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The supramolecular self-organization of an amphotropic cholesterol derivative

Micelles, liposomes and liquid-crystalline phases

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The cholesterol derivative tetraethoxycholesteryl semisuccinate is both a mesogen and an amphiphile. This combination of both molecular prerequisites permits two types of supramolecular self-organization: the formation of a thermotropic liquid-crystalline phase and of various forms of aggregates in contact with water or other solvents. Depending on the pH of the aqueous medium the compound self organizes in micelles or liposomes. At high concentrations lyotropic liquid-crystalline phases are obtained. The formation of liposomes and lyotropic phases is not restricted to water as a solvent but can also be induced in pure organic media such as water-free diethyleneglycol. Due to the broad range of supramolecular structures that depend both on molecular shape and on amphiphilic properties we propose to call the title compound a model for amphotropic phase behaviour.

1. Introduction

In life science the self-organization of matter in supramolecular assemblies is one of the basic prerequisites for all biological processes. Cellular liquid crystals are necessary tools for all chemical, electrical and mechanical functions of life [1]. Compartmentation of cells and subcellular organelles is enabled by the self-organization of amphiphilic lipid molecules in an aqueous surrounding. The driving force of this self-assembly, the hydrophobic effect, is also responsible for the formation of lyotropic liquid-crystalline phases and micelles, which are themselves important biological structures.

In materials science self-organization plays an important role in the field of thermotropic liquid crystals. They are interesting and potential candidates in new areas such as non-linear optics, optoelectronics, information storage or self-reinforcing plastics. The molecular basis for this type of self-organization are mesogens, which are composed of rigid, form-anisotropic (for example rod- or disc-like) molecules.

Despite the fact that supramolecular self-organization is one obvious connection between the two areas life sciences and materials sciences, the gap between the fields has not really been bridged [2]. Early classifications even termed some mesogenic molecules containing classical amphiphilic functional groups such as the carboxy group and aliphatic tails 'non-amphiphilic' [3]. Even now there exist only a few compounds which are able to self-organize both via their amphiphilic and mesogenic subunits. In this connection we present a simple derivative of cholesterol, tetraethoxy-

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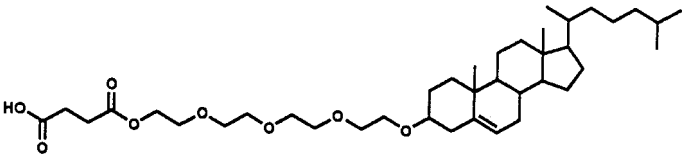
cholesteryl semisuccinate **1**, which is able to self-organize in a broad range of supramolecular structures (see figure 1), depending on both its amphilic properties (micelles, monomolecular layers, bilayer membranes, lyotropic liquid-crystalline phases) and its shape anisotropy and mesogenic structure (thermotropic liquid-crystalline phase).



Structural formula of tetraethoxycholesterolsemisuccinate **1**.

The formation of lyotropic liquid-crystalline phases and liposomes is not restricted to water as a solvent and occurs also in water-free diethyleneglycol (DEG). Therefore the title compound is a model for the ability to self-organize via two entirely different routes. Such a combination of mesogenicity (thermotropism) and amphiphilicity (lyotropism) in a single molecule could be termed amphotropism [4] as already mentioned in a review that includes a preliminary report on **1** [2].

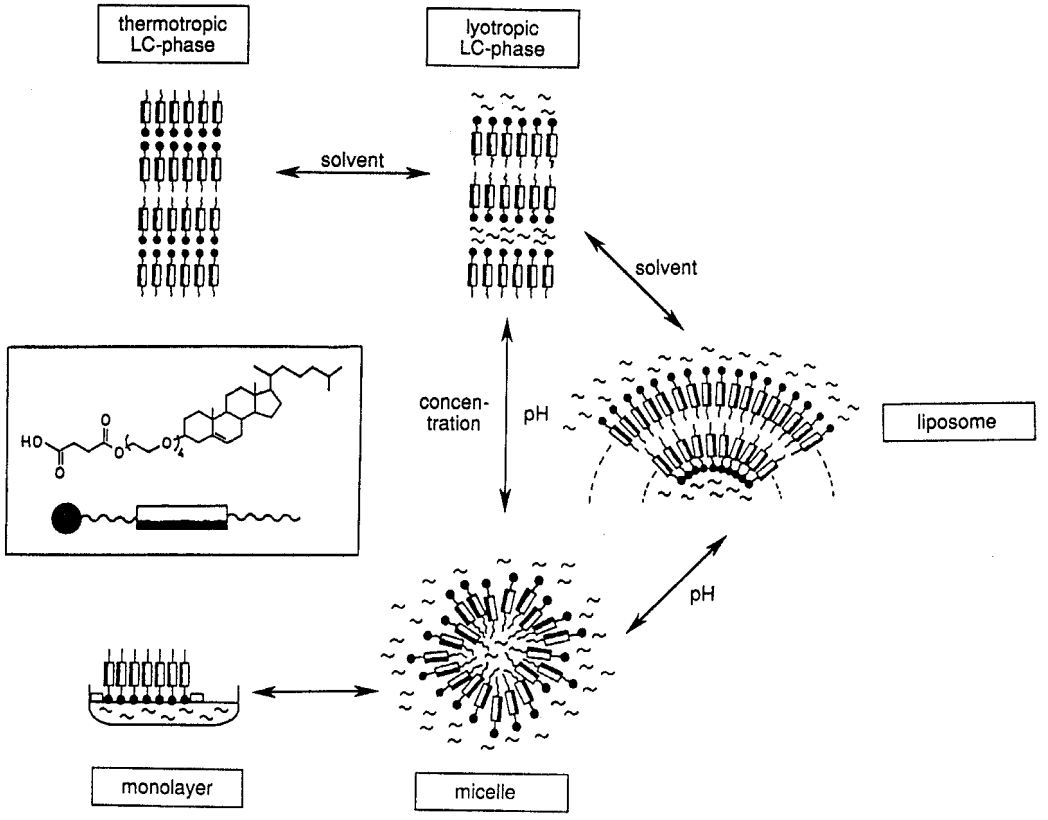


Figure 1. Schematic of molecular structure and supramolecular aggregates of **1**.

