

Orientation of Some Nitroxide Spin Labels in the Lamellar Mesophases of Aerosol-OT-Water and Decanol-Decanoate-Water Systems

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Abstract: The alignment of a number of nitroxide spin labels has been studied when contained in oriented smectic mesophases of Aerosol-OT-water (50 wt %) and decanol-decanoate-water. The small spin label 2,2,5,5-tetramethyl-3-pyrroline-1-oxyl-3-carboxamide (TMPO-CONH₂) (II) exhibited a unique orientation with the molecular plane parallel to the bilayers in the mesophases of Aerosol-OT-water and of decanol-decanoate-water systems, and was very probably located at the boundary between the water phase and the bilayers, i.e., around the polar groups of the mesogen. 12-Doxylstearic acid (VI), 5-doxylstearic acid (VIII), and their methyl esters (VII, IX) were also studied in the Aerosol-OT-water system. These spin labels were very probably aligned with their polar groups bent toward the water phase but with the main part of their hydrocarbon chains located between the two halves of the laminar bilayers parallel with the bilayers.

The molecular organization in a number of liquid crystal systems and some biological membranes has been investigated by the spin label technique.² The spin labels used in these studies were in general molecules of an elongated shape carrying a stable nitroxide group, such as the doxyl (*N*-oxyl-4',4'-dimethyloxazolidine) derivatives of 5 α -cholestan-3-one and various oxostearic acids. The preferred orientation of the spin labels, when included in the mesophases of rodlike molecules, was in general such that the long axis of the spin labels was parallel to the director, i.e., the long axis of the oriented molecules of the mesogen.

The lyotropic mesophase D of systems such as 1-decanol-sodium caprylate-water is composed of bilayers separated by water layers.³ The bilayers exhibit a high mobility with respect to each other; i.e., they slide on each other without much friction. This leads to a macroscopic ordering of the smectic phase, when it is forced between two closely separated, parallel, glass surfaces. Shearing forces and the contact with the glass surfaces arrange the microcrystalline regions in parallel layers on the supporting plates, thus repeating their molecular order on a macroscopic scale. The system then has a unique axis of orientation with the mesogen molecules perpendicular to the glass plates. Seelig⁴ has investigated a number of lyotropic mesophases such as the system decanol-decanoate-water by the spin label technique. The systems were studied between parallel plates by the use of a number of long chain compounds, which were spin labeled in various positions with a doxyl group, and by the use of some 3-doxyl derivatives of steroids. The molecular ordering of the hydrocarbon chains depended on the type of polar group at the end of the carbon chain of the spin label, the position of the doxyl group on the carbon chain, and the relation between the length of the spin label molecule and the thickness of the bilayer of the mesophase. In most cases the long axis of the labeled stearic acids was found to be preferentially oriented parallel to the long axis of the mesophase molecules.

The mesophases of egg lecithin and water has been studied between parallel glass plates by the use of spin labels such as 3-doxyl-5 α -cholestane. The long molecular axis of the label was oriented mainly perpendicular to the bilayers.⁵

A very high degree of ordering of the rigid spin label 3-doxyl-5 α -cholestane was observed by Luckhurst and Setaka⁶ in their study of the molecular organization in the smectic A mesophase of 4-*n*-butyloxybenzylidene-4'-acetaniline. The molecules of the mesogen were oriented parallel

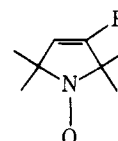
to the magnetic field when present in the nematic phase. The orientation was preserved and frozen in by slowly decreasing the temperature with the sample in the magnetic field, so that the system passed the transition from the nematic phase into the smectic A mesophase. The angular dependence of the ¹⁴N hfsc showed that the long axis of the steroid spin label was oriented parallel to the long axis of the mesophase molecules.

