four Esters of Cholesterol†

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Abstract—The techniques of differential scanning calorimetry, X-ray powder diffractometry, and positron annihilation have been used to study the polymorphic and mesomorphic behavior of the following esters of cholesterol: cholesteryl formate, cholesteryl butyrate, cholesteryl benzoate, and cholesteryl cinnamate. Each of these compounds exhibits a single mesophase of the cholesteric type. The solid phase formed from the melt for each ester was observed to be structurally different from the solid phase obtained from solution. Solvents from which the solution-grown samples were crystallized were as follows: cholesteryl formate and cholesteryl butyrate from acetone, cholesteryl benzoate from benzene, and cholesteryl cinnamate from 2-butanone.

1. Introduction

The esters of cholesterol belong to a group of compounds which has the unusual property of exhibiting one or more phases (mesophases) intermediate between the true solid state and the isotropic liquid state. In these phases the substance is liquid in mobility yet strongly anisotropic in some of its properties, a characteristic normally found only in solid crystals. For this reason these mesophases are called liquid crystals. This phenomenon is referred to as mesomorphism.

In addition to exhibiting mesomorphic behavior, many esters of cholesterol are known to occur in the solid state in more than one polymorphic form.[1,2] The basis of their polymorphism is related to the variety of ways in which their atoms can be packed in the crystal lattice.

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In this study the techniques of differential scanning calorimetry (DSC), X-ray powder diffractometry, and positron annihilation have been applied to the study of the polymorphic and mesomorphic behavior of four cholesteryl esters.

2. Experimental

A differential scanning calorimeter, Perkin–Elmer DSC-1B, was used to determine the phase transition temperatures and melting points of the cholesteryl esters. In each measurement the sample size was approximately 10 mg and the heating and cooling rates used were 5 °C per minute. The sample pans were always capped and the DSC was purged continuously at a slow and constant flow rate with dry nitrogen gas. Temperature calibration of the DSC was obtained using the melting points of standard calibration samples. A quantitative study of the heats of transition was not undertaken.

The X-ray data were obtained with a Norelco X-ray powder diffractometer. Each sample was ground into a fine powder before being packed into the specimen holder. The angular scanning range used was between 1.5 and 30° and the scanning speed was 1° per minute. All powder diffraction patterns were obtained at room temperature.

The mean lifetimes and intensities of positrons annihilating in the cholesteryl esters were experimentally studied by the usual method of delayed coincidences, using time-to-amplitude conversion. Positrons were obtained from the radioactive decay of Na^{22}. The detectors were Naton 136 plastic scintillators mounted on Amperex XP-1021 photomultipliers. The electronics consisted of a Chronetics Model 105 time-to-amplitude converter, a Stirrup Model 1405 fast-slow coincidence unit, and a Packard 400-channel pulse-height analyzer. The prompt coincidence curve, using a Co^{60} source, had a full width at half maximum of 0.45 nsec and a drop-off rate of 0.10 nsec. The accidental rate was always less than 0.001 of the coincidence rate in the peak channel. Each positron source was prepared by depositing approximately 10 μcuries of Na^{22} between Mylar films of 0.87 mg/cm^2 thickness.

To measure positron lifetimes as a function of temperature, the sample was packed around the Na^{22} source in an aluminum holder

which was in good thermal contact with a heated copper bar. The temperature of the sample was measured with a copper-constantan thermocouple.

The lifetimes of the τ_2 component were measured directly from the slope of the delayed component of the time spectra. The intensity, I_2, of the positrons annihilating with the τ_2 lifetime was obtained using the method of Green and Bell.[3]

TABLE 1   Information about Solvent, Purity, and Supplier of Samples

Compound	Solvent from which Crystallized	Purity	Supplier
Cholesteryl formate	Acetone	99.5%	Aldrich Chemical Co. Milwaukee, Wisconsin
Cholesteryl butyrate	Acetone	> 97%	K & K Laboratories Plainview, New York
Cholesteryl benzoate	Benzene	99%	Sigma Chemical Co. St. Louis, Missouri
Cholesteryl cinnamate	2-Butanone	98%	Eastman Organic Chemicals Rochester, New York

The compounds used in this study were obtained commercially and used without further purification. Table 1 lists the supplier, the solvent from which the sample was crystallized, and the supplier's statement of the sample purity.