2. Materials and methods

Tetraethoxycholesteryl semisuccinate **1** was synthesized by a standard esterification from 1·288 g of tetraethoxycholesterol [5] using a 1·5-fold excess of succinic

anhydride and triethylamine as base in 30 ml of CH_2Cl_2 as solvent. Unreacted succinic anhydride was quenched with diethyl amine after a reaction time of 2 hours. Another 50 ml of CH_2Cl_2 were added and the amines were extracted three times into 20 ml of an aqueous solution containing 0·1 mol of HCl and 0·1 mol of NaCl. The organic phase was dried with magnesium sulphate and the solvent was distilled off. The residue was chromatographed on silica gel with a solvent mixture of ethyl acetate, petroleum ether and tetrahydrofurane (2:2:1) containing 2 vol.% glacial acetic acid. The product fractions were collected, the solvent distilled off and the product dried at 10^{-2} torr and a temperature of 363 K. The material was re-dissolved in hexane and filtered through a 1·0 μm Teflon™ filter. Finally, the hexane was distilled off and the product dried again for two hours at 10^{-2} torr and a temperature of 393 K. 0·992 g of pure tetraethoxycholesteryl semisuccinate $C_{39}H_{66}O_8$ (MW 662·95) were obtained as a colourless grease-like material corresponding to a yield of 65 per cent.

Elemental analysis, calculated found: C 70·66 per cent, H 10·03 per cent, O 19·31 per cent; C 70·25, H 9·91 per cent. ^{1}H NMR (400 MHz in $CDCl_3$) chemical shifts δ: 6·04 ppm (broad) 1 H –COOH, 5·29 ppm 1 H –C=CH–CH_2–, 4·21 ppm 4 H –CO–CH_2–CH_2–O–, 3·60 ppm 12 H –(O–CH_2–CH_2)$_3$–O, 2·60 ppm 4 H –CO–CH_2–CH_2–CO–. The remaining signals of the cholesterol residue at 3·12 ppm and from 2·31 ppm to 0·62 ppm were not assigned in detail, their integrated intensities correspond to 44 protons.

All solvents were analytical grade. The ultra pure water used for all experiments was obtained by distillation followed by ion-exchange and filtration steps (Milli-Q; Millipore GmbH). The resistivity was better than 18·2 MΩ cm and the total organic content was less than 10 ppb (according to the manufacturer). Unoriented liquid-

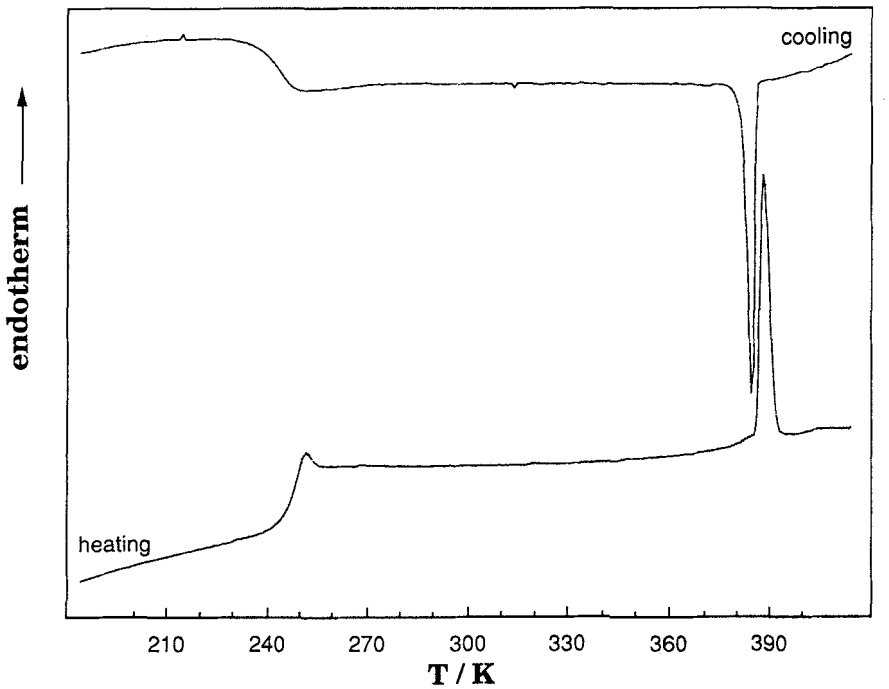
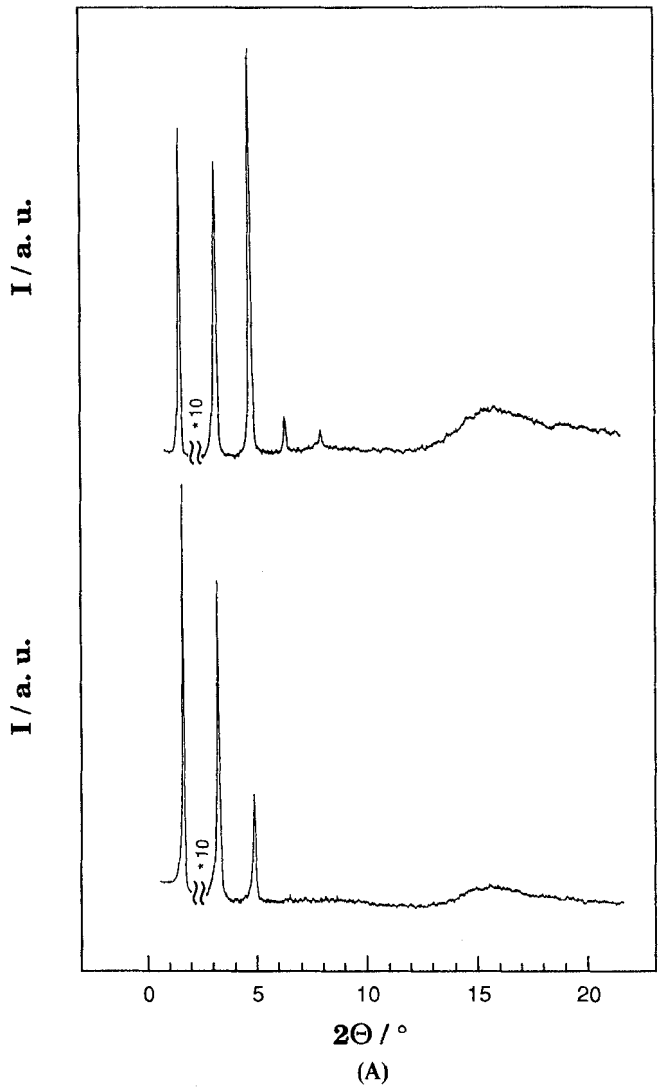


