MESOMORPHIC BEHAVIOUR OF SATURATED AND MONO-UNSATURATED C_{18} AND C_{22} FATTY ACID ESTERS OF CHOLESTEROL

Slavomir PIRKL
Department of Physics,
University of Chemical Technology, 532 10 Pardubice, The Czech Republic

Received May 5, 1993
Accepted September 20, 1993

The phase transitions and effective optical rotary power of saturated and monounsaturated aliphatic esters of cholesterol with 18 and 22 carbon atoms in the chain have been described. The effect of *cis/trans* isomerism on these properties is discussed.

Esters of cholesterol and long-chain acids represent an important group of nonpolar biologically active lipids and are also interesting as mesogenic compounds for some technical applications, e.g. temperature measurements or temperature mapping[1]. In both cases it is important to know their thermodynamic properties. In addition to it, for preparation of coloured temperature indicators it is desirable to know the so-called effective rotary power (ERP) which determines how a given ester affects the pitch of helix p in the mixture forming the cholesteric mesophase[2]. This quantity is defined as reciprocal value of pitch of helix (p^{-1}) and can be ascribed also to the compounds which do not form a cholesteric mesophase at the given conditions. In such cases ERP is determined by extrapolating the concentration dependence of the rotary power p^{-1} on composition of suitable binary mixture. Especially suitable for such extrapolation is the equation by Adams and Haas[2] and that by Kozawaguchi and Wada[3,4] which were derived under the presumption of nonexistence of specific mutual interactions of molecules. In the simplest case, the rotary power of mixture p^{-1} is the direct weighted mean of the ERP's of components, which was verified experimentally also for multicomponent mixtures of cholesterol esters (according to convention, the ERP values have opposite signs for left-handed and right-handed components).

The cholesteric pitch can easily be determined by measuring the wavelength λ_0 of the maximum of a selective light scattering on the layer of mesophase with planar texture. For the normal incidence it is $\lambda_0 = 2\,\overline{n}\,\,p$, where the mean refractive index $\overline{n} \cong n_0$. As the value $\overline{n}$ is changing only very little with composition of cholesterol esters, the change in λ_0^{-1} corresponds to that in rotary power p^{-1}. Many papers have shown that the value of refractive index is almost the same with all esters investigated and varies about the value of 1.5 (refs[1,5]). Hence in the case of spectrophotometry the results can directly be expressed with the help of the wavelength λ_0 and/or λ_0^{-1}, which in addition provides a better idea of the colour of mesophase. Then the quantity $\theta = \lambda_0^{-1}$ is called the optical rotary power or effective optical rotary power (EORP).

In the present communication we describe the phase transitions and effective optical rotary power (EOPR) of saturated and monunsaturated esters of cholesterol with the acyl chain length of 18 and 22 carbon atoms and the effect of *cis/trans* isomerism on these properties.

EXPERIMENTAL

Chemicals

Cholesteryl elaidate (CEl) and cholesteryl oleate (CO) (for biochemical purposes, Merck Co); the former was purified by two recrystallizations from acetone before use, the latter was used without any treatment. Cholesteryl stearate (CS), cholesteryl behenate (CBe), cholesteryl erucate (CE), cholesteryl brassidate (CBr), and cholesteryl chloride (CC) were synthesized by conventional methods from commercial cholesterol (Chemopharma Usti nad Labem) and the respective acids whose purity was above 99% (GC). Brassidic acid was obtained by the rearrangement of erucic acid[6].

The raw synthetic products were purified by column chromatography (Silica gel L 40/100, tetrachloromethane) and subsequent recrystallization from ethyl acetate until the constant temperatures of phase transitions. The absence of starting materials from the products was proved by TLC.

The samples of mixtures were prepared by heating the weighted components above the clearing temperature under vigorous stirring.

