

Raman studies of the C=C and C=O stretching modes in various cholesteryl alkanooates

S. Bresson, D. Bormann, and B. Khelifa

Laboratoire de Physicochimie des Interfaces et Applications, Université d'Artois, Faculté Jean Perrin, SP 18, rue Jean Souvraz, 62307 Lens Cedex, France

(Received 22 January 1997)

Cholesteryl alkanooates were previously mostly studied by biochemists, and more rarely by physicists. In this work we present Raman studies of the C=C and C=O stretching modes for various cholesteryl alkanooates after different treatments. The unicity of these bonds in these molecules permits us to propound the C=C stretching mode as an internal reference in regard to its independence from the alkyl chain length. The splitting of the C=O stretching mode leads us to conclude on the existence of two types of molecules, differing only by angle and bond-length variations, in the polycrystalline state. [S1063-651X(97)12906-3]

PACS number(s): 87.15.By, 87.10.+e

I. INTRODUCTION

Cholesterol derivatives present great interest in biological science, and many studies, using various physical techniques, have been made on systems where cholesteryl esters are implied [1–6]. Thus it is generally accepted that cholesteryl esters play a prominent part in understanding the genesis of atherosclerotic lesions [7–9]. These cholesterol derivatives, observed from the material science point of view, are very attractive in regard to their numerous phases [10,11]. One of the reasons for the physical interest is that these compounds can present various liquid crystal phases and in particular cholesteric phases [12]. Such liquid crystals are known to exhibit anisotropic electro-optical properties [13–16].

Raman investigations of various cholesterol liquid crystalline materials were carried out, with a biological background, by Bulkin and Krishnan [17]. Since this pioneering work, few cholesteryl ester studies were focused on some specific spectral ranges [18,19]. The few results about the C–H stretching spectral range and about the low-frequency behavior have opened an interesting research field [18,20,21]. The physical information extracted from these works was not sufficient to conclude about the intramolecular and intermolecular interactions. Although the first investigations using Raman spectroscopy are old, no decisive progress was registered since the late 1970s. In order to understand the behavior of the cholesteryl esters, we have undertaken to analyze these molecules using Raman spectroscopy by varying various parameters. This technique is very sensitive to the conformational changes and material organization.

We focused our attention on the cholesteryl alkanooates family, the rough formula is



where $n-1$ is the number of the alkyl chain carbons.

These molecules are composed of two characteristic groups: the steroid skeleton (rings A, B, C, and D) and the isopropyl group and the alkyl chain (Fig. 1).

A “knuckle joint” binds the two groups. This “knuckle joint” is constituted by the carbonyl group $O_3-C_{28}=O_{28}$. The double bond between C_{28} and O_{28} will be the object of the direct influence of the steroid skeleton and the alkyl chain at the same time.

X-ray studies on cholesteryl alkanooates were performed in the 1970s [22–28]. More recently Sawzik and Craven investigated the crystalline structure of cholesteryl acetate ($n=2$) by the same technique [29] and also by neutron diffraction [30]. The x-ray results obtained by these authors confirmed the previous works [22] that cholesteryl acetate is monoclinic with space group $P2_1$ in the crystalline state below 123 K. The unit cell contains four molecules arranged in two asymmetric units, implying the existence of two types of molecules, labeled (A) and (B). These molecules are only differentiated by a few conformation details. The neutron studies confirm and refine the x-ray results, and specify the bond length and angle variations between molecules (A) and (B) [30]. These studies show that the essential conformational differences are located on the steroid A and B rings and on the ester group. On one hand, there is a distortion from the chair conformation of A and B rings in the molecules (A) relative to the molecules (B). On the other hand, these authors notice a twisting of the “knuckle joint” group $O_3-C_{28}=O_{28}$ with various torsion angle and bond lengths for molecules (A) and (B). The $C_2-C_3-O_3-C_{28}$ torsion angle has a value of 151.3° in molecules (A), whereas in

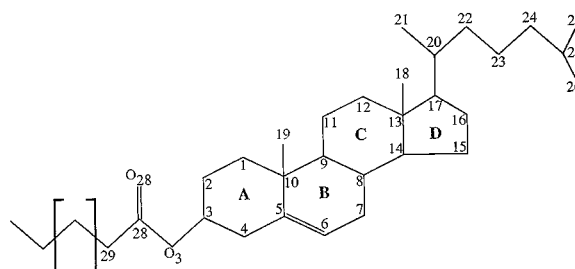


FIG. 1. The cholesteryl alkanooate molecules. The cholesteryl alkanooate molecules have a “knuckle joint” group: $O_3-C_{28}=O_{28}$. It separates the steroid (rings A, B, C, and D) and the isopropyl group and the alkyl chain beginning at C_{28} ($n=1$).

