

726. *The Mesomorphic Behaviour of the Fatty Esters of Cholesterol.*

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Fourteen fatty esters of cholesterol have been prepared, and their m. p.s and mesomorphic transition temperatures determined. These new values are compared with constants already recorded, and the differences discussed in terms of purity and method of determination of the transition points. The number of phases exhibited by these esters is discussed, and a probable resolution of the disagreement about this number expressed in previous publications has been reached. A brief comment has been made on the nature of the cholesteric phase, such that, although it may best be regarded as an individual phase type, it is more similar to the smectic than to the nematic mesophase.

THE phenomenon of mesomorphism was first discovered by Reinitzer,¹ who observed that cholesteryl benzoate melts at 145.5° to form a cloudy, viscous melt, which on further heating is transformed into the isotropic liquid at 178.5°. It is now recognised that the ester exhibits a cholesteric phase, and Reinitzer's observation was soon followed by the preparation of many organic compounds which exhibit mesomorphism. It soon became evident that most mesomorphic compounds have phases of either the smectic or nematic type, and that cholesteric phases are found exclusively in derivatives of cholesterol or in molecules of a similar shape or constitution. The cholesteric phase is therefore less common, but, despite this, its characteristic properties have been very carefully examined by Friedel.²

In recent publications³ which relate to the effects of molecular constitution on mesomorphic behaviour, twenty-one series of alkoxyarene-carboxylic acids and alkoxybenzylidenearylamines have been described. When the mesomorphic transition temperatures for any one of these series are plotted against the number of carbon atoms in the alkyl chain, it is found that smooth curves may be drawn through the points for like transitions. Thus, in a series which exhibits both smectic and nematic phases, the nematic-isotropic transition points lie on two smooth, falling curves, dependent on whether the number of carbon atoms in the alkyl chain is even or odd, whilst the smectic-nematic points constitute a rising curve, which, at or near its maximum, joins the nematic-isotropic transition point curve. On the other hand, the *n*-propyl 4'-*n*-alkoxydiphenyl-4-carboxylates,⁴ which show only smectic phases, give smectic-isotropic transition points which again lie on two curves, but these rise to a maximum early in the series, before falling, steeply at first and then more gradually, as the alkyl chain is lengthened. Because of this characteristic behaviour of smectic-nematic, nematic-isotropic, and smectic-isotropic transition points of homologous series, it would seem reasonable to expect that transitions involving the cholesteric phase would behave similarly and lie on smooth curves. Cholesteryl esters of the normal, open-chain, fatty acids afford an opportunity to verify this, and the transition temperatures of several esters of low and high molecular weight have been reported by Jaeger⁵ and Friedel,² respectively. If their transition temperatures (solid-cholesteric, smectic-cholesteric, and smectic- or cholesteric-isotropic) are plotted against the number of carbon atoms in the alkyl chain, it is found that a smooth curve cannot be drawn through the points. It is significant too that Jaeger and Friedel quote widely different constants for cholesteryl decanoate. Moreover, Friedel,² Lehmann,⁶ and Jaeger⁵ disagree about the number and nature of the phases which they observed in several cholesteryl esters. Table I summarises the information available for twelve cholesteryl esters. Monotropic transition temperatures are in parentheses.

¹ Reinitzer, *Monatsh.*, 1888, **9**, 435.

² Friedel, *Ann. Physique*, 1922, **17**, 273.

³ Gray, Brynmor Jones, *et al.*, *J.*, 1955, 4359, and preceding parts.

⁴ Gray, Hartley, and Brynmor Jones, *J.*, 1955, 1412.

⁵ Jaeger, *Rec. Trav. chim.*, 1906, **25**, 334.

⁶ Lehmann, *Z. phys. Chem.*, 1906, **58**, 750.

A re-examination of the mesomorphic properties of the fatty esters of cholesterol is obviously necessary in view of these disagreements and the incompleteness of the available information. The twelve esters in Table 1, together with cholesteryl heptanoate and palmitate, have therefore been prepared, and their transition temperatures (solid-cholesteric or smectic, smectic-cholesteric, and cholesteric-isotropic) are summarised in Table 2, where monotropic transitions are in parentheses, and, with the exception of the temperatures for the formate, are plotted against the number of carbon atoms in the ester alkyl chain in the Figure.