The liquid crystalline material present at room temperature in the sodium 1,4-bis(2-ethylhexyl)sulfosuccinate (Aerosol-OT)-water system was shown by Fontell to possess a lamellar structure in the range between 10 and 70% of Aerosol-OT.⁷ The AOT-water system has apparently not been previously studied with the spin label technique. Such a study has now been performed using a variety of spin labels. In addition to the AOT-water system, the 1-decanol-1-decanoate-water system, a lecithin-water system, and the thermotropic smectic A mesophase of 4-*n*-butyloxybenzylidene-4'-acetaniline were studied with the same spin labels, in order to compare the results obtained in the AOT-water system with those of the latter systems.

Experimental Section

Aerosol (AOT) (Fluka) was used as supplied after drying over night in vacuo at 102° over phosphorus pentoxide and storing in an *excicator*. The preparation of samples with various percentages of AOT in water was performed as described by Fontell.⁷ The actual composition of the decanol-decanoate-water system was almost the same as that used by Seelig,^{4a} i.e., 1-decanol 42 wt %, sodium 1-decanoate 28 wt %, and water 30 wt %. The lecithin-water system was prepared from β,γ -dipalmitoyl-DL- α -glycerylphosphatidylcholine (Sigma) and water as described by Chapman et al.⁸ 4-*n*-Butyloxybenzylidene-4'-acetaniline was synthesized⁹ from 4-aminoacetophenone and 4-*n*-butyloxybenzaldehyde, which was obtained by alkylating 4-hydroxybenzaldehyde.¹⁰

The following spin labels were used as supplied (Eastman): 2,2,5,5-tetramethyl-3-pyrroline-1-oxyl-3-carboxylic acid (TM-POC) (I), 2,2,5,5-tetramethyl-3-pyrroline-1-oxyl-3-carboxamide (TMPO-CONH₂) (II), 2,2,5,5-tetramethylpyrrolidine-1-oxyl-3-



I, R = COOH

II, R = CONH₂

IV, R = CN

Table I. Orientation of the Nitrogen 2p π Orbital of the Nitroxide Spin Labels with Respect to the Normal to the Bilayers of the Mesophase

Spin label	Mesogen		
	Aerosol-OT-H ₂ O Lamellar	Decanol-decanoate-H ₂ O Lamellar	4- <i>n</i> -Butyloxybenzylidene- 4'-acetoaniline Smectic A mesophase at 84°
TMPO-CONH ₂ (II) 2p π orbital perpendicular to the molecular plane	Parallel $S_{33} = 0.21$ (1)	Parallel $S_{33} = 0.15$ (2)	Perpendicular $S_{33} = -0.10$ (3)
12-Doxylstearic acid (VI) 2p π orbital parallel to long axis	Perpendicular $S_{33} = -0.20$ (5)	Parallel ^a $S_{33} = 0.23$ (6)	
5-Doxylstearic acid (VIII) 2p π orbital parallel to long axis	Parallel $S_{33}(I) = 0.27$ (5) $S_{33}(II) = 0.56$ (5)	Parallel $S_{33} = 0.60$ (6)	
3-Doxyl-5 α -cholestane (V) 2p π orbital perpendicular to long axis	Parallel $S_{33} = 0.04$ (7)	Perpendicular $S_{33} = -0.38$ (6)	Perpendicular ^b $S_{33} = -0.43$

^aReference 4a. ^bReference 6.

carboxamide (III). 3-Cyano-2,2,5,5-tetramethyl-3-pyrroline-1-oxyl (TMPO-CN) (IV) was prepared as described by Rozantsev.¹¹ The doxyl derivatives of 5 α -cholestan-3-one (V), 12-oxostearic acid (VI), 5-oxostearic acid (VIII), and the corresponding methyl esters (VII, IX) were prepared as described by Keana et al.¹² and by Waggoner et al.,¹³ respectively.

The concentration of the spin labels was about 1×10^{-3} M in the mesophase. A suitable amount of the nitroxide spin label dissolved in chloroform was placed in a test tube, the solvent was evaporated in vacuo, and the mesogen system was then added.