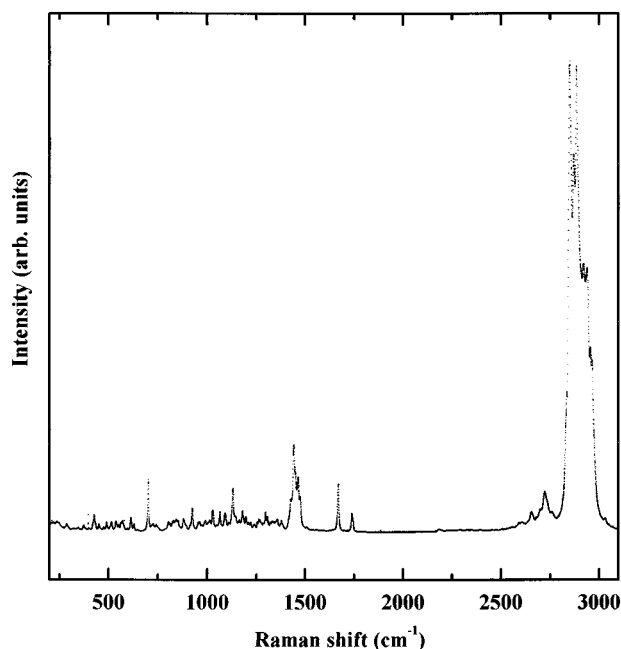


FIG. 2. Raman spectrum in the range 200–3100 cm^{-1} of the cholesteryl tetradecanoate. In the range 1550–1800 cm^{-1} , we observe few isolated peaks.

molecules (*B*) this angle equals 161.5° [30]. At the same time, $\text{C}_4\text{—C}_3\text{—O}_3\text{—C}_{28}$ pivots from an angle of 10.4° between molecule (*A*) conformation and the molecule (*B*) one [29]. The bond length $\text{O}_3\text{—C}_{28}$ is 1.350 Å for molecules (*A*) and 1.344 Å for molecules (*B*). More important is the bond length shift of $\text{C}_3\text{—O}_3$, it increases from 0.010 Å between molecules (*A*) and (*B*).

The Raman spectra of the cholesteryl alkanooates in the range 200–3100 cm^{-1} are very bushy (Fig. 2). Therefore, given that the $\text{C}=\text{O}$ double bond plays a major role in the ‘‘knuckle joint’’ group $\text{O}_3\text{—C}_{28}=\text{O}_{28}$, in this work, we have focused, our attention only on the 1500–1800- cm^{-1} range in order to characterize the influence of, on one hand, the alkyl chain length and, on the other hand, of the sample history. The stretching of the double bonds $\text{C}=\text{O}$ and $\text{C}=\text{C}$ are very interesting to analyze if we remark that they are unique in these molecules; this implies that these bonds are good candidates to be internal references. In the present paper, we present Raman spectroscopic results for the range 1550–1800 cm^{-1} for various cholesteryl alkanooates differing by the alkyl chain length and with different sample histories.

II. EXPERIMENTAL TECHNIQUE AND SAMPLE PREPARATION

The cholesteryl alkanooate spectra have been obtained with a DILOR (*XY*) Raman spectrophotometer. We used an excitation wavelength of 514.5 nm, and the beam was focused on the samples by microscope, the spot area was nearby 1 μm^2 . The signal was collected through a premonochromator and a charge-coupled-device (CCD) detector was used. With such an experimental configuration, the spectral resolution is of the order of 2 cm^{-1} , and the density of power is in order of a few mW to avoid the destruction of the samples.

TABLE I. Characteristics of the studied samples.