In nearly all cases, the constants given in Table 2 are higher, by 1–9°, than those reported by Jaeger (Table 1). These variations may be attributed to impurity in Jaeger's esters, since it is unlikely that these would be prepared from the highly pure materials, such as are now commercially available. In only two cases have lower transition temperatures been recorded, and these involve the monotropic cholesteric-isotropic transition points for cholesteryl formate and octanoate. Jaeger's method for determining monotropic transitions involved very sudden chilling of the isotropic liquid, and it is probable that the thermometer was not at equilibrium with the supercooled melt. The recorded constants

TABLE 1.

Cholesteryl ester	Temp. of transition to			Ref.	Number of phases	
	smectic	cholesteric	isotropic		Friedel	Lehmann
Formate	—	(<i>ca.</i> 90°)	96.5°	5	1	1
Acetate	—	(80–90°)	112.8	5	1	2
Propionate	—	93°	107.2	5	1	*
Butyrate	—	96.4	107.3	5	1	2
Valerate	—	91.8	99.2	5	1	2
Hexanoate	—	91.2	100.1	5	*	2
Octanoate	—	(<i>ca.</i> 101)	106.4	5	2	2
Nonanoate	78°	79	90.5	2	2	*
Decanoate	{ —	82.2	90.6 } { 55.5	5	2	2
Laurate	79	81	88	2	2	3
Myristate	72	78	83	2	2	3
Stearate	monotropic	—	?	2	1	*

* Not examined.

TABLE 2.

Cholesteryl ester	Temp. of transition to			Cholesteryl ester	Temp. of transition to		
	smectic	cholesteric	isotropic		smectic	cholesteric	isotropic
Formate	—	(60.5°)	97.5°	Octanoate ...	(69.5)	(96.5)	110
Acetate	—	(94.5)	116.5	Nonanoate ...	(77.5)	80.5	92
Propionate ...	—	102	116	Decanoate ...	(81.5)	85.5	92.5
Butyrate	—	102	116	Laurate	(83.5)	(90)	93
Valerate	—	93	101.5	Myristate	71	81	86.5
Hexanoate ...	—	99.5	101.5	Palmitate	(78.5)	79	83
Heptanoate ... (<92.5°)	—	(95.5)	114	Stearate	(75.5)	(79.5)	83

would then be too high to an extent dependent on the rate of cooling necessary to produce the mesophase before crystallisation occurred.

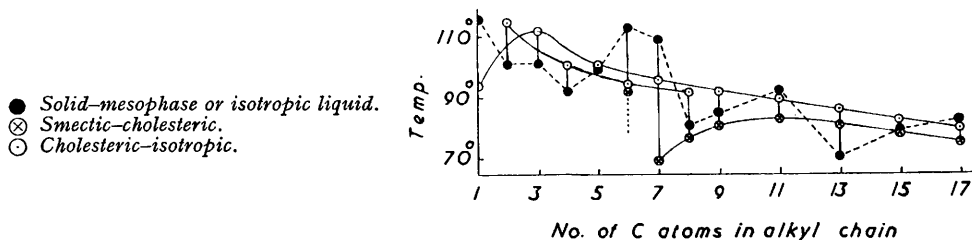
Friedel's constants (Table 1) are also, in general, lower than the present values, and in cholesteryl decanoate the difference is very great. Jaeger too records transition temperatures for this compound which are widely different from Friedel's. Impurity in Friedel's ester may again explain these discrepancies, and it is significant that he reports the presence of crystalline material in the cholesteric phases of cholesteryl laurate and myristate. Friedel explains this effect in terms of high-melting polymorphic modifications, but it is more likely that the crystals were high-melting impurity.