Figure 2. DSC heating and cooling scans of pure **1** (heating/cooling rate 10 K min^{-1}).

crystalline phases were characterized by small angle X-ray scattering (Siemens D-500 powder diffractometer using Copper K_α-radiation with a wavelength of 1·542 Å), by polarization microscopy (Leitz Ortholux II Pol-Bk) and by differential scanning calorimetry (Perkin–Elmer DSC 2c). Liposomal phases were characterized by video-enhanced phase contrast microscopy (Zeiss IM 35 microscope, Grundig FA 76 camera and Panasonic NV 9200 video recorder). Monolayer isotherms were measured with a home-built trough and surface tension measurements of the micellar solutions were carried out using a Fisher Surface Tensiomat equipped with a Pt/Ir ring. The error bars correspond to an average of 10–25 data points. The apparatus was not calibrated, instead the value for pure water is given in figure 5(A).

3. Results and discussion

This cholesterol derivative **1** is able to self-organize in a broad range of supramolecular structures as schematically depicted in figure 1. The liquid-crystalline



(A)

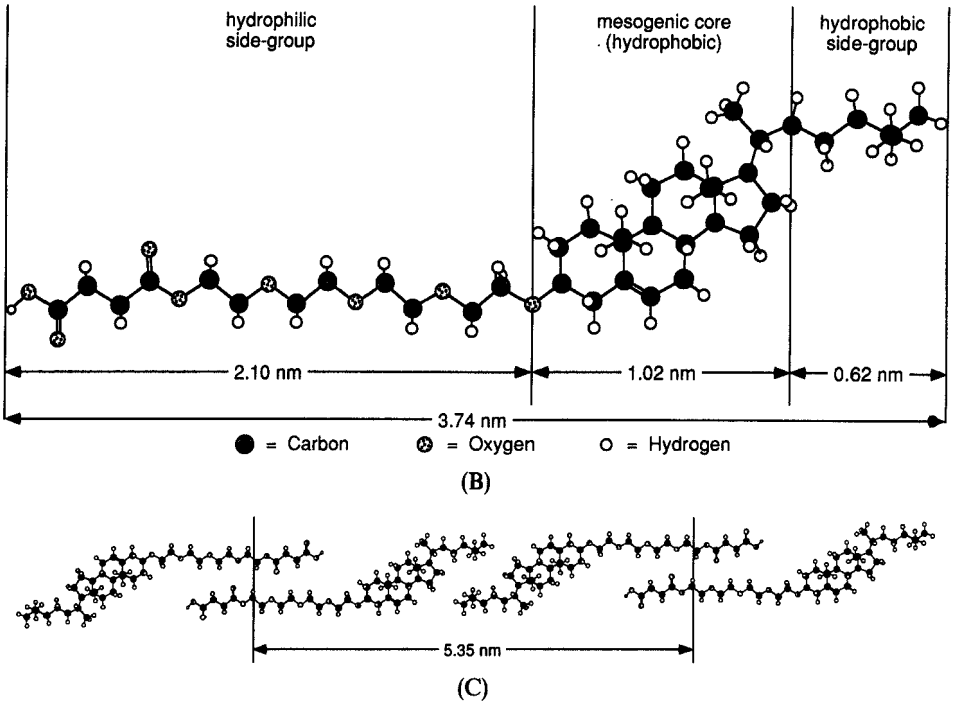
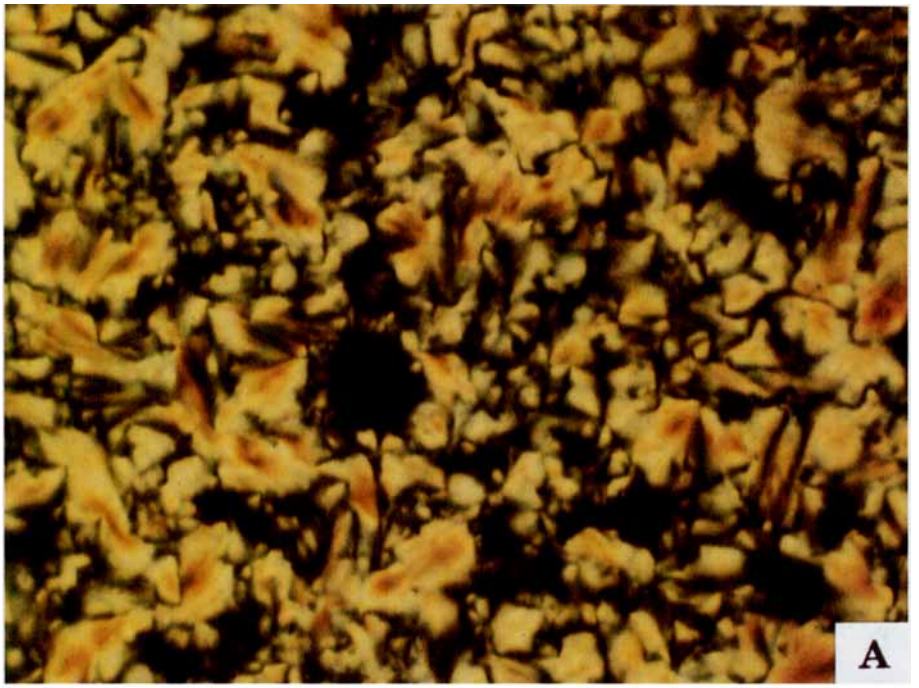
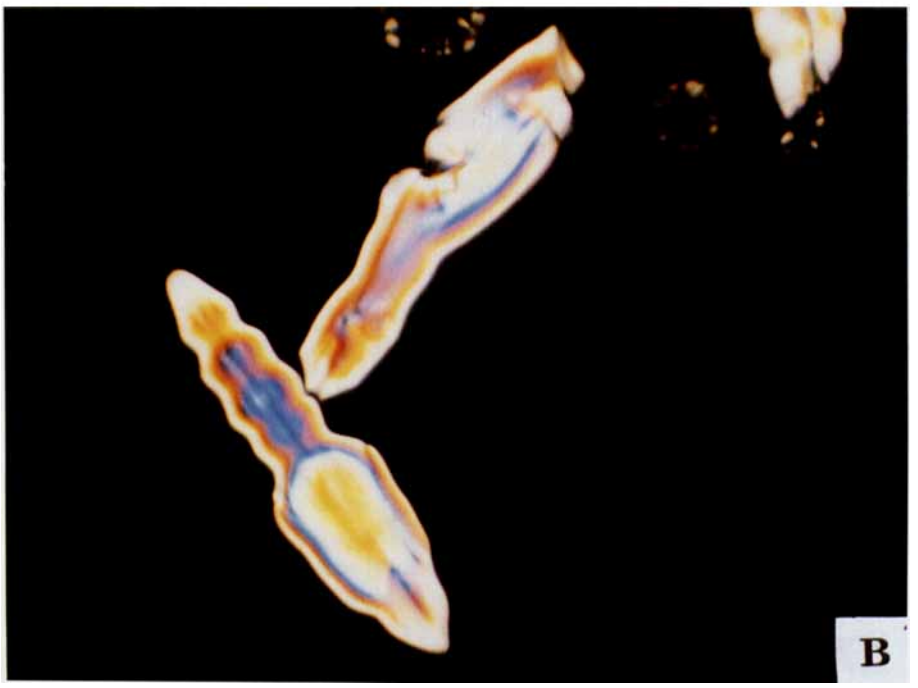


Figure 3. (A) X-ray diffractograms of **1** at 296 K (reflected intensity versus scattering angle). Upper trace thermotropic liquid crystal phase, lower trace lyotropic phase containing 30 wt.% water (the symbol *10 at $2\Theta = 2{\cdot}5°$ denotes a 10-fold increase in detector sensitivity). (B) Ball and stick representation of **1** indicating the estimated lengths of the molecule and its sub-units. (C) Schematic of the arrangement of the molecules in the bilayer smectic A phase assuming an extended conformation of the molecules and a non-tilted, interdigitated ordering that matches the observed layer spacing.

