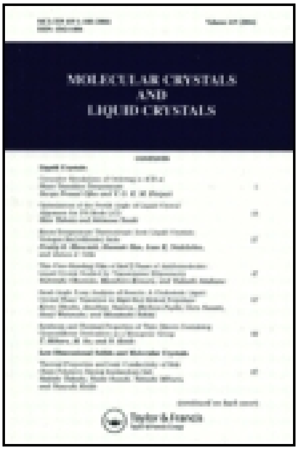

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Study of Cholesteryl Oleate-Cholesteryl Linoleate Binary Mixtures[†]

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The phase transitions for several mixtures of cholesteryl oleate and cholesteryl linoleate have been investigated by X-ray diffraction, differential scanning calorimetry and optical microscopy. Several physical quantities, *i.e.* structural and thermodynamical data, have been obtained. The results are consistent with previous investigations on pure compounds. The phase diagrams on heating, involving the solid-isotropic liquid transition, and on cooling, involving the isotropic liquid-cholesteric and cholesteric-smectic A phase transitions, have been derived. A region of nonmiscibility, in which two saturated solid solutions coexist in equilibrium, together with the presence of a eutectic, have been observed in the first phase diagram.

INTRODUCTION

Cholesteryl esters presents a biological interest as they are contained in serum lipoproteins and in the lipids of atherosclerosis lesions. As the two most abundant esters of cholesteryl in these biological systems are cholesteryl oleate (CO) and cholesteryl linoleate (CL), we have made recently an investigation of the structural properties of these two pure compounds.[1,2] In

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particular, the solid-isotropic liquid, isotropic liquid-cholesteric, and cholesteric-smectic A phase transitions were investigated by X-ray diffraction and other techniques. The two compounds showed similar physical behaviors but yielded some quantitatively different physical data.

This paper reports the results of an investigation of some physical properties of cholesteryl oleate-cholesteryl linoleate binary mixtures.

Pioneer work on these mixtures, as in general on cholesteryl esters, was performed by Small[3] by differential scanning calorimetry and optical examination. Then Krzewki and Porter[4] investigated the same mixtures by differential scanning calorimetry and polarizing microscopy.

In addition to these techniques, we have also utilized in our study X-ray diffraction.

EXPERIMENTAL DETAILS

Preparation of mixtures

The mixtures have been prepared by mixing during 30 min the pure esters (obtained from SERVA, with a claimed purity of 99%+) at 55°C after the melting process had occurred. The melt was then slowly cooled and stored at −2°C. In this way, mixtures of the following compositions in weight percent of CO were obtained: 10, 20, 22.5, 25, 30, 40, 50, 60, 70, 80 and 90.

X-ray diffraction

Part of the experiment was carried out by using a conventional X-ray powder diffractometer. Ni-filtered Cu Kα radiation (λ = 1.54 Å) was used. The divergence of the primary beam impinging on the sample was $\simeq 8'$. The generator-sample distance was $\simeq$18 cm and the sample-counter distance $\simeq$20 cm. The sample had thickness of $\simeq$1.5 mm and was held by two very thin Al sheets fixed to a circular hole in an Al matrix with diameter of $\simeq$1 cm. Heating was carried out by a hot stage (containing electrical resistors) whose temperature was controlled to ±0.1°C by an electronic device developed at G. E. N. G. in Grenoble. Part of the experiment was carried out with a Marconi–Elliot toroidal camera using the same radiation emerging from a Rigaku–Denki rotating anode generator. Similar sample holders were employed to those used in the diffractometer. Filling was performed under a nitrogen atmosphere. The heating and cooling rates were 1°C · min^{-1}.

Differential scanning calorimetry

The measurements were carried out by using a Perkin–Elmer Calorimeter, model DSC2. The scan rate was 2.5°C · min^{-1}. Al containers of 20 μl capacity were used.

Optical microscopy

Some optical observations, with crossed polarizers, were performed by using a polarizing microscope (Leitz Ortolux 2 Pol) equipped with a Mettler FP52 hot-stage. Scan rates of 1°C · min^{-1} were used during the heating and of 0.2°C · min^{-1} during the cooling.

INVESTIGATION OF THE SOLID–ISOTROPIC LIQUID TRANSITION

As substantial discrepancies exist in the data reported in the literature concerning the phase diagram obtained on heating mixtures of CO-CL, a brief summary of the available information on this subject seems suitable before presenting and discussing our experimental results.