3. Results

DSC thermograms were obtained for each of the cholesteryl esters over the approximate temperature range of 25 to 225 °C. For each compound measurements were made on solution-crystallized samples as well as on the melt-recrystallized material. The latter sample was obtained by a complete heating and cooling cycle of the former sample. As an example of the DSC data, Fig. 1 shows heating thermograms obtained for both the solution-crystallized and melt-recrystallized samples of cholesteryl butyrate.
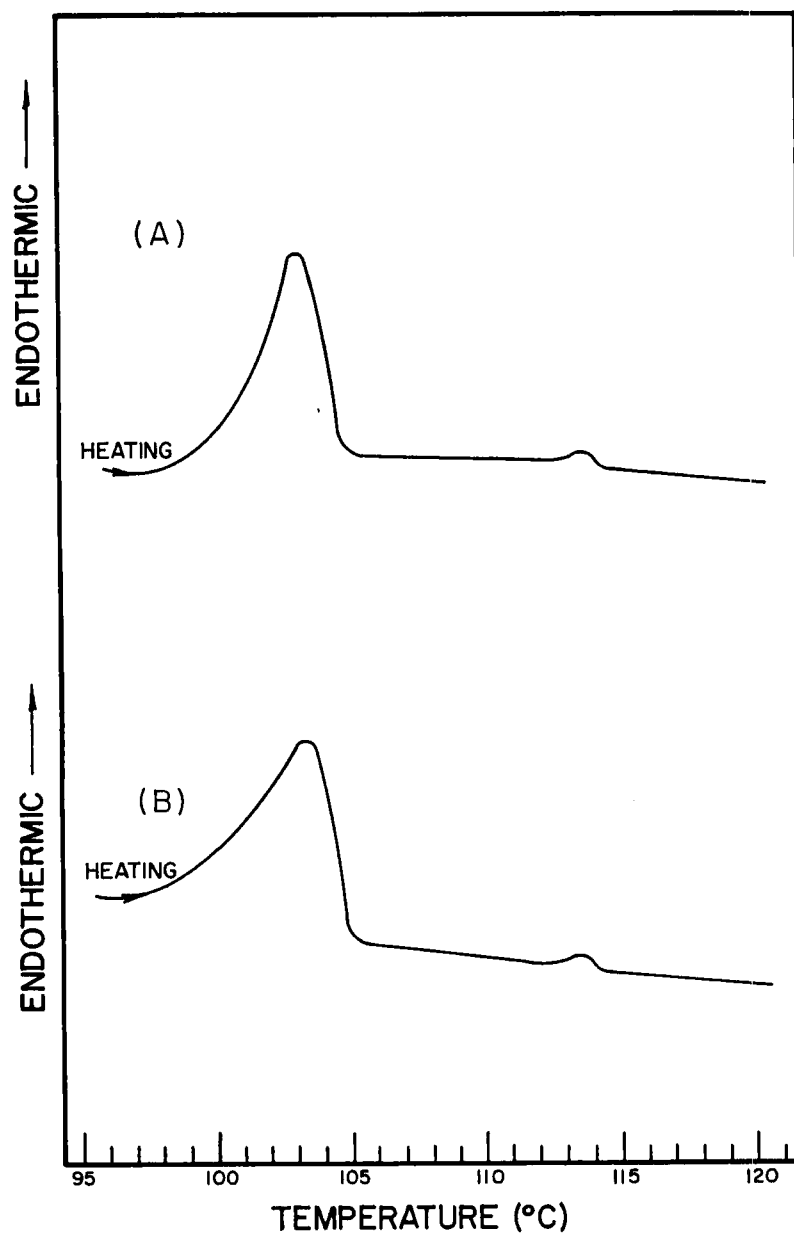
Figure 1.   Heating thermograms of samples of cholesteryl butyrate obtained from acetone solution (A) and from the melt (B).

Similarly, positron lifetime measurements were made over the same temperature range for both the solution-grown and the melt-recrystallized materials. Figure 2 shows typical time spectra obtained for samples of cholesteryl butyrate, and Fig. 3 shows τ_2 and I_2 as a function of temperature.

Finally, X-ray powder diffraction patterns were obtained for the solution-crystallized and the melt-recrystallized materials. An example of these data is given in Fig. 4.