Cholesteryl Alkanooates	<i>n</i>	Supplier	Purity (%)
Formate	1	Sigma	98
Acetate	2	Sigma	99
Pentanoate or valerate	5	Sigma	93
Nonanoate or perlagonate	9	Fluka	99
Tetradecanoate or myristate	14	Sigma	99
Behenate	22	Sigma	95

In this work, we analyze six cholesteryl alkanooates in different phases. These products were bought by the Aldrich-Sigma-Fluka society, and were in a polycrystalline form; the characteristics of the studied samples are given in Table I. The first work was performed on these samples without further treatment. In a second step we verified that the original samples were effectively in a polycrystalline state. For this aim, we recrystallized the samples using a classical solvent for such molecules: chloroform [31,32]. The cholesteryl alkanooates were dissolved, and the solvent was slowly evaporated at atmospheric pressure and room temperature, over a few days, to obtain a polycrystalline phase. The third step was to compare the polycrystalline and noncrystalline phases at room temperature. In order to obtain the noncrystalline phase, the polycrystallized samples were heated to 383 K to reach the isotropic liquid phase [10,11], and after 12 h they were cooled down to the room temperature. This cooling was slow to avoid a quenching of the samples, the rate was 5 K/h. These samples are labeled ‘‘melted’’ cholesteryl alkanooates.

III. RESULTS AND DISCUSSION

The spectra in the range 1550–1800 cm^{-1} of the different samples and after different treatments are shown in Figs. 3–5. The spectra of the samples without further treatment are shown in Fig. 3. Whatever the sample, we always observe a strong peak nearby 1670 cm^{-1} and a weaker peak at higher frequencies, split or not into two components. We notice the existence of a very weak peak near 1640 cm^{-1} in each spectrum for any sample.

The split mode frequencies, in the case of the formate $n=1$, are lowered of about 30 cm^{-1} with regard to the other samples, $n=2, 5, 9, 14$, and 22 (see Table II for the precise pointed values). For the cholesteryl alkanooates with a ‘‘real’’ alkyl chain ($n>1$) we observed a well-resolved doublet for the cholesteryl tetradecanoate and behenate samples; the reported frequencies values (Table II) are the pointed one. For the cholesteryl acetate samples, the doublet is weakly resolved; the pointed and fitted frequencies values are the same and are labeled ** in Table II. More striking is the fact that in the case of the cholesteryl pentanoate and nonanoate samples we observed only a broad peak in the same spectral region (1730–1740 cm^{-1}). The fitting is only successful with two components (fitted frequencies values labeled * in Table II). Our results show that for the studied series of cholesteryl alkanooates this weak peak always has two components.

In Fig. 4 we present the spectra after recrystallization. The appearance of these samples under a microscope was the same as for the nontreated samples: microcrystallites. Previ-

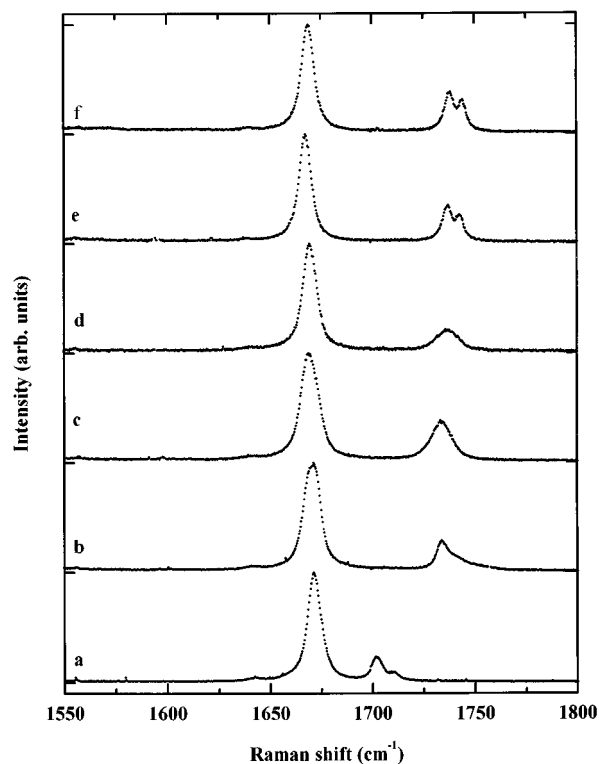


FIG. 3. Raman spectra of the cholesteryl formate (a), acetate (b), pentanoate (c), nonanoate (d), tetradecanoate (e), and behenate (f) without treatment.