Small[3] reports a quite complex phase diagram, involving several crystalline phases at room temperature, solid–solid phase transitions, and solid-mesophase transitions before finally melting occurs. Moreover, for CO concentrations smaller than 20%, the mixture appeared to be in a phase of the smectic type at room temperature. The author believed possible the existence of a eutectic and of monotropic mesophases in conditions of equilibrium, but doubting that the phase diagram could truly represent equilibrium conditions.

Krzewki and Porter[4] reported for the same kind of mixtures a phase diagram that was completely different, without making any reference to the previous work of Small. Complete miscibility of each ester in the other in both the liquid and solid phases and for the solid-solid transformation was obtained for each concentration. Moreover, solid-solid transitions were observed after quickly cooling the melt to −30°C and immediate successive reheating. Subsequent experiments[2,5] do not confirm this behavior for pure CL.

From analysis of the X-ray powder diffraction patterns of the solid phases of the two esters, it appeared in an our previous work[2] that the crystallographic organization of cholesteryl linoleate is not isostructural with that of cholesteryl oleate. From this fact, which contradicts the results of Ref. 4, it is expected that a limited miscibility should exist between the solid esters.

The X-ray diffraction patterns obtained for the solid phases are presented in Figures 1 and 2. For mixtures with a CO concentration larger than 40%, the stable solid phase is practically the same as that of pure CO. The crystal structure of the latter was determined by Craven and Guerina.[6] For this concentration range the diffraction patterns do not show any change till melting occurs. This type of solid solution will be called S_1.

The diffraction patterns for mixture with a CO concentration smaller than 22.5% are very similar to that of pure CL: small displacements in the low angle diffraction peaks are observed. Again for this concentration range, the diffraction patterns do not show any change till melting occurs. This type of solid solution will be called S_2.

For CO concentrations between 22.25% and 40%, the diffraction patterns observed are a combination of diffraction patterns of solid solutions S_1 and S_2. By storing the sample at −2°C for several weeks, the S_1 diffraction peaks become progressively more intense. By heating the samples corresponding to this concentration range, transitions from S_1 to $S_1 + S_2$ can be observed. All these results are summarized in the phase diagram reported in Figure 3. Solidus and liquidus temperatures were obtained by DSC using the intersection of the extrapolated leading edge and baseline and the return of the baseline, respectively.

These data were confirmed, within experimental accuracy, by X-ray diffraction and optical microscopy techniques. The confirmation of the solidus curve was obtained by observing the appearance of the X-ray diffraction peak at large angle, which characterizes the isotropic liquid phase, as is shown in Figure 4. Alternatively, the appearance of the first small isotropic regions was noted in optical observations. The confirmation of the

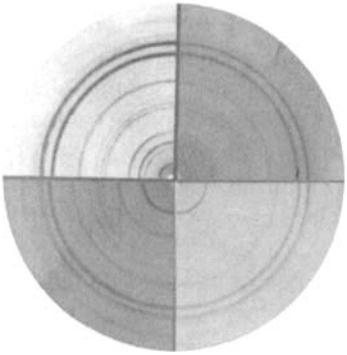


FIGURE 1 X-ray diffraction patterns obtained in the solid state: (*a*) pure cholesteryl oleate; (*b*) 50% CO-50% CL, an example of S_1 solid solution, (*c*) pure cholesteryl linoleate, (*d*) 20% CO-80% CL, an example of S_2 solid solution.

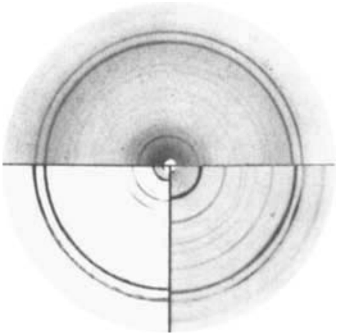


FIGURE 2 X-ray diffraction patterns obtained in the solid state: (*a*) pure cholesteryl oleate; (*b*) pure cholesteryl linoleate; (*c*) 25% CO—75% CL, an example of the combination of the diffraction patterns of the solid solutions S_1 and S_2.