A summary of the results for each ester is given in Table 2. The transition temperatures quoted are those obtained in the DSC measurements. Lack of really close agreement between transition temperatures as determined by DSC and positron lifetime measurements is not surprising because of differences in sample size and rates of heating and cooling.

The possible dependence of crystal habit on the type of solvent from which the solution-grown material was recrystallized was not studied. Experiments to investigate this possibility are underway and results will be reported in a subsequent paper.

CHOLESTERYL BUTYRATE

The two endotherms shown in the heating thermogram of Fig. 1 (A) at 99 and 113 °C correspond to the solid → cholesteric and cholesteric → isotropic liquid transitions respectively. When one compares the heating thermogram for the melt-recrystallized sample Fig. 1 (B) with that for the sample crystallized from acetone, two differences are observed. First, the melting point is about one degree higher for the solution-grown material, and second, the area under the melting endotherm is about 25% greater for the melt-recrystallized sample.

Both the positron lifetime data and the X-ray diffraction patterns also revealed significant differences between the solution-grown and melt-recrystallized samples. Positron annihilation measurements showed that the long-lived component of the positron lifetime (τ_2) increased from 1.70 nsec to 1.90 nsec and the intensity (I_2) decreased from 42 to 28% when the solution-grown material was melted and allowed to resolidify. This type of behavior has been observed previously[4,5] for other cholesteryl esters which exhibit two solid forms. The X-ray data of Fig. 4 clearly show a difference in the
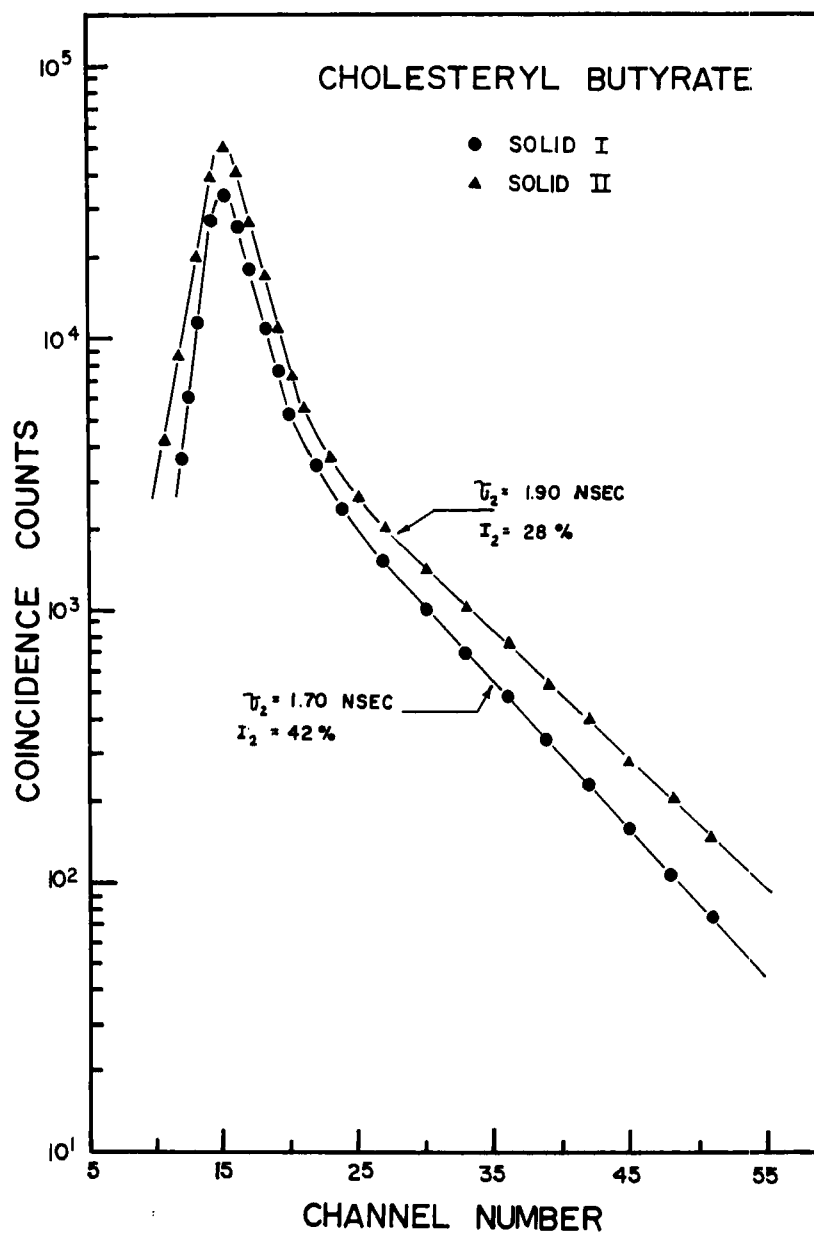
Figure 2.   Typical time spectra of positrons annihilating in samples of cholesteryl butyrate obtained from acetone solution (Solid I) and from the melt (Solid II).
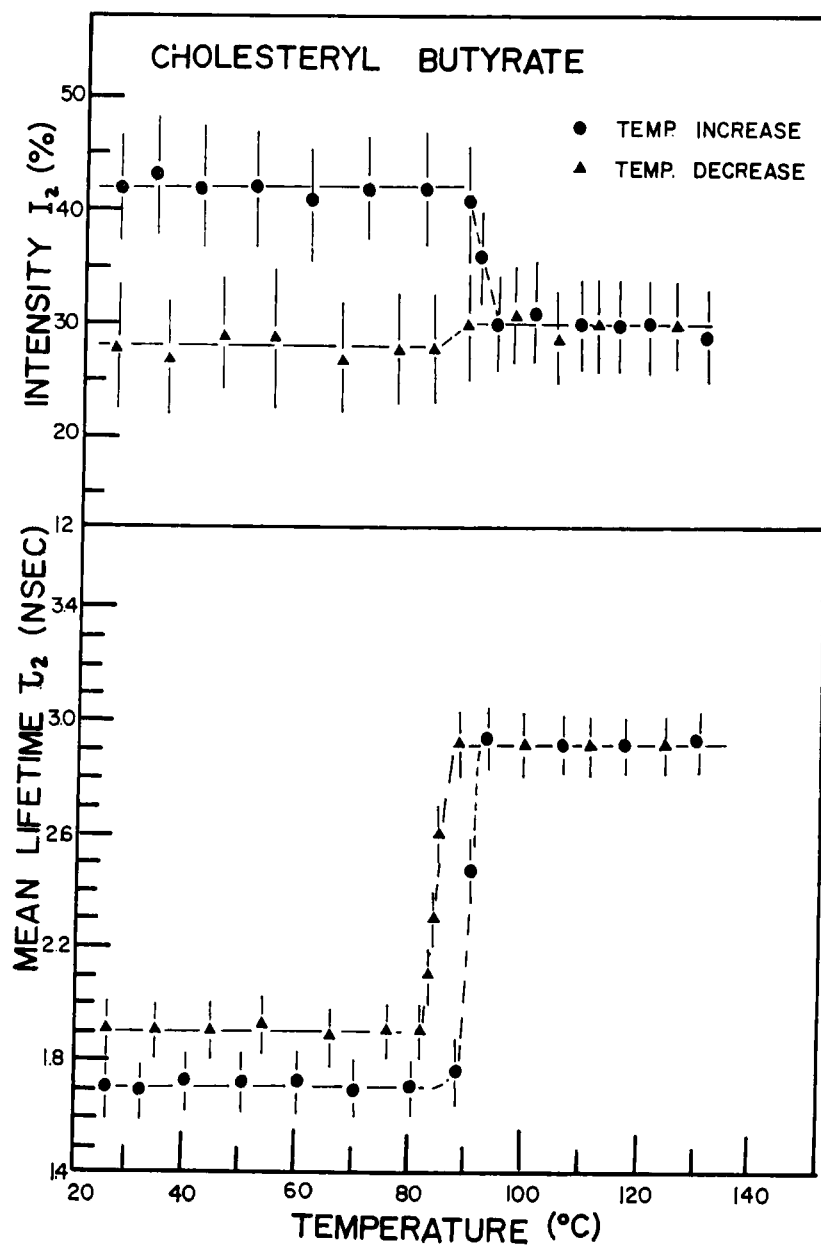
Figure 3. The mean lifetimes and intensities of positrons annihilating in cholesteryl butyrate as a function of temperature between 26 and 130 °C.