Measurement

The temperatures of phase transitions were measured with the help of a polarizing microscope with a special heating/cooling stage. The melting was monitored in nonpolarized light and the other phase transitions between crossed polarizers. The temperature of inversion of rotation of cholesteric mesophase was determined by the disappearance of finger print texture from the thin layer of mesophase between homeotropically orienting surfaces.

The selective scattering of light (λ_0) at the thin layers of cholesteric mesophase with planar texture was measured spectrophotometrically.

RESULTS AND DISCUSSION

Phase Transitions

With all the aliphatic esters of cholesterol we found the formation of smectic mesophase type A (SmA), mostly monotropic one. This mesophase is highly stable and exists for a long time without crystallization even after deep undercooling of CO and CE. The cholesteric mesophase was observed with complete certainty only with CSt, CBe, CO, and CEl. In the case of CE and CBr we observed signs of formation of cholesteric mesophase only in a very narrow temperature interval (0.2 to 0.3 °C) below the temperature of transition to isotropic liquid. In contrast to the results published earlier[7] we succeeded in observing the formation of smectic and cholesteric mesophases also with CBe and cholesteric mesophase with CE. The measured t_i value of CBe agrees well with the presumed value of 73 °C (ref.[7]).

Cholesteryl chloride only forms a monotropic cholesteric mesophase unable of sufficiently long existence at deep undercooling. Except for CE (Fig. 1*b*) the esters investigated form the mesophases only when undercooled (Fig. 1*a*). The temperatures of phase transitions are given in Table I. From the results it is obvious that:

TABLE I
Temperatures of transitions to isotropic liquid (t_i) and temperatures of transitions between smectic and cholesteric mesophases (t_{sc})

Cholesteryl ester	*n* [a]	M.p., °C	t_i, °C	t_{sc}, °C
Stearate CSt	18	78.0 – 79.0	79.0 – 79.2	76.0[b]
Elaidate CEl (*trans*)	18	65.2 – 66.5	52.6 – 52.7[b]	49.0[b]
Oleate CO (*cis*)	18	49.0 – 50.8	49.8 – 50.1[b]	45.4[b]
Behenate CBe	22	89.4 – 89.6	75.0[b,c]	72.5[b,c]
Brassidate CBr (*trans*)	22	63.3 – 64.5	56.4 – 56.6[b]	[d]
Erucate CE (*cis*)	22	47.0 – 47.7	50.6 – 50.8	[d]
Chloride CC	–	96.0 – 97.0	67.8 – 68.0[b]	–

[a] *n* Number of carbon atoms in the acyl chain. [b] Monotropic transition. [c] With regard to the fact that CBe is unable of a sufficiently long existence in the undercooled state the error of measurement is ±0.3 °C. [d] The formation of cholesteric mesophase is probably in the temperature interval ca 0.3 °C below the transition to isotropic liquid.

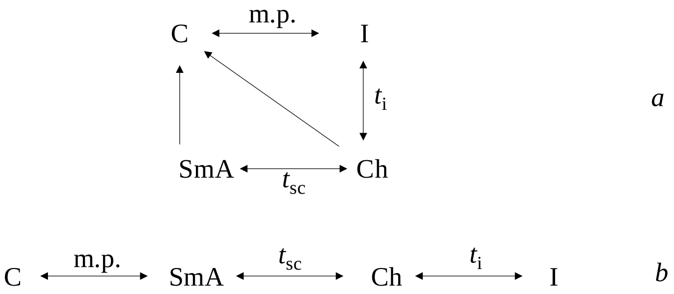

FIG. 1
Scheme of phase transitions of cholesterol esters: *a* for CS, CBe, CO, CEl, CBr; *b* for CE. C, crystal; Ch cholesteric mesophase; SmA smectic mesophase A; I isotropic liquid

1) The melting point, t_i, and t_{sc} are increased in the order *cis*-isomer < *trans*-isomer < saturated chain, the increase being the most distinct at the melting point. As a consequence, the unsaturated *cis*-isomers form enantiotropic cholesteric mesophase, whereas the *trans*-isomers only form the monotropic mesophase. This trend is more pronounced with the esters containing a higher number of carbon atoms.