Despite these variations from previously recorded values, it is felt that the constants in Table 2 are accurate and reliable, since the specimens used appeared to be pure as indicated by the combustion analyses and the sharp, clear-cut reversibility of the isotropic-cholesteric and cholesteric-smectic transitions. Added confidence is given by the smooth

[1956]

Fatty Esters of Cholesterol.

curve relations shown by the transition temperatures in the Figure. Thus, the smectic-cholesteric points lie on one curve which rises at first and then falls gradually, with a maximum at cholesteryl laurate. This curve is similar to smectic-nematic curves observed in other homologous series, but it is noteworthy that, whereas in most of these cases nematic properties do not exist in the last two or three members of the series, here cholesteric phases persist in all the long-chain esters examined. The cholesteric-isotropic transitions lie on two curves, of which the lower falls steeply at first and then more gradually through the points for esters with an even number of carbon atoms in the alkyl chain. However, the upper curve, for the esters with an odd number of carbon atoms in the chain, rises steeply from the acetate to the butyrate, and then falls gradually through the points for the hexanoate, octanoate, decanoate, laurate, myristate, palmitate, and stearate. The first section of this curve cannot be drawn accurately, but the significant feature is that the curve does show a maximum. This immediately recalls the smectic-isotropic curves for the *n*-propyl 4'-*n*-alkoxydiphenyl-4-carboxylates⁴ which behave similarly. It was suggested that this behaviour in the diphenyl esters may be due to their very weak terminal intermolecular cohesions compared with the lateral and planar cohesions, and similar factors may operate in the cholesteryl esters. This cannot at present be confirmed however. If the cholesteric-isotropic transition point for cholesteryl formate were included in the Figure and considered as the origin of the curve for the esters with an even number of carbon atoms in the chain (zero being considered as an even number), this curve too would



rise at first and have a maximum at the propionate. This point has not been included in the Figure, since the terminal cohesions arising from the formate grouping will not be comparable with those originating from groups with even-number carbon chains. However, the other cholesteric-isotropic curve is so similar to the smectic-isotropic curve for the diphenyl esters that it is a logical step to relate cholesteric- and smectic-isotropic transitions, and to consider whether the cholesteric phase may not simply be a polymesomorphic modification of the smectic phase. This point of view is strengthened by other features which the two phases have in common—both phases appear from the isotropic liquid in characteristic bâtonnets which coalesce to form focal-conic groups arising from layer structures in the mesophases. On the other hand, definite differences do exist, and whilst the smectic phase is optically positive, the cholesteric phase is optically negative and has other distinctive properties (intensely iridescent coloration, high optical-rotatory power) which are not found in smectic phases. At any rate, the cholesteric phase appears to be more closely related to the smectic phase than it is to the nematic phase, which gives no bâtonnets or focal-conic groups, is optically positive, and has no iridescence or optical-rotatory power. Despite this, Friedel asserted that the cholesteric phase is nematic in type, but he based his arguments entirely upon the fact that he had never observed more than one smectic phase in a given compound, and that the smectic phase is most commonly found in conjunction with the nematic phase. Since cholesteryl esters exhibit smectic phases at lower temperatures and cholesteric phases at higher temperatures, he concluded that the latter are of nematic type. Friedel's reasoning can no longer be considered valid, because polymesomorphism⁷ is now well established, and many compounds, *e.g.*, the 6-alkoxy-5-nitro-2-naphthoic acids⁸ and the 4'-alkoxy-3'-nitrodiphenyl-4-carboxylic

⁷ Vorländer, *Trans. Faraday Soc.*, 1933, **29**, 913; Bernal and Crowfoot, *ibid.*, p. 1032.

⁸ Gray and Brynmor Jones, *J.*, 1955, 236.

acids,⁹ exhibit two or even three smectic-type phases. Moreover, the smectic-cholesteric transition point curve is similar in shape, not only to smectic-nematic curves, but also to smectic-smectic transition point curves, which also have a maximum. It is quite possible then to regard the cholesteric phase as being smectic in type, but as possessing additional characteristics which arise from the particular molecular structure of cholesteric compounds and not necessarily from the manner of orientation of these molecules relative to one another in the mesophase. However, until the molecular orientation in the cholesteric phase is known precisely, it is best to classify the mesophase as a distinct phase type, but, if this is indeed the case, its properties are more similar to those of the smectic phase, and the shape of the cholesteric-isotropic transition point curve in the Figure is an added feature which relates the phases to one another.