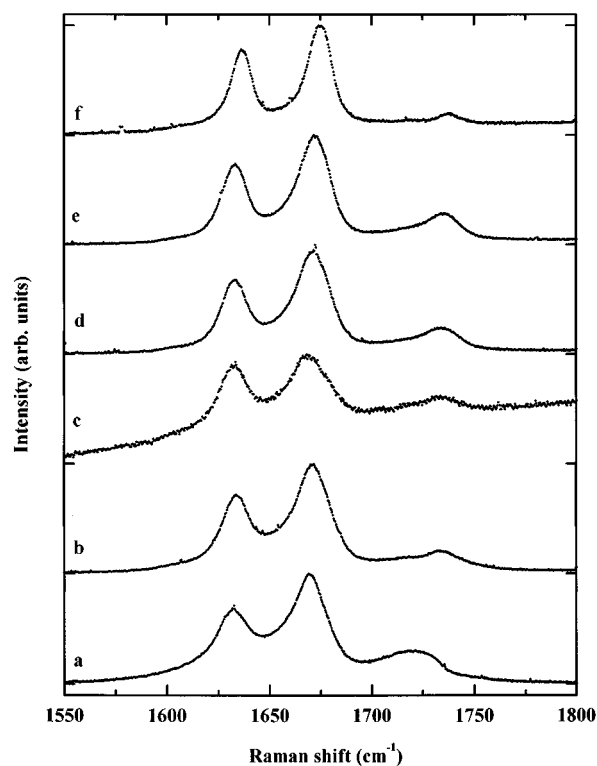


FIG. 5. Raman spectra of the "melted" cholesteryl formate (a), acetate (b), pentanoate (c), nonanoate (d), tetradecanoate (e), and behenate (f). There is a broadening of the C=C stretching mode and a disappearing of the C=O stretching doublet.

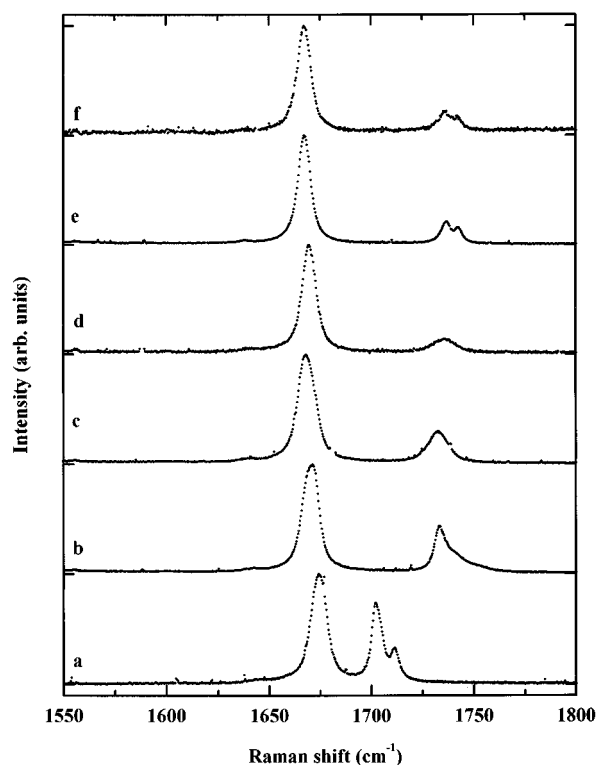


FIG. 4. Raman spectra of the recrystallized cholesteryl formate (a), acetate (b), pentanoate (c), nonanoate (d), tetradecanoate (e), and behenate (f).

ous observations on nontreated samples are available for these recrystallized samples. The peaks are pointed or fitted at the same frequencies (Table II) as for the nontreated samples, taking into account the experimental and calculation errors (1.5 cm^{-1}). We remark that the intensity ratio between the strong 1670-cm^{-1} peak and the weak doublet is not conserved after the recrystallization.

The "melted" samples spectra are reported in Fig. 5. These spectra differ greatly from the recrystallized and nontreated sample spectra. The peak nearby 1670 cm^{-1} is as strong as before melting, but he is broad (the half-width of about 15 cm^{-1}). In place of the doublets we observe weak peaks with a half-width of about 15 cm^{-1} , it is not possible to fit them with two components, so in Table II we present the frequency distribution ranges. These broad peaks have a central frequency which corresponds approximately to the doublet one. That is true for all the samples except for cholesteryl formate, whose spectrum shows a frequency shift of about $+10 \text{ cm}^{-1}$ between the weak peak frequency and the doublet one. The very weak peak observed on the nonmelted samples at about 1640 cm^{-1} is not observed but a strong broad peak (labeled "SB peak" in Table II) appears for each cholesteryl alkanate around 1635 cm^{-1} .