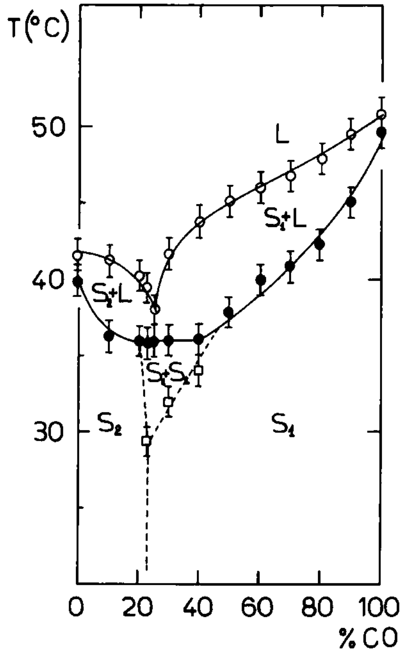


FIGURE 3 Phase diagrams obtained by heating: S_1, solid solution of CL in CO; S_2, solid solution of CO in CL; L, isotropic liquid phase. Phase boundaries represented by broken lines are only approximate.

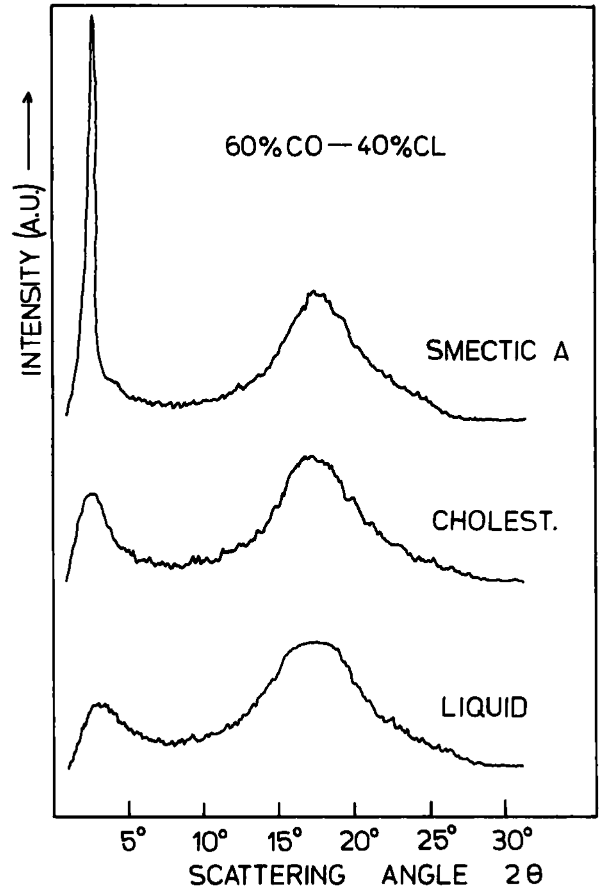


FIGURE 4 X-ray diffusion patterns obtained from the mixture with 60% CO—40% CL concentration in the isotropic liquid, cholesteric and smectic A phases.

liquidus curve was obtained by observing the complete disappearance of all the diffraction peaks characterizing the solid phases or the complete disappearance of any birefringent element. These procedures for determining the liquidus and solidus curves were applied also to the pure compounds, for which the two corresponding temperatures are reported.

A minimum in the liquidus curve and the presence of a horizontal segment in the solidus curve, which characterize a eutectic, must be emphasized. This behavior was expected on the basis of the presence of a region of nonmiscibility in the temperature range corresponding to solid phases. Moreover this eutectic observation confirms the above mentioned predictions by Small. The phase diagram obtained is in contradiction with the results of Ref. 4, as was expected on the basis of the data reported in Ref. 2 and according to the above discussion. In fact the sample treatments were different in the two experiments: in Ref. 4, the blends were homogenized by heating at 95°C for 30 min in a nitrogen atmosphere, within a sealed sample holder in the DSC unit, whereas in our experiments, the samples never reached a temperature higher than 55°C, fearing a possible degradation of the material involving the double bonds. Further discussion of this will be presented later in this publication.

INVESTIGATION OF THE PHASE TRANSITIONS INVOLVING MONOTROPIC MESOPHASES

The X-ray diffraction technique was used to investigate the isotropic liquid-cholesteric and the cholesteric-smectic A phase transitions in the different mixtures. Figure 4 reports the X-ray diffusion patterns obtained for the mixture with 60% CO concentration, in the isotropic liquid, cholesteric, and smectic phases. In this last phase, a pattern typical of a disordered smectic mesophase was obtained, i.e. a narrow low angle diffraction peak associated with the interlayer distance and a broad peak associated with the lateral intermolecular short range organization. The temperature dependence of the position Q_p in Q units ($Q = 4\pi/\lambda \cdot \sin\theta$, λ being the X-ray wavelength) of this diffraction peak, which becomes diffuse in the cholesteric mesophase, was similar to that observed in the case of the pure compounds. Moreover the same behavior was observed for all the compositions. Therefore the conclusion was reached that the smectic mesophase is of type A for all the mixtures, as for the pure compounds.