The ESR measurements were performed on a Varian E-9 spectrometer with the samples contained in capillary tubes (~ 1 mm i.d.), or between two flat quartz plates mounted on a quartz rod. The space between the plates was about 0.05 mm. The orientation of the samples relative to the magnetic field was measured by the use of a sample holder placed in an axially movable angular measuring device fixed to the upper stack of the ESR cavity.

Alignment of Spin Labels in Oriented Smectic Liquid Crystals

The coordinates of the nitroxide group of the spin labels are conventionally taken so that the xy plane coincides with the molecular plane containing the three bond axes of the nitrogen atom, and so that the NO axis constitutes the x direction. The z direction then coincides with the axis of the 2p π orbital containing the unpaired electron. The nitrogen hyperfine tensors of the nitroxide spin labels are approximately axially symmetric having the positive values:¹⁴ $A_{zz} \approx 31$ G $>$ $A_{yy} \approx A_{xx} \approx 5$ -6 G.

The oxazolidine ring attaches the NO group rigidly to the hydrocarbon chain so that the nitrogen 2p π orbital will be oriented parallel to the long molecular axis of the doxyl stearic acid if the chain is frozen in a planar all-trans configuration. The nitrogen 2p π orbital is oriented perpendicular to the long molecular axis of the rigid 3-doxyl-5 α -cholestane molecule, and is perpendicular to the ring plane of the small nitroxide labels, i.e., I, II, and IV.

The orientational order S_{33} of the 2p π orbital (z axis or axis with index 3) of the spin label with respect to the director, i.e., the long axis of the molecules of an oriented smectic mesophase, is defined by¹⁵

$$S_{33} = (\frac{1}{2}) \langle 3 \cos^2 \theta_3 - 1 \rangle \quad (1)$$

where θ_3 is the angle between the z axis and the director. When the motion is isotropic, S_{33} is zero, whereas if the z axis is completely aligned along the director, the maximum of S_{33} is unity since θ_3 is zero. If the z axis is perpendicular to the director, the minimum value is $-1/2$. The orientational order can be obtained from ESR measurements according to the equation^{4,6}

$$S_{33} = (\bar{A}_{\parallel} - a_{\text{iso}}) / (A_{zz} - a_{\text{iso}}) \quad (2)$$

where \bar{A}_{\parallel} is the splitting constant observed when the director is parallel to the magnetic field, and a_{iso} is the isotropic splitting constant. The sign of S_{33} is obtained under the reasonable assumption that all the quantities of eq 2 have a positive sign for the nitroxides concerned. In general when the director makes an angle ϕ with the magnetic field, the following equation for the hyperfine splitting is valid,⁶

$$A_{\text{obsd}} = (\bar{A}_{\parallel}^2 \cos^2 \phi + \bar{A}_{\perp}^2 \sin^2 \phi)^{1/2} \quad (3)$$

neglecting the small anisotropy in the partially averaged \bar{g} tensor. From the invariance of the trace of a tensor on coordinate transformations, it follows that

$$2\bar{A}_{\perp} + \bar{A}_{\parallel} = 2A_{xx} + A_{zz} = 3a_{\text{iso}} \quad (4)$$

If both \bar{A}_{\perp} and \bar{A}_{\parallel} can be obtained experimentally, a_{iso} can be determined from eq 4. In the case where A_{zz} could not be determined experimentally, A_{zz} was calculated from eq 4 by use of the relation $A_{xx}/A_{zz} = \text{constant} = 0.188$ and the experimental data on a_{iso} .^{4a}

Results

The results to be described are also collected in Table I.