A further feature of the results in Table 2 is that the first six esters exhibit only one phase (cholesteric) and the remainder two phases (smectic and cholesteric). With the exception of cholesteryl stearate, these facts agree with Friedel's observations, but in several cases Lehmann⁶ reports the existence of an additional phase. It seems possible to explain these discrepancies in terms of the appearances which the cholesteric phase may adopt on a glass supporting surface. If a sample of one of these esters is melted on a glass slide and pressed down under a cover slip to obtain a thin section, observations on the number and the appearance of the phases can readily be made visually or by means of a microscope with crossed nicols, while the specimen is heated or cooled. According to Friedel, the cholesteric phase appears from the isotropic liquid in bâtonnets which coalesce to a focal-conic pattern, but, on the slightest displacement of the cover slip, this is immediately changed to the Grandjean plane structure, which consists of large homogeneous areas with stepped edges and contains only traces of focal-conic groups. When this change occurs, the sample at once exhibits intensely iridescent colours and exceedingly high optical-rotatory power. These facts have been confirmed in this study, and determinations of the transition temperatures have been made as described in the experimental section. When these measurements are made, the temperature of the mounted sample is slowly raised, and, at the melting point, the sample gives the focal-conic cholesteric phase, which, unless disturbed, remains until the cholesteric-isotropic transition temperature is reached, and the field of view between crossed nicols then becomes extinct. From previous experience all nematic- and smectic-isotropic transition temperatures have been found to be precisely reversible within the limits of experimental error, and it was expected that the cholesteric-isotropic transition would behave similarly. When the isotropic liquid is cooled, however, the bâtonnets and focal-conic groups appear at a lower temperature than the cholesteric-isotropic transition, except in cholesteryl formate where the two values are coincident. The extent of this variation between the two temperatures is shown in Table 3.

TABLE 3.

Ester	Temp. A *	Temp. B *	Diff.	Ester	Temp. A *	Temp. B *	Diff.
Formate	60.5°	60.5°	0°	Octanoate	96.5°	89°	7.5°
Acetate	94.5	93.5	1	Nonanoate	92	91	1
Propionate	116	115	1	Decanoate	92.5	88.5	4
Butyrate	113	112	1	Laurate	90	87.5	2.5
Valerate	101.5	100.5	1	Myristate	86.5	83	3.5
Hexanoate	101.5	100	1.5	Palmitate	83	80.5	2.5
Heptanoate	95.5	93.5	2	Stearate	79.5	76.5	3

* Temp. A = temperature of focal-conic cholesteric-isotropic transition.

Temp. B = temperature of isotropic-focal-conic cholesteric transition.

The isotropic-cholesteric transition temperatures given in Table 3 are average values, since, unlike the cholesteric-isotropic transition temperature which is reproducible to 0.2°, these transitions may vary over 1° in a series of measurements. The differences between the two temperatures vary irregularly along the series between 0° and 7.5°, although the larger differences tend to occur with the longer-chain esters. At first it was thought that supercooling of the cholesteric phases caused this irregular behaviour, although

• Gray, Brynmor Jones, and Marson, unpublished work.

this effect has never been found in smectic or nematic phases. The following observations, however, rule out this possibility. If the isotropic liquid obtained from cholesteryl formate is cooled on a slide and observed visually, the phase appears as a light grey wave-front, which, on examination under a microscope between crossed nicols, is seen to consist of bâtonnets and focal-conic groups. A similar set of observations on an ester such as cholesteryl octanoate shows that the phase appears as a dull, blue-grey, opaque wave-front which is well-defined to the eye, but is optically extinct between crossed nicols. On further cooling, a much less well-defined wave-front appears. This is translucent to the eye, but in fact consists of minute focal-conic groups. This behaviour was found in all esters except the formate, which gives no discrepancy between the cholesteric-isotropic and isotropic-cholesteric transition temperatures. With this exception then, the cholesteric phase appears from the isotropic liquid in a completely homœotropic condition, and the focal-conic groups appear at a lower temperature. The values given in the column headed Temp. *B* in Table 3 are therefore in fact homœotropic cholesteric-focal-conic cholesteric transition temperatures. The temperature at which the homœotropic cholesteric phase appears is in all cases the same as that at which the focal-conic cholesteric-isotropic transition occurs. That both the homœotropic and focal-conic conditions are cholesteric in type is shown by the fact that cover-slip displacements give the Grandjean plane structure in each case. Moreover, if the homœotropic phase is converted into the Grandjean structure before the development of focal-conic groups begins, these groups never appear on further cooling. The two wave-fronts were also observed by Lehmann who considered that the homœotropic and focal-conic conditions represented two distinct mesophases. It seems doubtful whether this is a legitimate conclusion, because the homœotropic to focal-conic cholesteric transitions are not exactly reproducible, the distribution of the transition points is irregular along the homologous series of esters, and the invasion of the homœotropic state by the focal-conics is a slow process, often incomplete at a much lower temperature when the smectic phase appears. Further, the change is irreversible and the focal-conic structure passes directly to the isotropic liquid on heating of the sample. This is quite unlike polymesomorphic changes in other systems which have been examined,^{8,9} and until more data concerning this change are available, it would seem best to consider the presence of only one cholesteric phase, which may adopt a homœotropic or a focal-conic texture, depending on the molecular orientation on the glass surface and the way in which this orientation changes with falling temperature.