state of matter was discovered slightly over 100 years ago with the synthesis of some cholesterol fatty acid esters [6, 7]. While the function of cholesterol in the living body, as well as its role as an amphiphile in model membrane systems, have been extensively investigated [8], it was only recently discovered that some derivatives of cholesterol are able to form bilayer membranes and liposomes by self-organization of the pure compounds in water [5, 9, 10].

The phase behaviour of liquid crystals is either induced by temperature (thermotropism) or by the influence of a given solvent (lyotropism). The vast majority of liquid crystals belongs to either one of these groups though some exceptions have been reported previously (see, for example, 10–17]). There is, however, still an argument as to whether the thermotropic phase of some substances is truly dependent on their mesogenic properties or whether it is a pseudo-thermotropic lyotropic phase [11]. In the case of compound **1**, the observed liquid-crystalline phase is a true thermotropic phase, since other cholesterol derivatives like cholesteryl myristate, a classic non-amphiphilic thermotropic liquid crystal, also exhibit the same liquid-crystalline phase (S_A). The phase behaviour of tetraethoxycholesteryl semisuccinate **1** was analysed by calorimetry, X-ray diffraction and polarization microscopy. Figure 2 shows the heating and cooling DSC scans of pure **1**. It exhibits a thermotropic liquid-crystalline phase ranging from 245 K to 386 K. On cooling the compound turns into a glass at 245 K instead of crystallizing, a behaviour normally only observed with polymeric liquid