(1) TMPO-CONH₂ (II) in the Aerosol-OT-Water System. In the range between 30 and 70 wt % of Aerosol-OT in water, the mesophase doped with the small spin label TMPO-CONH₂ (II) exhibited ESR spectra with axially symmetric pseudo-powder patterns when contained in circular capillary tubes. The hyperfine splittings were estimated by measuring the separation of the outer or inner hyperfine extremas¹⁶ (Figure 1 and Table II). The maximum of \bar{A}_{\parallel} (minimum of \bar{A}_{\perp}) was observed with 30 wt % H₂O at the lowest temperature of the recording on a fluid system, i.e., $\bar{A}_{\parallel} = 24.0$ G, $\bar{A}_{\perp} = 10.7$ G at -10° . \bar{A}_{\parallel} was found to decrease continuously with increasing temperature up to 80° , or an increasing water content of the system up to 70 wt %. These findings corresponded to an increasing orientational order upon decreasing the temperature or decreasing the water content. At temperatures greater than 80° , the spectra changed toward isotropic spectra, and at temperatures below 0 to -10° the spectra changed toward a true powder spectrum.

When the Aerosol-OT-water system doped with TMPO-CONH₂ (II) was placed as a thin layer between parallel quartz plates, the ESR spectra showed three sharp lines with no further overlaps in the range between 40 and 55 wt % of H₂O (Figure 2). The splitting constant A_{obsd} was

Table II. Weight Percent H₂O in Aerosol-OT, and Splitting Constants \tilde{A}_{\parallel} and \tilde{A}_{\perp} in Gauss

t°	30		40		55		60		64		70	
	\tilde{A}_{\parallel}	\tilde{A}_{\perp}	\tilde{A}_{\parallel}	\tilde{A}_{\perp}	\tilde{A}_{\parallel}	\tilde{A}_{\perp}	\tilde{A}_{\parallel}	\tilde{A}_{\perp}	\tilde{A}_{\parallel}	\tilde{A}_{\perp}	\tilde{A}_{\parallel}	\tilde{A}_{\perp}
-10	24.0	10.7		<i>a</i>		<i>a</i>		<i>a</i>		<i>a</i>		<i>a</i>
0	22.0	11.6	21.6	11.8								
23	19.6	12.5	19.9	12.5	19.0	13.0	18.9	13.0	18.3	13.3	17.4	13.5
40	18.6	12.9	19.0	12.9	18.3	13.3	18.4	13.3	17.8	13.5	17.4	13.7
58	17.9	13.4	18.1	13.3	17.4	13.6	17.5	13.6	17.2	13.8	17.0	14.1
78	17.1	13.6	17.4	13.6	16.8	13.9	16.9	13.9	16.6	14.0		

^a Superposition of axial pseudo-powder spectrum and other types of spectra.

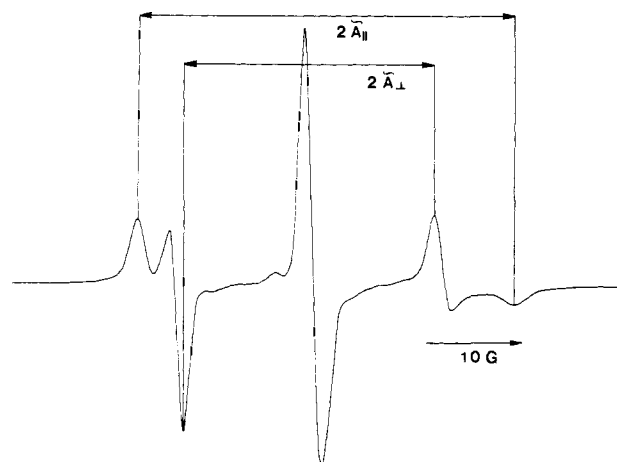


Figure 1. ESR spectrum of the spin label TMPO-CONH₂ (II) in the Aerosol-OT-H₂O (50 wt %) system in a capillary tube at room temperature.

found to be a function of the angle ϕ between the long axis of the mesogen molecules perpendicular to the surface of the quartz plates and the magnetic field. The maximum value was obtained when $\phi = 0^{\circ}$, i.e., $A_{\text{obsd}}(0^{\circ}) = 19.2$ G, the minimum value $\phi = 90^{\circ}$, i.e., $A_{\text{obsd}}(90^{\circ}) = 13.2$ G, at room temperature. These values are very close to those obtained from the axial pseudo-powder spectrum observed in a circular capillary tube at the same temperature i.e., $\tilde{A}_{\parallel} = 19.4$ G and $\tilde{A}_{\perp} = 12.9$ G.