2) The thermal stability of mesophases is decreased by introducing a double bond, being lower with the *cis*-isomer than with the *trans*-isomer.

Effective Optical Rotary Power (EORP)

All the aliphatic esters of cholesterol investigated are white crystalline solids at room temperature and form the cholesteric mesophase either in the undercooled state or in a very narrow temperature interval, hence a measurement of selective scattering of light is impossible. Therefore EORP was determined by extrapolating the concentration dependences of λ_0^{-1} in mixtures of individual left-handed esters of cholesterol with right-handed cholesteryl chloride. A typical example of phase diagram of such a mixture is presented in Fig. 2 and others were published earlier[8,9]. For the determination of EORP the most significant part of this diagram is the dependence of t_n giving the composition of completely compensated mixtures with infinite pitch of helix ($p^{-1} = 0$).

The other points for construction of concentration dependences of λ_0^{-1} or p^{-1} were obtained by measuring the temperature dependences of λ_0 for mixtures with varying content of cholesteryl chloride (e.g. see Fig. 3). Unfortunately, the selective scattering of light could not be measured for cholesteryl behenate because the cholesteric mesophase is not sufficiently stable in the undercooled state.

With respect to considerably different temperature intervals of existence of cholesteric mesophase in mixtures of different compositions it is advantageous to construct

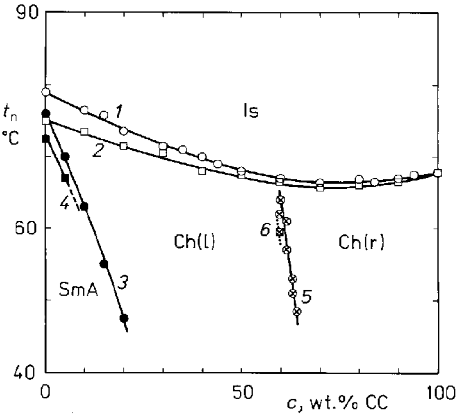

FIG. 2
Effect of composition on temperatures of phase transitions in the mixtures of CSt–CC (circles) and CBe–CC (squares). *1*, *2* temperature of transition to isotropic liquid; *3*, *4* temperature of transition of cholesteric mesophase to smectic mesophase; *5*, *6* temperature of formation of compensated mixture

the concentration dependences for the reduced temperature differing from t_i by a constant value. In this way it is also possible to compare the mesophases with the same parameter of arrangement[10]. However, it turned out that in this case the agreement between experiment and theory is somewhat worse than that in the case of isothermal concentration dependences.

Typical examples of the concentration dependences of λ_0^{-1} are presented in Figs 4 and 5 (for others see refs[8,9]). In this case, according to the convention, the values of pitch of left-handed and right-handed helices were plotted as positive and negative, respectively. This procedure has the advantage in that it enables determination of λ_0 value by extrapolation even in the long-wave spectral region which is not easily accessible experimentally. The concentration dependences are linear in a wide range if the concentration is expressed in wt.%. Only the experimental points in the region of low concentrations are affected by the pre-transition phenomena near the change of the cholesteric mesophase into the smectic one and deviate from the linear dependence. In accordance with the theory by Adams and Haas[2] we get the equation (*1*),

$$\lambda_0^{-1} = \sum w_i \lambda_i^{-1}, \qquad (1)$$

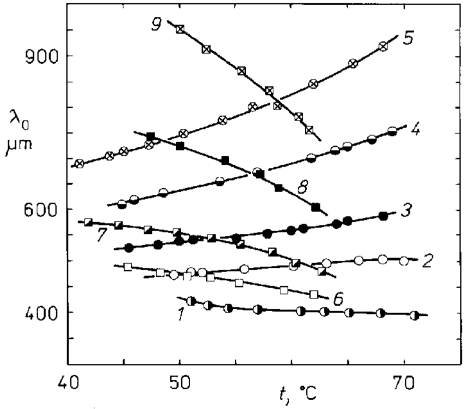

FIG. 3
Temperature dependence of wavelength of maximum reflectivity λ_0 for CSt–CC mixtures with variable CC concentration (wt.%): *1* 20, *2* 30, *3* 35, *4* 40, *5* 44, *6* 94, *7* 90, *8* 84, *9* 80