The strong peak near 1670 cm^{-1} observed in every sample spectrum is assigned to the $\text{C}_6=\text{C}_5$ stretch vibration. In the case of the cholesteryl alkanates considering the $\text{C}_6=\text{C}_5$ position inside the six membered ring *B* we can, in a

TABLE II. The frequencies values of the C=C and C=O stretching modes for the studied samples in three cases: without treatment, after recrystallization, and after melting, labeled "melted" (*: fitted frequencies values, *: the pointed and the fitted frequency values are equal).

Cholesteryl alkanooates	C=C stretching frequencies (cm ⁻¹)			C=O stretching frequencies (cm ⁻¹)			SB peak
	Without treatment	Recrystallized	Melted	Without treatment	Recrystallized	Melted	Melted
Formate	1674	1675.5	1672	1704 1713	1704 1713	1720–1730	1634
Acetate	1673	1673	1672.5	1736** 1743**	1736** 1743**	1715–1725	1635
Pentanoate	1669	1670.5	1670	1730.5* 1734.5*	1733* 1737*	1730–1740	1634
Nonanoate	1671	1671.5	1673	1735.5* 1740*	1735.5* 1739.5*	1733–1739	1634
Tetradecanoate	1668.5	1669.5	1674	1738 1744.5	1739 1745	1733–1739	1634.5
Behenate	1669	1669.5	1676.5	1738 1744.5	1738 1744.5	1735–1745	1637.5

first approximation, admit that C₆=C₅ is equivalent to an alkyl-trisubstituted ethylene. It is well known that in this case the Raman spectra has a strong band in the range 1665–1680 cm⁻¹ [33,34]. This peak is a good candidate to be an internal reference for further investigations because it is a strong isolated band resulting from the stretch of the unique carbon-carbon double bond of these molecules.

In the polycrystalline samples (not melted) we always observed a doublet, resolved or not, assigned to the C₂₈=O₂₈ stretch vibration. This doublet is in the spectral range 1730–1745 cm⁻¹ for $n > 1$. For cholesteryl formate ($n = 1$) it is shifted to lower frequencies. C₂₈=O₂₈ is the double bond of an ester function; it is established that the stretch vibration in esters is mostly in the range 1725–1750 cm⁻¹ [35–37]. In the ester family, formate esters are characterized by a lowered C=O stretch vibration frequency of about 15–20 cm⁻¹ [35–39]. This shift results from a mass effect; the C₂₉ atom of the cholesteryl alkanooates ($n > 1$) is replaced by a hydrogen atom in cholesteryl formate, and so the force constant of the C=O double bond decreases [33,40,41]. For the cholesteryl formate the observed shift is of -30 cm⁻¹ and not -15 cm⁻¹. The splitting into two components of this C=O stretching mode could be surprising in those materials. Such a behavior is characteristic of bond angle effects. This result implies the existence of two geometries of the "knuckle joint" group O₃-C₂₈=O₂₈: the double-bond-single-bond angle has two different values. This indicates that for some molecules the ester group is strained. Our interpretation is confirmed by x-ray and neutron diffusion studies [22,25,29,30,32]. These works revealed the existence of two types of molecules in the crystalline cholesteryl esters. Indeed the corresponding bond angles in these two types of molecules (A) and (B) are different. In the cases where the doublet is resolved we can suppose that the bond angle variations between the molecules of types (A) and (B) are more significant than in the cases where the doublet is not resolved.

Our results do not permit us to conclude that the C=C stretching mode has a dependence on the alkyl chain length.

Nevertheless it is reasonable to suppose that the C=C bond mode is insensitive to the alkyl chain length, seeing that this bond is in the steroid. Considering the C=O mode, the vibration frequency seems to be independent of the alkyl chain length for $n \geq 14$. The splitting of the C=O mode is the signature of the existence of the molecules (A) and (B), and the ester group O₃-C₂₈=O₂₈ is the linkage between the steroid and alkyl chain parts of the molecules; these double interests justify our further investigations in polarized Raman.