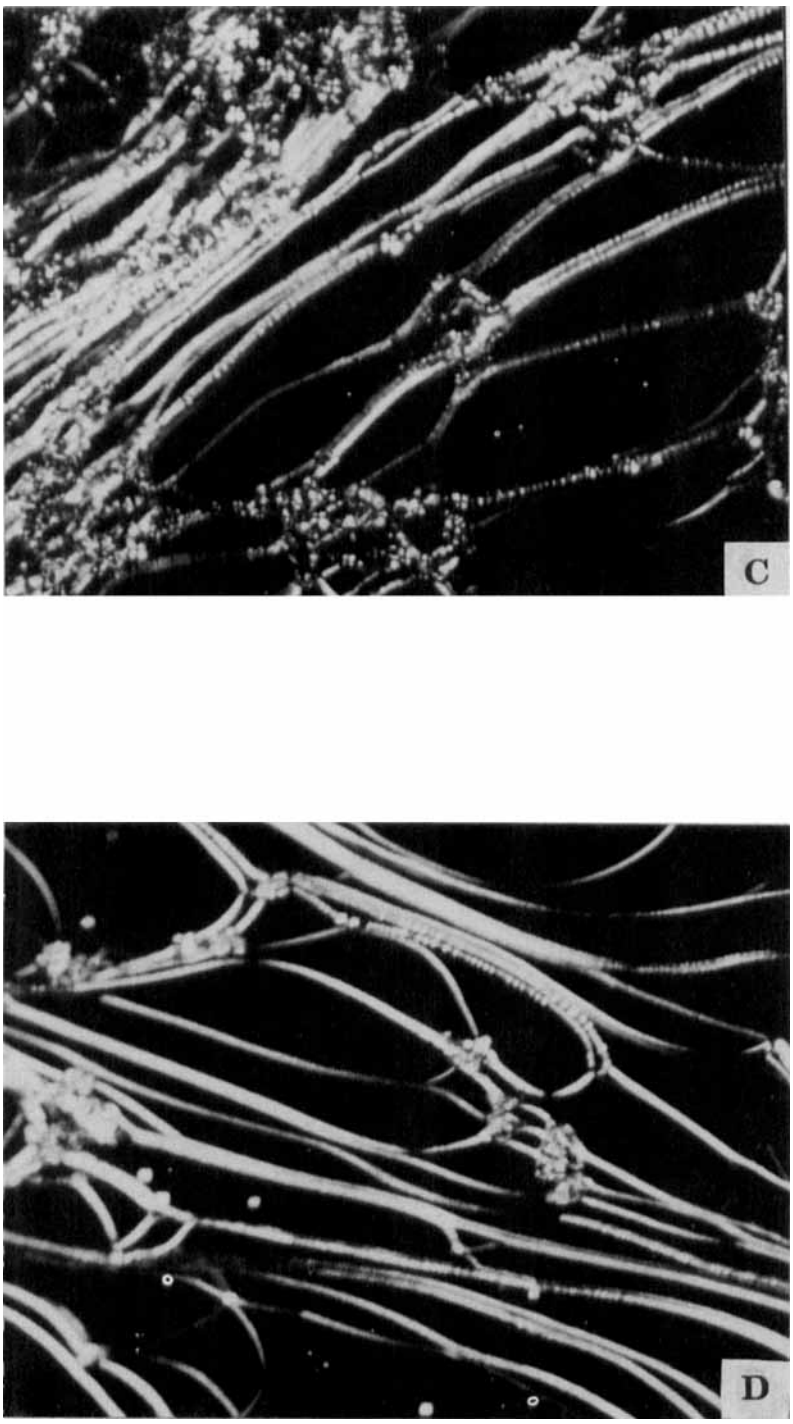


Figure 4. Birefringent textures of liquid crystal phases. (A) and (B) correspond to the thermotropic phase, (C) and (D) to lyotropic phases. (A) Smectic fans at 303 K after cooling from the isotropic melt. (B) Bâtonnets at 385 K after cooling from the isotropic melt. (C) Oily streaks of a sample with 30 wt.% water. (D) Oily streaks of a sample with 30 wt.% diethyleneglycol.