TABLE 2   Summary of the Experimental Results

Compound	Phase	Range of Stability of Phase (°C)	X-Ray Powder Diffraction Data		τ_2 (nsec)	I_2 (%)
			Long Spacing (Å)	Short Spacing (Å)		
Cholesteryl formate $C_{28}H_{46}O_2$	Solid I (obtained from acetone solution)	Below 96	27.6 (w), 15.8 (m), 13.8 (vw), 10.7 (vw)	6.41 (w), 5.99 (vw), 5.57 (vw), 5.34 (w), 5.01 (vw), 4.44 (vw), 4.04 (vw)	1.64±0.10	32±5
	Liquid	Above 96			2.80±0.10	22±5
	Cholesteric[a]	58 to 46			2.80±0.10	22±5
	Solid II (obtained from the melt of Solid I)	Below 97	27.6 (s), 14.0 (w), 7.38 (vw)	6.37 (vw), 5.99 (w), 5.57 (m), 5.25 (vw), 5.04 (vw), 4.96 (vw), 4.67 (vw), 4.04 (vw)	1.92±0.10	32±5
Cholesteryl butyrate $C_{31}H_{52}O_2$	Solid I (obtained from acetone solution)	Below 99	18.0 (w), 10.4 (s), 7.69 (vw), 7.31 (vw)	5.91 (vw), 5.07 (m), 4.85 (w), 4.75 (vw), 4.27 (vw)	1.70±0.10	42±5
	Cholesteric	99 to 113			2.92±0.10	30±5
	Liquid	Above 113			2.92±0.10	30±5
	Solid II (obtained from the melt of Solid I)	Below 98	14.5 (m), 8.59 (vw), 7.98 (vw)	6.42 (w), 5.13 (s), 4.82 (w), 4.02 (vw)	1.90±0.10	28±5

Compound	Phase	Temperature				
Cholesteryl benzoate $C_{34}H_{50}O_2$	Solid I (obtained from benzene solution)	Below 147	13.4 (w), 9.80 (w), 9.10 (w), 8.30 (w)	6.15 (m), 5.72 (vw), 5.28 (s), 5.01 (vs), 4.72 (vs), 4.40 (m)	1.41 ± 0.10	45 ± 5
	Cholesteric	147 to 186			3.20 ± 0.10	30 ± 5
	Liquid	Above 186			3.20 ± 0.10	30 ± 5
	Solid II (obtained from the melt of Solid I)	Below 147	25.2 (w), 22.6 (m), 19.6 (vw), 11.8 (w)	5.87 (vw), 5.75 (vw), 5.54 (vw), 5.18 (vs), 4.85 (s), 4.60 (vw), 4.40 (vw), 4.37 (vw)	1.89 ± 0.10	38 ± 5
	Solid I (obtained from 2-butanone solution)	Below 165	28.5 (w), 14.3 (w), 12.6 (m), 10.8 (vw)	6.30 (w), 5.47 (vw), 4.96 (m), 4.80 (w), 4.53 (vw)	1.35 ± 0.10	16 ± 5
Cholesteryl cinnamate $C_{36}H_{52}O_2$	Cholesteric	165 to 217			2.90 ± 0.10	30 ± 5
	Liquid	Above 217			2.90 ± 0.10	30 ± 5
	Solid II (obtained from the melt of Solid I)	Below 165	12.6 (w), 10.5 (vw), 9.50 (vw)	6.70 (vw), 5.60 (w), 5.50 (vw), 4.96 (m), 4.80 (w), 4.51 (vw)	1.80 ± 0.10	22 ± 5