The broadening of the C=C stretching mode and the disappearing of the C=O stretching doublet indicate a lower order in the "melted" samples. This result is in agreement with the phase transition studies [10,11,31]. These works re-

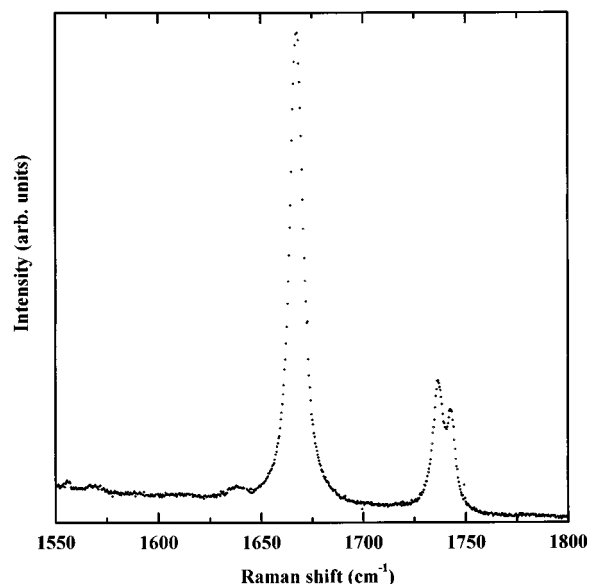


FIG. 6. Raman spectrum of the recrystallized "melted" cholesteryl behenate. This spectrum is the same as the spectra of Figs. 3 and 4.

port that, after melting and cooling the polycrystalline cholesteryl alkanooates, they are in a polymorphic phase [29,31,42]. In order to verify the reversibility of these structural transitions, we dissolved the "melted" samples and then recrystallized them as previously described. For example, in Fig. 6 we present the corresponding spectrum, for behenate, which is identical to the nontreated and recrystallized sample spectrum. This result is valid for each cholesteryl alkanooate studied. This fact shows that the thermal history of the samples must be taken into account before any conclusion. Our results obtained for different cholesteryl alkanooates after various treatments demonstrate the high sensibility of the studied spectral range to the molecule geometry and the structural order.

IV. CONCLUSION

In this work we have presented systematic Raman studies of cholesteryl alkanooates in the spectral range 1550–1800 cm^{-1} . The stretching modes of the $\text{C}_5=\text{C}_6$ and $\text{C}_{28}=\text{O}_{28}$ bonds are in this range. In regard to the unicity of these bonds in the molecules, and because the C=C stretch frequency shows independence from the alkyl chain length, we propose to consider this mode an internal reference. The split stretch mode of $\text{C}_{28}=\text{O}_{28}$ permits us to conclude on the existence of two types of molecules (A) and (B) in the polycrystalline phase. These molecules differ from each other only by bond angle variations. For $n \geq 14$ the frequency of the $\text{C}_{28}=\text{O}_{28}$ mode seems to be independent of the alkyl chain length in opposite of the cases where $n < 14$.