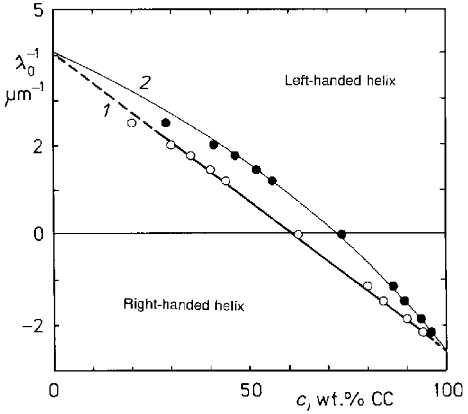

FIG. 4
Dependence of reciprocal value of wavelength of maximum reflectivity on CC content in CSt–CC mixture at reduced temperature (t_i − 10) °C. Solid lines are for theoretical dependences calculated from: *1* Eq. (*1*), *2* Eq. (*2*). $A = 1.65$, $\lambda_{CSt}^{-1} = 4.08$, and $\lambda_{CC}^{-1} = -2.53$. ○ Experimental values for wt.% CC; ● experimental values for mole % CC

where w_i is the mass fraction of the i-th component and λ_i^{-1} are the EORP values of pure components.

When the amounts of components are expressed in mole %, the concentration dependence of λ_0^{-1} is curved in the whole range investigated and can better be described by Eq. (*2*) according to Kozawaguchi and Wada[4],

$$\lambda_0^{-1} = [(1-x)\,A\ \lambda_a^{-1} + x\ \lambda_b^{-1}]/[A\,(1-x) + x]\,, \qquad (2)$$

where x is mole fraction, A is a material constant, and λ_a^{-1} and λ_b^{-1} are the EORP values of pure components.

The extrapolation according to both equations (*1*) and (*2*) leads to the same EORP values of the investigated esters of cholesterol. However, the agreement between experiment and theory is better for isothermal dependences than that for the dependences at reduced temperature. The results are given in Table II. It is evident that the EORP value decreases in the order: saturated chain > *trans*-isomer > *cis*-isomer, as well as with decreasing length of molecule. This is in accordance with the observation of decreasing ERP with decreasing number of carbon atoms in fatty acid residue in homologous series of cholesteryl alkanoates[11,12]. As the effect of number of carbon atoms upon

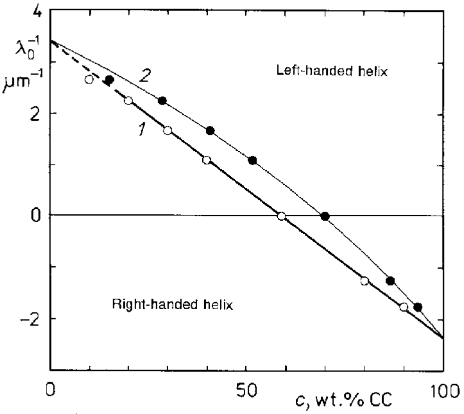

FIG. 5
Dependence of reciprocal value of wavelength of maximum reflectivity on CC content in CEl–CC mixture at 45 °C. Solid lines are theoretical dependences calculated from: *1* Eq. (*1*), *2* Eq. (*2*). $A = 1.57$, $\lambda_{CEl}^{-1} = 3.42$, $\lambda_{CC}^{-1} = -2.35$. ❍ Experimental values for wt.% CC; ● experimental values for mole % CC