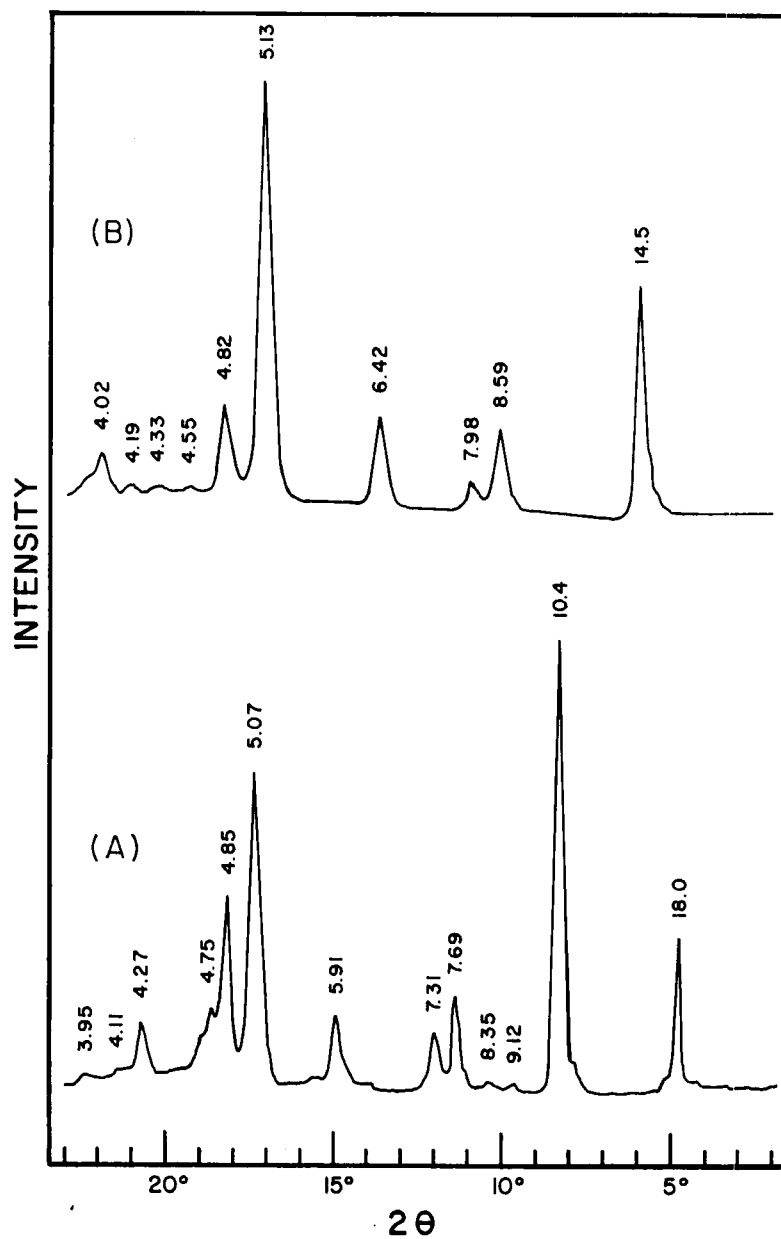(a) Mesophase obtained only on supercooling.

Figure 4.   X-ray powder diffraction patterns of samples of cholesteryl buty-
rate which crystallized from acetone solution (A) and from the melt (B).

d-spacings exhibited by the two samples, further evidence that the two solid forms are structurally different.

The following sequence of changes of state is proposed for cholesteryl butyrate:

$$C_1' \xrightarrow{\;99°\;} M \underset{110°}{\overset{113°}{\rightleftarrows}} L$$

$$M \underset{98°}{\overset{90°}{\updownarrow}} C_2'$$
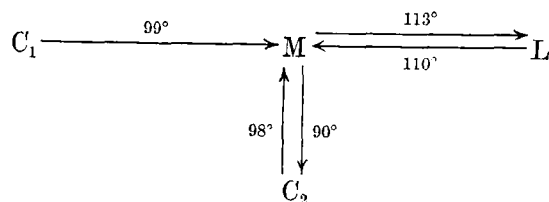
where C_1 is the solid phase formed from solution, C_2 is the solid obtained from the melt, M is the cholesteric mesophase, and L is the isotropic liquid. These results are in good agreement with those reported by Barrall *et al.*[1] except for the melting point of C_2 which differs by approximately 6 degrees.
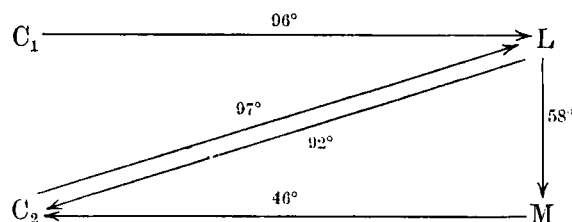
CHOLESTERYL FORMATE

Of the esters included in this study, cholesteryl formate is the only one which exhibited a monotropic cholesteric mesophase. The solid form crystallized from acetone melted at 96 °C, going directly into the isotropic liquid. On cooling DSC measurements showed an isotropic liquid → cholesteric transition occurring at 58 °C followed by a cholesteric → solid change at 46 °C. However, in the positron measurements the sample was cooled at a much slower rate, and it was observed that the isotropic liquid solidified at 92 °C without having passed through the cholesteric phase. This result is in agreement with recent findings of Davis *et al.*[6]

On the second heating cycle the melt-recrystallized solid melted at 97 °C and showed an increase of approximately 20% in the area of the melting endotherm.