The angular dependence of the experimentally observed splitting constant A_{obsd} was in excellent agreement with the values obtained from eq 3, as shown in Figure 3, curve a. With $A_{zz} = 34$ G obtained from the true powder spectrum in a water solution at 77 K and with $a_{\text{iso}} = 15.2$ G, the orientational order S_{33} was equal to 0.21.

The results indicated that the $2p \pi$ orbital of the spin label TMPO-CONH₂ (II) was preferably aligned parallel to the long axis of the mesogen molecules, which means that the molecular plane was oriented perpendicular to the long axis of the mesogen molecules in the Aerosol-OT-water system (50 wt % H₂O).

(2) **TMPO-CONH₂ (II) in the Decanol-Decanoate-Water System.** The results obtained with this system were very similar to those obtained with TMPO-CONH₂ in the Aerosol-OT-water system (1). The splitting constants obtained from the axial pseudo-powder spectrum taken with the sample in a capillary tube were $\tilde{A}_{\parallel} = 18.2$ G and $\tilde{A}_{\perp} = 13.2$ G. The values observed from the angular dependence measurement obtained with the sample between parallel quartz plates were $A(0^{\circ}) = 17.8$ G and $A(90^{\circ}) = 13.8$ G which correspond to $S_{33} = 0.15$. Thus, the $2p \pi$ orbital is preferably aligned parallel and the molecular plane perpendicular to the long axis of the mesogen molecules.

(3) **TMPO-CONH₂ (II) in 4-*n*-Butyloxybenzylidene-4'-acetoaniline.** The system was studied at 84° in the smectic

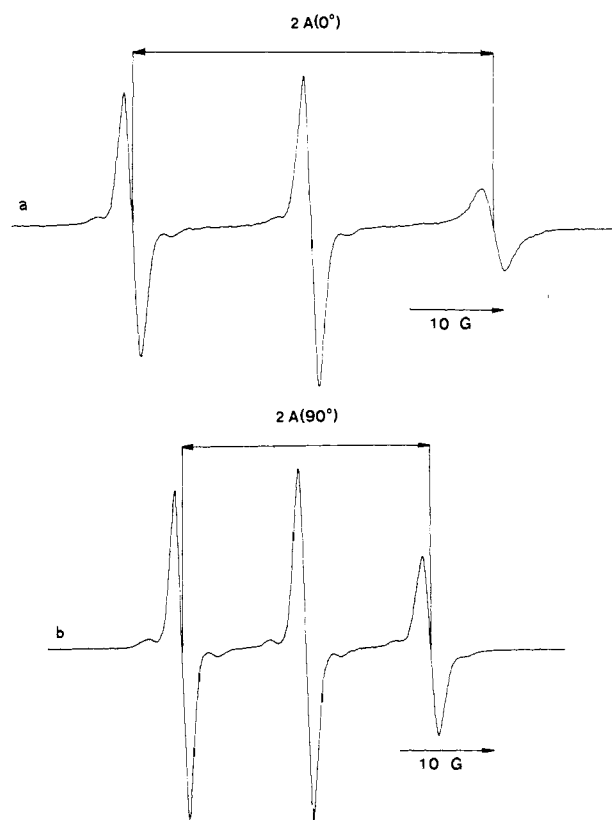


Figure 2. ESR spectra of the spin label TMPO-CONH₂ (II) in the Aerosol-OT-H₂O (50 wt %) system in a thin layer between parallel quartz plates at room temperature. Orientation of the sample with the angle ϕ between the normal to the bilayers of the mesophase and the magnetic field: (a) $\phi = 0^{\circ}$; (b) $\phi = 90^{\circ}$.