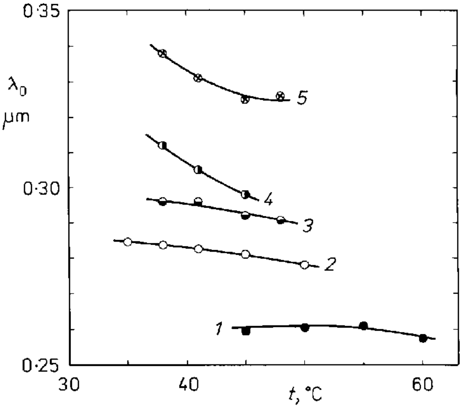

FIG. 6
Temperature dependence of fictitious values of wavelength of maximum reflectivity λ_0 obtained by extrapolation for investigated cholesterol esters: *1* CSt, *2* CBr, *3* CEl, *4* CE, *5* CO

the EORP values decreases with increasing number of C atoms, it can be presumed that the EORP for CBe will be close to EORP for CSt. In such a case the effect of number of C atoms upon EORP decreases in the order: *cis*-isomer > *trans*-isomer > saturated chain.

The EORP values found in this work belong to the highest ones so far published and correspond to a fictitious selective scattering of light in the ultraviolet region of spectrum. The EORP value for cholesteryl chloride agrees well (within ±2%) with the values found by Hanson[13].

As it could be expected EORP is also a function of temperature and its value increases with increasing temperature. Figure 6 represents the change of corresponding fictitious wavelength values of selective scattering λ_0 with temperature. Like in the case of left-handed cholesterol esters forming a stable cholesteric mesophase[5] the λ_0 value increases with decreasing temperature. The change is more distinct with *cis*-isomers than with *trans*-isomers.

TABLE II
Extrapolated EORP values at various temperatures (°C)

Cholesteryl ester	$t_i - 10$		45	
	λ_0, μm	λ_0^{-1}	λ_0, μm	λ_0^{-1}
Stearate	0.245	4.08	0.260	3.85
Elaidate	0.283	3.53	0.292	3.42
Oleate	0.322	3.10	0.330	3.03
Behenate	[a]	[a]	[a]	[a]
Brassidate	0.272	3.68	0.281	3.56
Erucate	0.289	3.46	0.299	3.34
Chloride	0.402	2.49	0.424	2.35

[a] CBe is unable of a sufficiently long existence in the undercooled state.

REFERENCES

1. Kelker H., Hatz R.: *Handbook of Liquid Crystals*. Verlag Chemie, Weinheim 1980.
2. Adams J., Haas W.: Mol. Cryst. Liq. Cryst. *30*, 1 (1975).
3. Kozawaguchi H., Wada M.: Jpn. J. Appl. Phys. *14*, 651 (1975).
4. Kozawaguchi H., Wada M.: Jpn. J. Appl. Phys. *14*, 657 (1975).
5. Demus D., Wartenberg G.: Pramana *1*, 363 (1975).
6. Doree C., Pepper C.: J. Chem. Soc. *1942*, 447.

7. Ginsburg G. S., Small D. M.: Biochim. Biophys. Acta *664*, 98 (1981).
8. Pirkl S., Myl J. in: *Advances in Liquid Crystal Research and Applications* (L. Bata, Ed.), p. 657. Pergamon Press, Oxford 1980.
9. Pirkl S.: Sci. Papers Univ. Chem. Technol., Pardubice *37*, 25 (1977).
10. Finkelmann H., Stegemeyer H.: Ber. Bunsenges. Phys. Chem. *78*, 869 (1974).
11. Baessler H., Labes M. M.: J. Chem. Phys. *52*, 631 (1970).
12. Adams J. E., Haas W., Wysocki J. J. in: *Liquid Crystals and Ordered Fluids* (J. F. Johnson and R. S. Porter, Eds), p. 463. Plenum Press, New York 1970.
13. Hanson H., Dekker A. J., van der Woude F.: Mol. Cryst. Liq. Cryst. *42*, 15 (1977).

Translated by J. Panchartek.