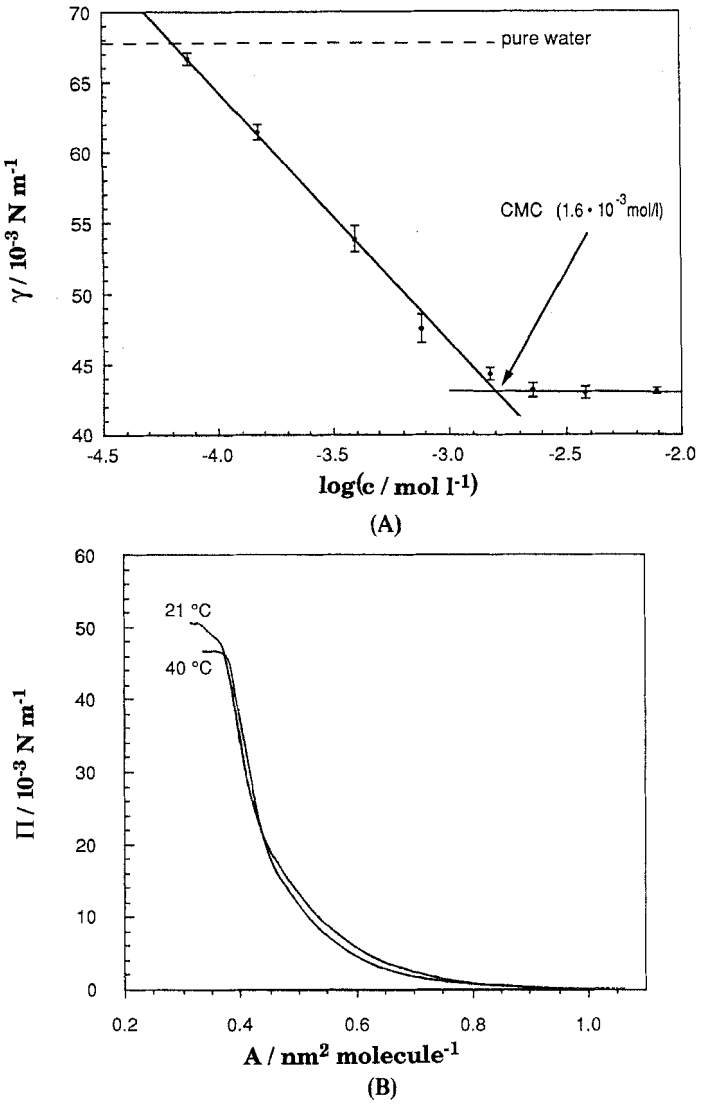


Figure 5. Determination of molecular aggregation with water. (A) Determination of the critical micelle concentration at pH 9·3 (surface tension not scaled with respect to pure water). (B) Surface pressure versus area isotherm of a monolayer of **1** on pure water at pH 5·5.

crystals. Attempts to prepare these so-called ordered glasses from non-polymeric liquid crystals by rapid cooling are not new, but in most systems these glasses undergo recrystallization upon heating (see, for example, [18–20]). The cholesterol derivative **1** shows a completely reversible glass transition (with preservation of order in the glassy state), which is independent of the cooling rate. Attempts to induce crystallization by prolonged annealing at temperatures slightly above the glass transition temperature did not produce any significant changes in the DSC scan. The enthalpy of the phase transition at 386 K is 5·1 kJ mol^{-1} and is in the range expected for a smectic to isotropic transition.

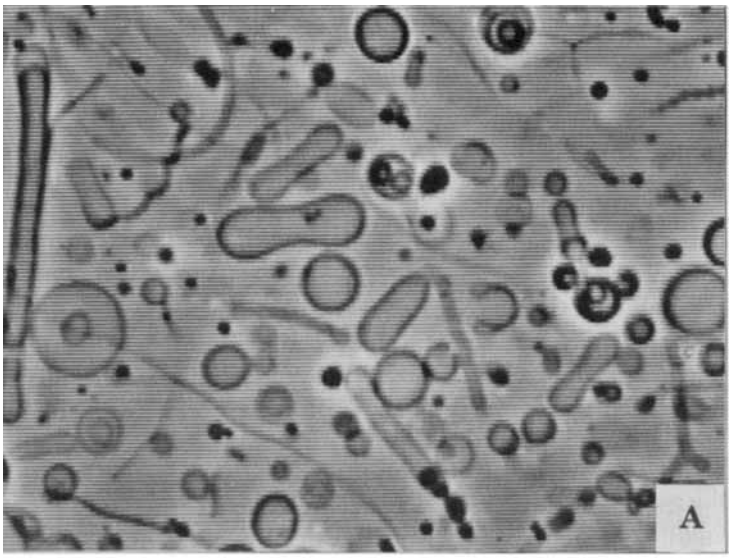
X-ray diffraction supports the view that the observed mesophase belongs to the bilayer-smectic type. The upper trace of figure 3 (A) shows five orders of reflection for the smectic layer spacing of 5·35 nm. From space filling models a molecular length of 3·74 nm is calculated assuming a maximum extended conformation. Since the layer spacing is larger than one but smaller than two molecules, the smectic layers must be bilayers in which two molecules are arranged either in an interdigitated way (see figure 3 (C)) or highly tilted or we have to assume a non-extended conformation. If we assume an interdigitated structure the observed layer spacing is matched when both the hydrophilic and hydrophobic side-groups are interdigitated as shown in figure 3 (C).

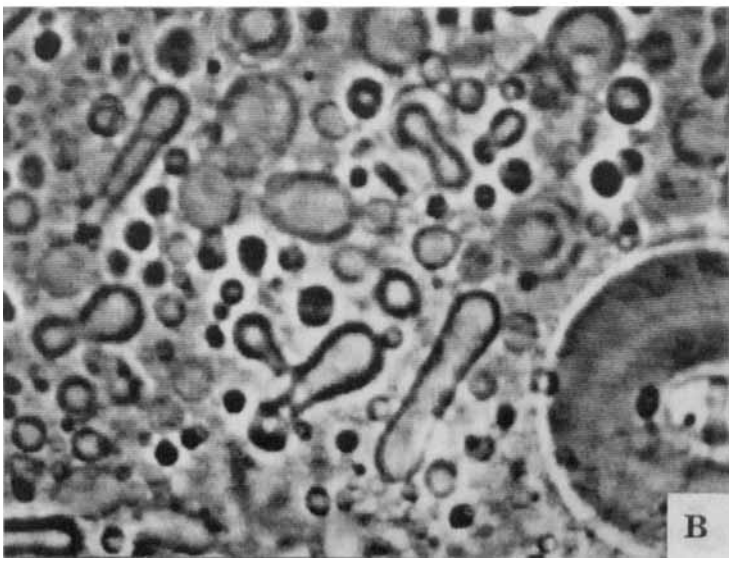
A smectic phase is also in agreement with phase-contrast microscopy. Figure 4 (A) shows the characteristic texture, smectic fans and a homeotropic background, of **1** at room temperature after rapid cooling from the isotropic state. Cooling the isotropic melt to 385 K results in the formation of bâtonnets (see figure 4 (B)). If the compound is cooled slowly between two hydrophilic glass plates, separated by less than approximately 50 μm, only the homeotropic texture is observed. Upon shearing birefringence is observed immediately, but the texture returns to homeotropic within minutes, since the carboxylic terminal groups of **1** anchor strongly to the glass thus favouring homeotropic alignment.