A mesophase after the mesogen had been aligned in the nematic phase in the magnetic field.⁶ The angular measurements performed with the aligned sample in a capillary tube gave $A(0^{\circ}) = 12.9$ G and $A(90^{\circ}) = 15.3$ G, and $a_{\text{iso}} = 14.6$ G, corresponding to $S_{33} = -0.10$. The result indicated that the TMPO-CONH₂ label was aligned so that the $2p \pi$ orbital was perpendicular and the molecular plane parallel to the long axis of the mesogen molecules. The angular dependence of the experimentally observed splitting constants was in excellent agreement with the values obtained from eq 3, as shown in Figure 3, curve b.

(4) **The Spin Labels I, III, and IV in the AOT-H₂O, the Decanol-Decanoate-H₂O Systems, and in 4-*n*-Butyloxybenzylidene-4'-acetoaniline.** Only nearly isotropic ESR spectra were obtained with the sample contained in a capillary tube with no indication of any pronounced ordering of these spin labels when included in Aerosol-OT-water, or the decanol-decanoate-water systems. No ordering could be observed with any of these spin labels, nor with TMPO-CONH₂ (II), when included in the lecithin-water system.

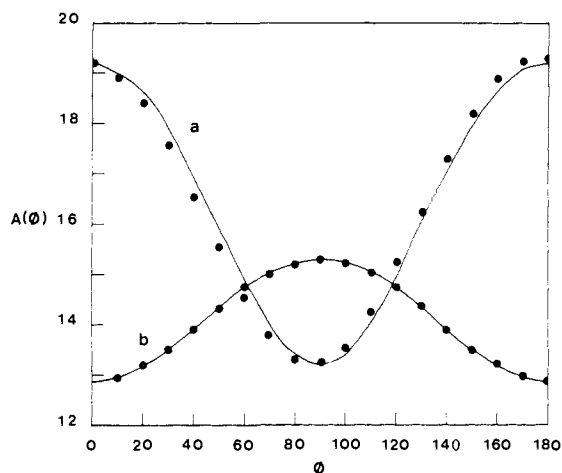


Figure 3. The splitting constant A_{obsd} as a function of the angle ϕ between the normal to the bilayers of the mesophase and the magnetic field in the ESR spectra of TMPO-CONH₂ (II) in the Aerosol-OT-H₂O (50 wt %) system with the sample in a thin layer between parallel quartz plates at room temperature (a); in 4-*n*-butyloxybenzylidene-4'-acetoaniline at 84° (capillary tube) (b). (●) Experimentally obtained values, (—) curves drawn according to eq 3 with $\bar{A}_{\parallel} = 19.2$ G, $\bar{A}_{\perp} = 13.2$ G, and $\bar{A}_{\parallel} = 12.9$ G, $\bar{A}_{\perp} = 15.3$ G, respectively.

However, a small angular dependence of the hyperfine splittings was observed for some of the systems when the samples were placed in a thin layer between parallel plates. The following values were observed with the spin label III: contained in the Aerosol-OT-water system, $A(0^\circ) = 14.9$ G, $A(90^\circ) = 15.5$ G; contained in the decanol-decanoate-water system, $A(0^\circ) = 13.4$ G, $A(90^\circ) = 15.9$ G. TMPO-CN (IV) contained in the decanol-decanoate-water system exhibited the values: $A(0^\circ) = 13.0$ G, $A(90^\circ) = 15.5$ G. Although the angular dependence was very small, the observed values indicated that the orientation of the $2p \pi$ orbital of the spin labels III and IV was parallel to the bilayers, and therefore quite different from that of TMPO-CONH₂ (II) both in the Aerosol-OT-water and the decanol-decanoate-water systems.