A similar behavior was observed for all the compositions at the different phase transitions during the cooling process. In particular, a transition from the isotropic liquid to the cholesteric mesophase, with phase coexistence, was observed by the different experimental techniques: for each mixture, the blue phase was observed only by optical microscopy, but not in a reproducible way as far as the start point was concerned, due to difficulties in distinguishing it from the normal cholesteric mesophase. As a consequence, in Figure 5, which reports the phase diagram obtained during the cooling process, only the disappearance of the blue phase is represented in the form of full circles. The order of magnitude of the temperature range of existence of the blue phase was found to be 0.5°C, when the careful procedure suggested by Keyes, Nicastro and McKinnon[7] was followed, including the slow cooling rate of 0.2°C · min^{-1}. By going on with the cooling process from the cholesteric mesophase, a transition to the smectic A mesophase was observed, with a phase coexistence region. The results are summarized in Figure 5.

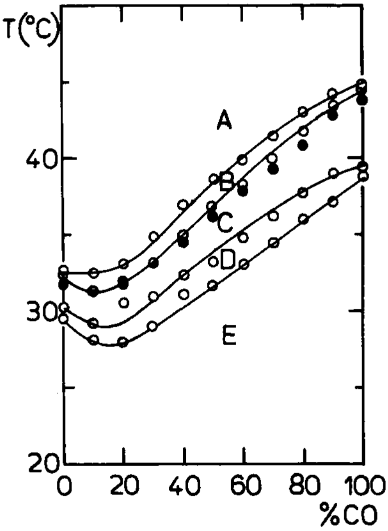


FIGURE 5 Phase diagram obtained by cooling: A, isotropic liquid phase; B, coexistence region of isotropic liquid and cholesteric phases; C, cholesteric mesophase, D, coexistence region of cholesteric and smectic A mesophases, E, smectic A mesophase. Full circles (●) represent the temperature of disappearance of the blue phase.

Minima in the transition temperatures were observed in a composition range around about 20% CO, which corresponds to the region of the eutectic in Figure 3. The smectic A phase shows a high stability, no transition to the solid phase was observed with the exception of concentrations higher than 80% CO. Table I reports the transition temperatures for the different phase transitions and for the different compositions, as obtained by reading the peak position on the differential scanning calorimetry traces. Figure 6 reports the transition enthalpies associated with the different phase transitions. It must be emphasized that the enthalpy associated with the solid-isotropic liquid phase transition is always larger, as it should be, than the sum of the enthalpies associated with the isotropic liquid-cholesteric and cholesteric-smectic A transitions. The temperature dependence of the different structural parameters during the cooling process appeared to be similar to that for the pure compounds, including pretransitional effects in the in-plane coherence length, and therefore are not reported here. Figure 7 reports the interlayer and the intermolecular lateral distances in the smectic A phase, as a function of mixture composition. The first quantity, which was obtained from the low angle peak position by using the Bragg's law, appears to increase slightly on increasing the CO percentage. The second quantity, which was obtained from the large angle diffuse peak by using the modified Bragg equation[8]

TABLE I

Temperatures (in °C) of the solid-isotropic liquid, isotropic liquid-cholesteric and cholesteric-smectic A transitions for cholesteryl oleate-cholesteryl linoleate binary mixtures

CO Wt%	Solid-isotropic liquid	Isotropic liquid-cholesteric	Cholesteric-smectic
0	41.0	32.5	30.5
10	37.5	31.0	28.1
20	38.1	31.0	28.5
30	38.5	33.0	29.7
40	38.4	36.1	31.5
50	39.5	36.5	32.0
60	40.9	38.1	33.1
70	42.7	39.1	33.1
80	44.3	41.4	36.1
90	46.1	42.8	37.7
100	49.7	44.4	38.8