Examination of the X-ray and positron data in Table 2 for cholesteryl formate indicates that the solid form obtained from solution is structurally different from the solid form obtained from the melt. Positron τ_2 values were 1.64 nsec and 1.92 nsec for the solution-grown and melt-recrystallized samples respectively.

The path of changes of state postulated for cholesteryl formate is as follows:

Two solid forms for cholesteryl formate have not been previously reported, although Porter et al.[7] obtained calorimetric data which indicated the likelihood of a metastable crystal structure formation for the formate ester.

CHOLESTERYL BENZOATE

Both the solid crystallized from benzene and the melt-recrystallized sample of the benzoate ester were observed to go into the cholesteric mesophase at 147 °C and to have a cholesteric → isotropic liquid transition at 186 °C. Upon cooling the cholesteric → solid transition supercooled by several degrees. However, the thermogram of the melt-recrystallized material showed a 25% larger heat of fusion than the solid obtained from solution.

The τ_2 lifetime in the sample obtained from solution was 1.41 nsec while that in the material obtained from the melt was 1.89 nsec. It should be pointed out that an earlier study[8] of positron lifetimes in cholesteryl benzoate also revealed different τ_2 values for samples obtained from solution and from the melt. At that time the change in τ_2 was attributed to oxidation of the melted sample. In the present work the possibility of oxidation was eliminated by use of a vacuum oven.
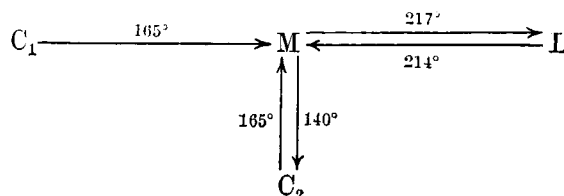
X-ray powder diffraction data in Table 2 also show that the solid obtained from the melt is structurally different. This second solid form of cholesteryl benzoate has not been previously reported.

The phase of transitions of cholesteryl benzoate can be summarized as follows:

$$C_1 \xrightarrow{147^\circ} M \underset{186^\circ}{\overset{186^\circ}{\rightleftarrows}} L$$

$$M \underset{140^\circ}{\overset{147^\circ}{\updownarrow}} C_2$$
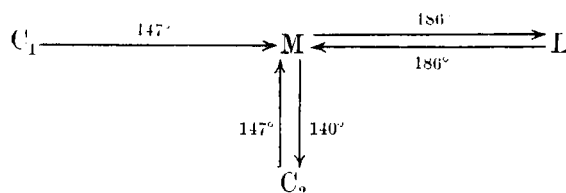
CHOLESTERYL CINNAMATE

Cholesteryl cinnamate exhibited a cholesteric mesophase over the range from 165 to 217 °C. Its DSC thermograms showed identical solid → cholesteric transition temperatures for the sample crystallized from 2-butanone and the melt-recrystallized sample, but the latter had a 20% greater heat of transition. The τ_2 lifetime and the intensity, I_2, of positrons annihilating in a sample of this ester obtained from solution (1.35 nsec and 16%) differed significantly from the values obtained for the melt-recrystallized material (1.80 nsec and 22%). In addition the solution-grown solid exhibited much larger d-spacings in the X-ray diffraction patterns. These results indicate that two distinct solid forms exist for cholesteryl cinnamate. This is in disagreement with Barrall et al.[2], who recently studied this compound by differential scanning calorimetry and found that the solid phase formed from the melt does not appear to be different from the solid formed from solution. On the other hand, we agree within 2.5 degrees with their reported transition temperatures. The proposed path of changes of state is as follows:

$$C_1 \xrightarrow{165^\circ} M \underset{214^\circ}{\overset{217^\circ}{\rightleftarrows}} L$$

$$M \underset{140^\circ}{\overset{165^\circ}{\updownarrow}} C_2$$

4. Discussion

The results given in Table 2 reveal that for all four compounds investigated the positron lifetimes and intensities showed no change at the cholesteric ⇌ isotropic liquid transitions in agreement with previous results for other esters.[4,5,8] Thus, measurements on the mesomorphic behavior of these esters were limited entirely to differential scanning calorimetry. In general our results for meso-morphic transition temperatures are in good agreement with those tabulated by Barrall et al.[2]