An angular dependence of the hyperfine splittings was observed also in the smectic A phase of 4-*n*-butyloxybenzylidene-4'-acetoaniline with the spin labels I, III, and IV, although the magnitude of the angular dependence was rather different for these three spin labels. The largest variation of the angular dependence was observed with the spin label I at 84°, i.e., $A(0^\circ) = 11.6$ G and $A(90^\circ) = 15.9$ G which give an orientational order $S_{33} = -0.17$ with $a_{\text{iso}} = 14.5$ G. The angular dependence was of the same type as that observed with TMPO-CONH₂ (II) (3) in the actual mesophase (4-*n*-butyloxybenzylidene-4'-acetoaniline), suggesting that the molecular plane of the spin labels I, II, III, and IV was oriented parallel to the long axis of the mesogen molecules.

(5) 12-Doxylstearic Acid (VI), 5-Doxylstearic Acid (VIII), and Their Methyl Esters (VII, IX) in the Aerosol-OT-Water (50 wt %) System. The angular dependence of the splitting constant when the sample with 12-doxylstearic acid was contained between parallel quartz plates was such that the maximum value was observed for $\phi = 90^\circ$, i.e., $A(90^\circ) = 15.8$ G, and the minimum value for $\phi = 0^\circ$, i.e., $A(0^\circ) = 10.8$ G, at room temperature. With $A_{zz} = 30.7$ G and $a_{\text{iso}} = 14.1$ G, the orientational order S_{33} was equal to -0.20 . A nearly identical result was obtained with the methyl ester of 12-doxylstearic acid (VII): $A(0^\circ) = 10.9$ G, $A(90^\circ) = 15.9$ G. Figure 4, curve a, gives the splitting constants A_{obsd} as a function of the angle ϕ between the long axis of the mesogen molecules and the magnetic field for the 12-doxylstear-

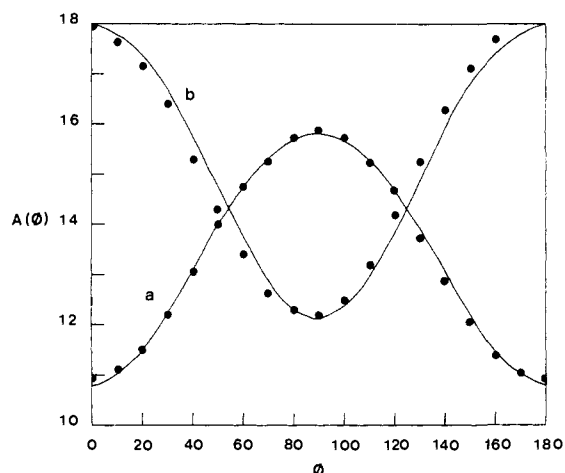


Figure 4. The splitting constant A_{obsd} as a function of the angle ϕ between the normal to the bilayers of the mesophase and the magnetic field in the ESR spectra of 12-doxylstearic acid (VI) in the Aerosol-OT-H₂O (50 wt %) system (a); in the decanol-decanoate-H₂O system (b), with the samples contained between parallel quartz plates in a thin layer at room temperature. (●) Experimentally obtained values, (—) curves drawn according to eq 3 with $\bar{A}_{\parallel} = 10.8$ G, $\bar{A}_{\perp} = 15.8$ G, and $\bar{A}_{\parallel} = 18.0$ G, $\bar{A}_{\perp} = 12.2$ G, respectively.

ic acid spin label. The agreement between A_{obsd} and the values obtained according to eq 3 was excellent.

The results obtained indicated that the $2p \pi$ orbital and the long molecular axis of 12-doxylstearic acid were preferably aligned parallel to the bilayers.