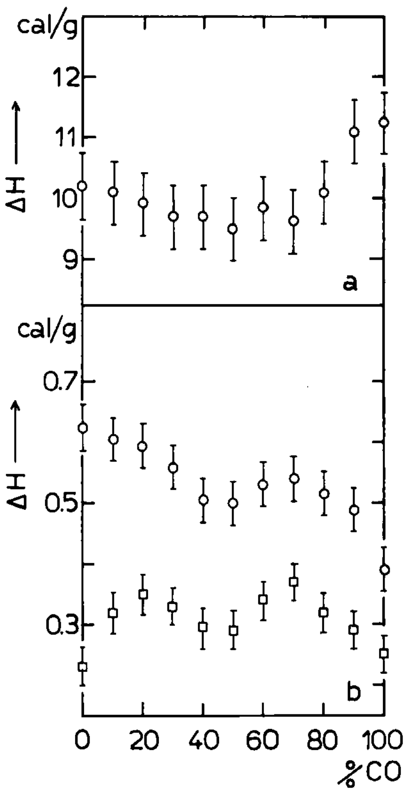
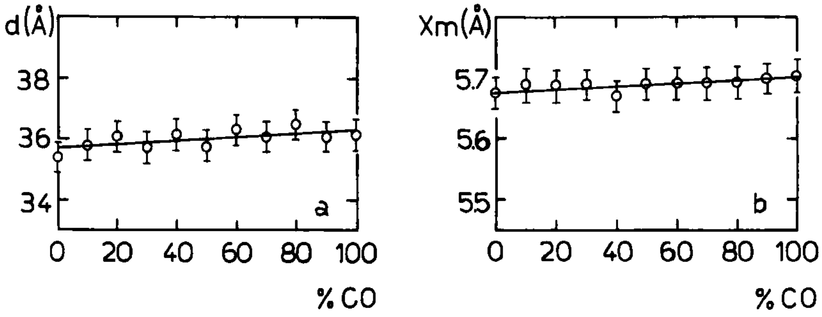


FIGURE 6 Enthalpies associated with the different phase transitions: (*a*) solid-isotropic liquid phase; (*b*) empty circles–isotropic liquid-cholesteric phase; (*b*) empty squares–cholesteric-smectic A phase.



(*a*) (*b*)

FIGURE 7 Interlayer (*a*) and intermolecular lateral (*b*) distances as a function of mixture composition.

$$1.117\lambda = 2 \cdot X_m \cdot \sin\theta$$

appears to increase slightly as the CO percentage is increased.

DISCUSSION

The striking result of the present investigation was the presence of the nonmiscibility region in the temperature range corresponding to solid phases, in agreement with previous data of Small[3] and in disagreement with the results of Krzewki and Porter,[4] who found complete miscibility between solid CO and CL.

We cannot explain the discrepancy, and in fact, as mentioned before, the results of the present experiments are in agreement with those of previous experiments on pure compounds. Actually the structure of CL was not solved due to difficulties in crystallization; however, in Ref. 2, it was observed by X-ray diffraction that CL has a different crystallographic structure to that of CO.

A possible reason for the contradiction could be that the purity of our samples is less than that claimed; however, the full width at half maximum of the DSC peak associated with the melting of pure linoleate was typical of a pure compound, i.e. $\Delta T_{1/2} \simeq 1.3$°C, as reported in Ref. 2. It would be interesting to observe whether a small percentage of added impurity could introduce a substantial change in the crystallographic structure and in the phase diagram of the mixtures, without introducing an appreciable modification of the melting point. If this were the case, it would be interesting to investigate this more carefully, particularly in view of the possible biological implications. However, in addition to the data of Ref. 2, the nonmiscibility region of the solid phases is confirmed by the observation of eutectic in Figure 3 and of a minimum in the phase diagram of Figure 5.

References

1. G. Albertini, B. Dubini, S. Melone, M. G. Ponzi Bossi and F. Rustichelli, *Mol. Cryst. Liq. Cryst.*, **70**, 169 (1981).
2. B. Dubini, S. Melone, M. G. Ponzi Bossi, F. Rustichelli and G. Torquati, submitted to *Mol. Cryst. Liq. Cryst.*
3. D. M. Small, in "Surface Chemistry of Biological Systems," (Ed. M. Blank), Plenum Press, N. Y. 1970, p. 55.
4. R. J. Krzewki and R. S. Porter, *Mol. Cryst. Liq. Cryst.*, **21**, 99 (1973).
5. D. Armitage, *Phys. Lett.*, **65A**, 68 (1978).
6. B. M. Craven and N. G. Guerina, *Chem. and Phys. of Lip.*, **24**, 91 (1979).
7. P. H. Keyes, J. Nicastro and E. M. McKinnon, *Mol. Cryst. Liq. Cryst.*, **67**, 59 (1981).
8. A. De Vries, *Mol. Cryst. Liq. Cryst.*, **10**, 219 (1970).