In Lehmann's observations we therefore find that cholesteryl acetate to cholesteryl hexanoate are described as having two phases. Friedel reports that cholesteryl octanoate and decanoate show a smectic and a cholesteric phase, whereas the two phases observed in these esters by Lehmann, who did not detect the smectic phase, are the homœotropic and focal-conic cholesteric modifications. The presence of a smectic phase in the octanoate and decanoate has been fully substantiated in this investigation. In cholesteryl laurate and myristate, where Lehmann did observe smectic properties, he therefore reports three phases—homœotropic cholesteric, focal-conic cholesteric, and smectic.

Friedel makes no reference to this homœotropic condition of the cholesteric phase. It is quite possible that he did not notice its existence, particularly if he examined the phases only under a microscope where the homœotropic state is not visible. This would also explain why he does not record a cholesteric phase for cholesteryl stearate, for, as shown by the values in Tables 2 and 3, the temperature falls to within 1° of the cholesteric-smectic transition point before focal-conic groups begin to develop in the specimen. If the homœotropic state had not been observed, it would be easy to confuse this development of the groups with the appearance of the smectic phase itself.

The above facts seem to account for the differences in the number of phases observed by Friedel and Lehmann in the cholesteryl esters, and also to explain discrepancies between their conclusions and those reached in the present study.

The iridescent colours which are seen when the Grandjean plane structures of cholesteric phases are observed between crossed nicols have been discussed in detail by Friedel,² who noticed that the reflected light may vary from infrared to ultraviolet, and changes with temperature. In the cholesteryl esters the wavelength of the reflected light increases as

3738 *The Mesomorphic Behaviour of the Fatty Esters of Cholesterol.*

the temperature falls, and in most cases a greater or lesser part of the solar spectrum is traversed. In this examination, it has been further observed that the wavelength of the reflected light appears to depend also on the length of the ester alkyl chain. For example, the acetate reflects light of colour changing from green to red as the temperature falls, the propionate from violet to yellow, whilst the butyrate to decanoate reflect in the visible violet or at the most in the blue region of the spectrum. Of the above esters, the octanoate, nonanoate, and decanoate exhibit smectic behaviour, and when this phase appears it is preceded by a narrow rainbow in which the solar spectrum is completed, *i.e.*, from blue to red. This effect may be caused by disturbance of the Grandjean planes as they change to the focal-conic smectic state. In cholesteryl laurate to stearate, a further shift to shorter wavelengths is noticeable, for their cholesteric phases reflect no visible colour—the reflected light is presumably ultraviolet. These esters also exhibit smectic phases, but no rainbow effect precedes this phase's appearance. It would appear that in these esters, the disturbances involved in the orientation changes are unable to bring the wavelength of the reflected light into the visible part of the spectrum. The opposite of this is observed in cholesteryl formate. This ester reflects no visible colour of light from its cholesteric phase, but here the wavelength of the reflected light is probably in the infrared region. Cover-slip displacements caused the appearance of flashes of red reflected light.

All compounds which exhibit cholesteric phases behave in this general way, but some may show the reverse sequence of colour changes as the temperature falls, *i.e.*, changing from long to short wavelengths.