Due to its amphiphilic structure **1** can also self-organize in water or protic organic solvents using the driving force of the hydrophobic effect. As already shown in figure 1 micelles, monomolecular layers at the air/water interface and liposomes are formed. Depending on the pH of the aqueous medium the terminal carboxylic group allows for protonation/deprotonation and thus regulates hydrophilicy, solubility and aggregation behaviour. At pH 9·3 (equivalence point) the sodium salt of compound **1** shows a critical micelle concentration of $1{\cdot}6 \times 10^{-3}$ mol l^{-1} (figure 5 (A)). At higher concentrations the micellar phase shows birefringence, indicating the formation of a lyotropic phase. A lamellar lyotropic phase can also be obtained directly by extensively mixing pure **1** with water or diethyleneglycol. Figures 4 (C) and (D) show the textures of the lyotropic lamellar phases of **1** with 30 wt.% of these two solvents. Interestingly 30 wt.% water does not significantly increase the bilayer repeat distance as seen by X-ray diffraction (see lower trace of figure 3 (A)). This seems to indicate that the water is incorporated in the hydrophilic headgroup region and does not separate the layers at this concentration.

At pH 5–6 **1** is water insoluble and shows a different aggregation behaviour. At the air/water interface **1** forms stable monomolecular layers (see figure 5 (B)) that can be compressed into a tightly packed state. However, attempts to deposit multilayers of **1** on to solid supports by the Langmuir–Blodgett technique, either from pure water or from a $CdCl_2$ containing subphase, proved unsuccessful.

Swelling of a thin film of **1** in contact with water or water-free DEG leads to the formation of stacks of bilayer membranes and giant liposomes (see figures 6 (A) and (B) as documented by phase contrast microscopy. In addition figure 6 (D) shows an electron micrograph (freeze etching technique) of a multilamellar liposome of an identical preparation in water. The spherically closed structure of the lipid bilayer was confirmed by a dye entrapment experiment. Liposomes were prepared by injecting a pentane solution of the title compound into an aqueous solution of pyrenetetrasulfonic acid tetrasodium salt at 323 K. After removal of the non-encapsulated dye by gel permeation chromatography, the liposomal fractions were investigated by phase contrast (see figure 6 (C)) and fluorescence microscopy. It was confirmed that the dye molecules were completely entrapped within the liposomes inner compartments.





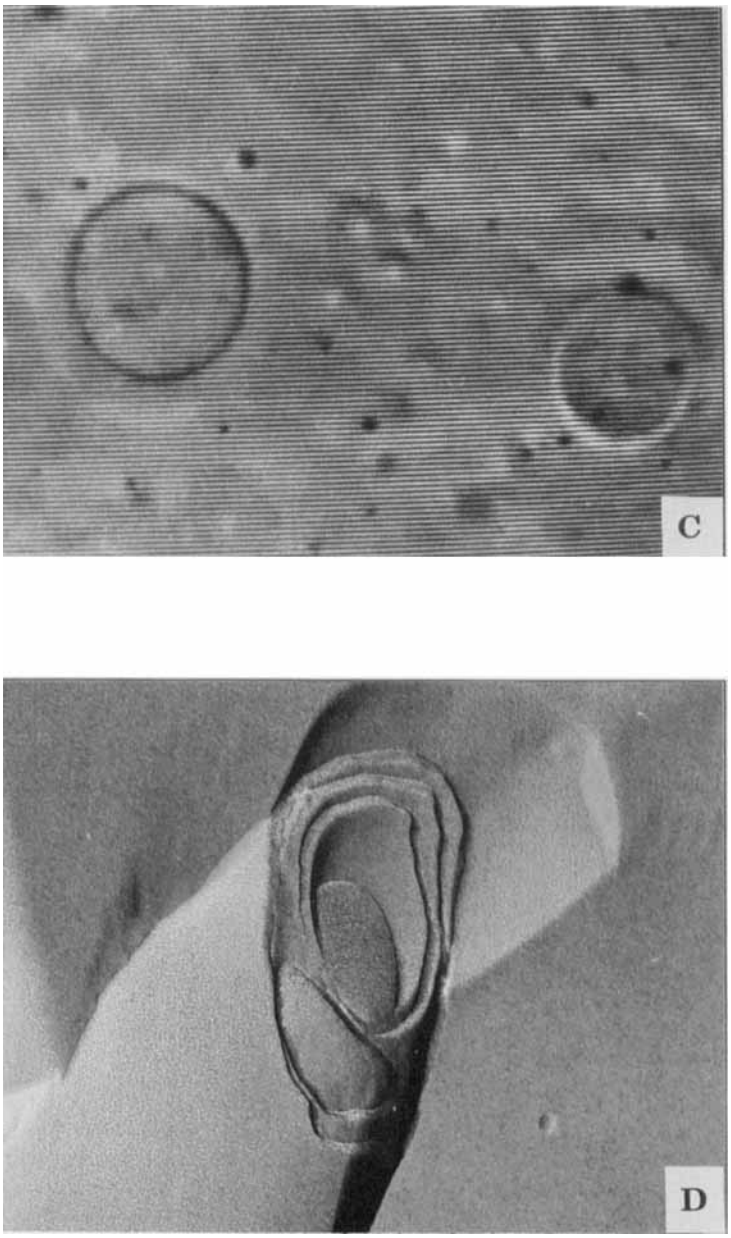


Figure 6. Characterization of liposomal aggregates. (A), (B) and (C) are optical phase-contrast micrographs. (A) After swelling of a thin film of **1** in excess water. (B) After swelling of a thin film of **1** in excess diethyleneglycol. (C) Liposome preparation by pentane injection method. (D) Freeze-fracture electron micrograph of a multilamellar liposome after swelling of a thin film of **1** in water.