-
- [1] S. C. Goheen, L. J. Lis, and J. W. Kauffman, *Chem. Phys. Lipids* **20**, 253 (1977).
- [2] R. Faiman, *Chem. Phys. Lipids* **18**, 84 (1977).
- [3] S. Fowler Bush, Henry Levin, and Ira W. Levin, *Chem. Phys. Lipids* **27**, 101 (1980).
- [4] R. Faiman and K. Larsson, *J. Raman Spectrosc.* **5**, 3 (1976).
- [5] P. Lecacheux, G. Menard, H. N. Quang, N. Q. Dao, A. G. Roach, and D. Dron, *Spectrochim. Acta A* **52**, 1619 (1996).
- [6] P. Lecacheux, G. Menard, H. N. Quang, Q. Dao, P. Weinmann, and N. Jouan, *Appl. Spectrosc.* **50**, 1253 (1996).
- [7] C. R. Loomis, M. J. Janiak, D. M. Small, and G. G. Shipley, *J. Mol. Biol.* **86**, 309 (1974).
- [8] D. M. Small and G. G. Shipley, *Science* **185**, 222 (1974).
- [9] B. Lundberg, *Chem. Phys. Lipids* **14**, 309 (1975).
- [10] G. W. Gray, *J. Chem. Soc.* **3**, 3733 (1956).
- [11] R. D. Ennulat, *Mol. Cryst. Liq. Cryst.* **8**, 247 (1969).
- [12] P. G. de Gennes, in *The Physics of Liquid Crystals*, edited by W. Marshall and D. H. Wilkinson (Clarendon, Oxford, 1974).
- [13] I.-C. Khoo and S.-T. Wu, in *Optics and Nonlinear Optics of Liquids Crystals* (World Scientific, Singapore, 1993).
- [14] J.-C. Lee and S. D. Jacobs, *J. Appl. Phys.* **68**, 6523 (1990).
- [15] Z. Sekkat *et al.*, *Opt. Commun.* **111**, 324 (1994).
- [16] I. P. Il'chishin and A. Y. Vakhnin, *Liq. Cryst.* **265**, 687 (1995).
- [17] B. J. Bulkin and K. Krishnan, *J. Am. Chem. Soc.* **93**, 5998 (1971).
- [18] B. J. Bulkin and K. Krishnan, *J. Am. Chem. Soc.* **94**, 1109 (1971).
- [19] B. J. Bulkin, J. O. Lephardt, and K. Krishnan, *Mol. Cryst. Liq. Cryst.* **19**, 295 (1973).
- [20] G. Vergoten, G. Fleury, M. Tasumi, and T. Shimanouchi, *Chem. Phys. Lett.* **19**, 191 (1973).
- [21] G. Vergoten and G. Fleury, *Mol. Cryst. Liq. Cryst.* **30**, 223 (1975).
- [22] J. A. W. Barnard and J. F. Lydon, *Mol. Cryst. Liq. Cryst.* **26**, 285 (1974).
- [23] W. L. McMillan, *Phys. Rev. A* **6**, 936 (1972).
- [24] J. H. Wendorff and F. P. Price, *Mol. Cryst. Liq. Cryst.* **22**, 85 (1973).
- [25] J. H. Wendorff and F. P. Price, *Mol. Cryst. Liq. Cryst.* **25**, 71 (1974).
- [26] B. M. Craven and G. T. Detitta, *J. Chem. Soc. Perkin Trans.* **2**, 814 (1976).
- [27] N. G. Guerina and B. M. Craven (unpublished).
- [28] N. G. Guerina and B. M. Craven (unpublished).
- [29] P. Sawzik and B. M. Craven, *Acta Crystallogr. B* **35**, 895 (1979).
- [30] H.-P. Weber, B. M. Craven, P. Sawzik, and R. K. McMullan, *Acta Crystallogr. B* **47**, 116 (1991).
- [31] A. K. Zeminder, B. Jha, S. Paul, and R. Paul, *Mol. Cryst. Liq. Cryst.* **48**, 183 (1978).
- [32] M. P. McCourt, N. Li, W. A. Pangborn, R. Miller, C. M. Weeks, and D. L. Dorset, *J. Phys. Chem.* **100**, 9842 (1996).
- [33] N. B. Colthup, L. H. Daly, and S. E. Wiberley, in *Introduction to Infrared and Raman Spectroscopy*, 3rd ed. (Academic, New York, 1990).
- [34] H. L. McMurry and V. Thornton, *Anal. Chem.* **24**, 318 (1952).
- [35] D. Lin-Vien, N. B. Colthup, W. G. Fateley, and J. G. Grasselli, in *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules* (Academic, New York, 1991).
- [36] R. Dollish, W. G. Fateley, and F. F. Bentley, in *Characteristic Raman Frequencies of Organic Compounds* (Wiley, New York, 1974).
- [37] H. W. Thompson and P. Torkington, *J. Chem. Soc. London* **2**, 640 (1945).
- [38] C. J. Pouchert, in *The Aldrich Library of FT-IR Spectra* (Aldrich Chemical, Milwaukee, 1985).
- [39] C. J. Pouchert, in *Vapor Phase* (Aldrich Chemical, Milwaukee, 1989).
- [40] S. Bratoz and S. Besnainou, *C. R. Acad. Sci.* **248**, 546 (1959).
- [41] J. O. Halford, *J. Chem. Phys.* **24**, 830 (1956).
- [42] V. A. Usol'tseva and I. G. Chistyakov, *Russ. Chem. Rev.* **32**, 502 (1963).