However, positron lifetimes in each ester were different in the sample formed from solution and the melt-recrystallized sample. The τ_2 values were always larger in the sample obtained from the melt. The I_2 values were either the same or smaller in the melt-formed sample, except in the case of cholesteryl cinnamate. Since X-ray powder diffraction patterns also showed a difference in the two samples of each ester, it is concluded that two solid forms are exhibited by each of the four esters studied. The existence of two solid forms has not been previously reported for the benzoate and cinnamate esters.

In previous studies of cholesteryl acetate[4] and cholesteryl propionate,[5] it was found that these two esters also exhibit two solid forms. However, in these compounds the second solid can be obtained by a solid–solid transition below the melting point. X-ray diffraction data for the two solid forms of the acetate and propionate esters showed that in each case the solid form with the larger τ_2 lifetime gave a pattern whose long d-spacings were intense relative to the short d-spacings. The form with the smaller τ_2 lifetime showed just the opposite behavior in its X-ray tracing. No such correlation seems to exist for the four esters included in the present study.

Figure 5 is a plot of the τ_2 lifetimes of the two solid forms of each cholesteryl ester as a function of their respective melting points. It is seen that the τ_2 lifetimes in the solution-crystallized samples decrease with increasing melting point. This dependence of τ_2 on melting point is in agreement with the results reported previously for the benzoate, stearate, and myristate esters.[8] Such a correlation might be expected because the melting point of a compound depends
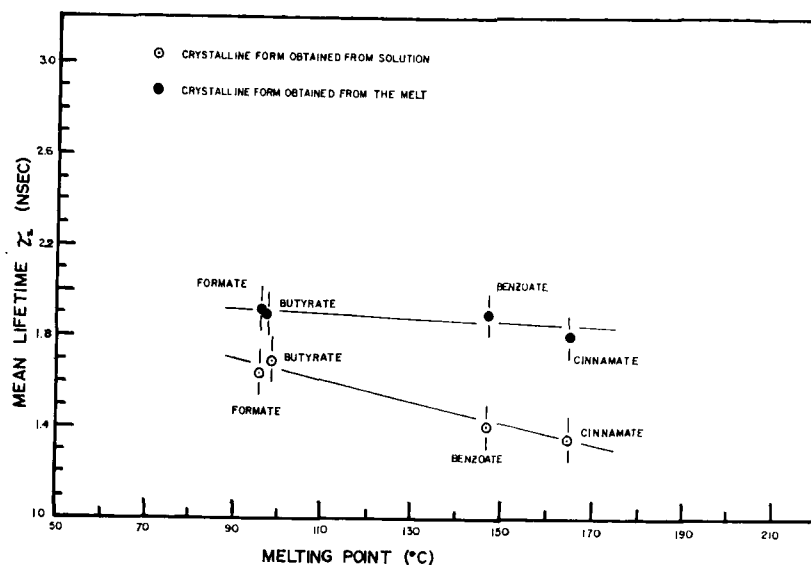
Figure 5. τ_2 lifetime as a function of melting point for solution-grown and melt-recrystallized samples.

upon the intermolecular attractive forces whose strength will depend on the way in which the molecules are packed together in the crystal lattice.

On the other hand, the melt-recrystallized samples all exhibit approximately the same τ_2 lifetime, practically independent of melting point. This might be interpreted as an indication that in the melt-formed samples, the different ester chains are no longer structurally arranged in such a way as to provide a unique electronic environment (as far as the positronium atom is concerned) for each ester.

In conclusion it is clear that the technique of positron lifetime measurement indicates that as the isotropic liquid forms from the cholesteric phase it maintains an order which is similar to that of the mesophase. Most probably the helical stack is still evident to a great extent with only the size of each swarm being diminished at the cholesteric–isotropic transition.

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