4. Summary and conclusions

The broad range of supramolecular aggregates that are formed by tetraethoxycholesterol semisuccinate **1** and described here is easily visualized on the basis of its molecular structure. The formation of thermotropic mesophases is well-known from similar ethers of cholesterol. All experimental data point to a bilayer smectic A phase. The lyotropic phase behaviour is strongly influenced by the carboxylic acid group. At low pH the carboxylic acid group is protonated and the compound is water-insoluble. Therefore, it can be spread at the air/water-interface and compressed to a tightly packed monolayer. When bulk material is brought into contact with water, the solvent penetrates into the bilayers and gives rise to a lamellar lyotropic structure. When more water is added, the bilayers finally separate and form spherically closed membranes (liposomes). At high pH the carboxylic acid forms a salt, rendering the compound water-soluble and resulting in the formation of micellar aggregates. The lyotropic phases are not restricted to water as a solvent. A lamellar phase and the formation of liposomes is also observed in diethyleneglycol.

Tetraethoxycholesteryl semisuccinate is one example for a molecule which achieves its tailor-made physical properties from the combined properties of its sub-units. In other words, its designed molecular architecture leads to the intended formation of various supramolecular assemblies. In the life sciences it is easily visualized that the complexity of the biological functions is related to the size of the functional entity, being either macromolecules (proteins or DNA), aggregates of macromolecules or aggregates of macromolecules and many small molecules (see, for example, [21, 22]). In this report we have discussed one example in the area of materials science, where a similar correlation of functionality (aggregation behaviour) and molecular size holds true. The combination of mesogenicity and tunable amphiphilicity in a single molecule requires a certain molecular size and leads to the observed amphotropism.

We thank C. Fahn of the Physics department E22 of the Technical University of Munich (Germany) for assistance in preparing the freeze-fracture electron micrograph and M. Schmidt for help with the X-ray measurements.

References

[1] BROWN, G. H., and WOLKEN, J. J., 1979, *Liquid Crystals and Biological Structures* (Academic Press).

[2] RINGSDORF, H., SCHLARB, B., and VENZMER, J., 1988, *Angew. Chem.*, **100**, 117; 1988, *Angew. Chem. Int. Ed.*, **27**, 113.

[3] GRAY, G. W., 1974, *Liquid Crystals and Plastic Crystals*, vol. 1 edited by G. W. Gray and P. A. Winsor (Ellis Horwood Ltd), p. 103.

[4] Amphotropism, amphotropic: a combination of two driving forces for the molecular self-organization. The expressions are derived from: amphi-, amph[o]- [fr. Gk. *ἀμφί-,ἀμφ[ό]-*] both, on both sides, of both kinds; and -tropic [fr. Gk. *τροπή, τροπος*] that which turns, turn, direction. Amphotropic is also used for a subclass of murine retroviruses. There are three types of oncoviruses, ecotropic ones that affect only murine cells, xenotropic ones that affect only non-murine cells and amphotropic ones, that affect both. Additionally in a recent publication (FULLER, S., HOPWOOD, J., RAHMAN, A., SHINDE, N., TIDDY, G., ATTARD, G. S., HOWELL, O., and SPROSTON, S., 1992, *Liq. Crystals*, **12**, 521) the term amphitropic has been used in this context.

[5] PATEL, K. R., LI, M. P., SCHUH, J. R., and BALDESCHWIELER, J. D., 1984, *Biochim. biophys. Acta*, **797**, 20.

[6] REINITZER, F., 1888, *Mh. Chem.*, **9**, 421.

[7] LEHMANN, O., 1890, *Z. Kristallogr.*, **18**, 464.

[8] GIBBONS, G. F., MITROPOULOS, K. A., and MYANT, N. B., 1982, *The Biochemistry of Cholesterol* (Elsevier Biomedical Press).
[9] BROCKERHOFF, H., and RAMSAMMY, L. S., 1982, *Biochim. biophys. Acta*, **691,** 227.
[10] ABID, S. K., and SHERRINGTON, D. C., 1987, *Polym. Commun.*, **28,** 16.
[11] MARKUS, M. A., and FINN, P. L., 1985, *Molec. Crystals liq. Crystals Lett.*, **2,** 159.
[12] KELLER-GRIFFITH, R., RINGSDORF, H., and VIERENGEL, A., 1986, *Coll. Polym. Sci.*, **264,** 924.
[13] BODEN, N., BUSHBY, R. B., FERRIS, L., HARDY, C., and SIXL, F., 1986, *Liq. Crystals*, **1,** 109.
[14] LÜHMANN, B., and FINKELMANN, H., 1986, *Coll. Polym. Sci.*, **264,** 189.
[15] BRUCE, D. W., DUNMUR, D. A., LALINDE, E., MAITLIS, P. M., and STYRING, P., *Nature, Lond.*, **323,** 791.
[16] BAZUIN, C. G., GUILLON, D., SKOULIOS, A., and NICOUD, J.-F., 1986, *Liq. Crystals*, **1,** 181.
[17] ZIMMERMANN, H., POUPKO, L., LUZ, Z., and BILLARD, J., 1989, *Liq. Crystals*, **6,** 151.
[18] SORAI, M., YOSHIOKA, H., and SUGA, H., 1984, *Liquid Crystals and Ordered Fluids*, vol. 4, edited by A. C. Griffin and J. F. Johnson (Plenum Press), p. 233.
[19] ROSTA, L., 1985, *Molec. Crystals liq. Crystals*, **127,** 195.
[20] KUNIHISA, K. S., and SATOMI, Y., 1986, *Molec. Crystals liq. Crystals*, **141,** 1.
[21] LUISI, P. L., 1983, *Chimia*, **37,** 73.
[22] SRERE, P. A., 1984, *Trends biochem. Sci.*, **9,** 387.