EXPERIMENTAL

Determination of Transition Temperatures.—The m. p.s and mesomorphic temperatures of the cholesteryl esters were determined by using the microscope heating block¹⁰ in conjunction with the projection system already described.¹¹ When observed microscopically between crossed nicols, the cholesteric phases are seen to consist of focal-conic groups of very variable size and definition, whilst the smectic phases are mosaics of minute focal-conic groups which have a marked tendency to become homœotropic. Such detail is not seen when the projector is used, but the difference between the images obtained from the two phases is perfectly distinct. In the case of enantiotropic transitions, the sequence of changes is, therefore, solid-smectic-focal-conic cholesteric-isotropic. On cooling, the isotropic-homœotropic cholesteric phase change is not visible when either projector or microscope is used, and this transition was obtained by visual observation of the mounted sample in the heating block. The temperature at which the focal-conic groups first appear on the screen is readily observed, as is the reversal to the smectic phase at a temperature exactly the same as that obtained on heating the sample. The sequence of changes on cooling is isotropic-homœotropic cholesteric-focal-conic cholesteric-smectic-solid. Monotropic transitions were determined by gradually cooling the specimen until the transition occurred. If supercooling was sufficient, the temperature was then slowly raised to obtain the reversal. The temperatures were coincident in all cases. The usual accuracy ($\pm 0.25^\circ$) can be claimed for all transitions, except those of the type homœotropic cholesteric-focal-conic cholesteric, which, as described in the discussion, are not quite reproducible, and average values have been quoted.

Preparation of Materials.—*Cholesteryl formate.* Pure cholesterol (1.0 g.) and 90% w/w formic acid (d 1.2; 50 ml.) were heated at 100° for 3 hr. The solution, together with some oil which separated, was poured into water (50 ml.), and the solid filtered off. The ester was crystallised from glacial acetic acid and ethanol until the transition temperatures were constant.

Cholesteryl acetate—cholesteryl hexanoate. Cholesterol (1.9 g.), pyridine (20 ml.), and the pure acid anhydride (2 mols.) were heated at 100° for 2 hr. The liquors were poured into water (50 ml.) and the solid filtered off, washed with dilute hydrochloric acid, and crystallised from glacial acetic acid and ethanol until no variation in the transition temperatures was observed.

Cholesteryl heptanoate—cholesteryl stearate. Pure acid anhydrides were not available for the preparation of these esters. The pure acids were converted into the acid chlorides by thionyl chloride, and distilled twice. Cholesterol (1.9 g.), redistilled *NN*-dimethylaniline (15 ml.), and the acid chloride (2 mols.) were heated at 100° for 1 hr., oily esters separating. The mixture

¹⁰ Gray, *Nature*, 1953, **172**, 1137.

¹¹ Gray and Ibbotson, *Nature*, 1955, **176**, 1160.

Cholesteryl ester	Found		Formula	Calc.	
	C, %	H, %		C, %	H, %
Formate	81.15	11.2	$C_{28}H_{46}O_2$	81.2	11.1
Acetate	81.2	11.2	$C_{28}H_{46}O_2$	81.3	11.2
Propionate	81.5	11.3	$C_{30}H_{50}O_2$	81.45	11.3
Butyrate	81.5	11.4	$C_{31}H_{52}O_2$	81.55	11.4
Valerate	81.7	11.6	$C_{35}H_{54}O_2$	81.7	11.5
Hexanoate	81.9	11.5	$C_{35}H_{56}O_2$	81.8	11.6
Heptanoate	81.9	11.5	$C_{34}H_{58}O_2$	81.9	11.65
Octanoate	82.1	11.7	$C_{35}H_{60}O_2$	82.0	11.7
Nonanoate	82.0	11.7	$C_{36}H_{62}O_2$	82.1	11.75
Decanoate	82.3	11.9	$C_{37}H_{64}O_2$	82.2	11.85
Laurate	82.4	12.0	$C_{39}H_{68}O_2$	82.4	12.0
Myristate	82.6	12.2	$C_{41}H_{72}O_2$	82.55	12.1
Palmitate	82.65	12.2	$C_{45}H_{76}O_2$	82.7	12.2
Stearate	82.9	12.3	$C_{45}H_{80}O_2$	82.8	12.3

was poured into water (50 ml.) and extracted with ether, and the extract washed with 2N-sulphuric acid until the dimethylaniline had been removed. Washing was continued with water, aqueous sodium hydrogen carbonate, and water, the ether solution dried (Na_2SO_4), and the solvent removed. The residual esters were crystallised from glacial acetic acid and ethanol until the transition temperatures were constant.

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