Two partially overlapping signals were observed when the sample contained 5-doxylstearic acid as shown in Figure 5. The relative intensities of the two signals, I and II, were dependent on the amount of water present in the actual sample, but in general the intensity of signal I was the major one. The angular dependence of the splitting constant was of the same type for both signals. Thus the maximum values were observed at $\phi = 0^\circ$ with $A_1(0^\circ) = 19.8$ G and $A_{11}(0^\circ) = 25.5$ G, and the minimum values were observed at $\phi = 90^\circ$ with $A_1(90^\circ) = 12.6$ G and $A_{11}(90^\circ) = 10.3$ G. The corresponding orientational order S_{33} was 0.27 for signal I and 0.56 for signal II.

Only one signal was observed with a sample containing the methyl ester of 5-doxylstearic acid (IX). The angular dependence of the splitting constant was of a type similar to that observed for 5-doxylstearic acid (VIII). The maximum value was $A(0^\circ) = 17.0$ G and the minimum value was $A(90^\circ) = 13.6$ G, which gave an orientational order $S_{33} = 0.13$.

These results indicated that the $2p \pi$ orbital of 5-doxylstearic acid (VIII) and its methyl ester (IX) was preferentially aligned perpendicular to the laminar bilayers in the Aerosol-OT-water system.

(6) 12-Doxylstearic Acid (VI), 5-Doxylstearic Acid (VIII), and 3-Doxyl-5 α -cholestane (V) in the Decanol-Decanoate-Water System. The results obtained in these systems were essentially the same as those reported by Seelig.^{4a} The orientational order S_{33} was 0.23 for 12-doxylstearic acid and 0.60 for 5-doxylstearic acid in this mesophase. The angular dependence of the observed hyperfine splittings of 12-doxylstearic acid is given in Figure 4, curve b, together with the values observed in the Aerosol-OT-water (50 wt %) system described in (5), curve a. It is evident from Figure 4 that the orientations of 12-doxylstearic acid were quite different in these two systems.

The result obtained here with 3-doxyl-5 α -cholestane, i.e., $S_{33} = -0.38$, was in good agreement with that given by Seelig^{4a} for 3-doxyl-5 α -androstan-17 β -ol (-0.34) and 3-

doxyl-5 α -androstande (-0.28), indicating that the steroid spin labels were preferentially aligned with their long molecular axes parallel to the long axis of the mesogen molecules.

(7) **3-Doxyl-5 α -cholestane (V) in the Aerosol-OT-water (50 wt %) System.** The angular dependence of the splitting constant showed that the maximum value was obtained for $\phi = 0^\circ$ ($A(0^\circ) = 15.6$ G) and the minimum value for $\phi = 90^\circ$ ($A(90^\circ) = 14.6$ G). With $A_{zz} = 32.5$ G and $a_{iso} = 14.9$ G, which gives $S_{33} = 0.04$, a rather low degree of ordering, it can be concluded that the alignment of the $2p \pi$ orbital, if any, was parallel and that of the long molecular axis was perpendicular to the long axis of the mesogen molecules.

(8) **Destruction of Spin Labels by Ascorbic Acid.** In order to locate the position of the spin labels in the mesophase systems, a few qualitative experiments were made with the method described by Kornberg and McConnell for the inside/outside localization of spin labels in liposomes.¹⁷ The addition of an excess of ascorbic acid (0.1 mg/ml of mesophase) to the TMPO-CONH₂-Aerosol-OT-water system resulted in an instantaneous disappearance of the ESR signal, whereas the ESR spectrum could be detected in nearly half of its original intensity for about 30 min after the addition of ascorbic acid in the experiments performed with the 12-doxylstearic acid-Aerosol-OT-water system. A similar result with a delayed destruction was also observed with 12-doxylstearic acid in the decanol-decanoate-water system.

An instantaneous disappearance of the ESR signal was also observed with spin label III in the Aerosol-OT-water and the decanol-decanoate-water systems and with TMPO-CN (IV) in the decanol-decanoate-water system.

Discussion

An orientation in lamellar mesophases has hitherto been observed only with spin labels of a rather elongated shape. The orientation of the small spin label TMPO-CONH₂ (II) therefore seems to be quite unexpected. TMPO-CONH₂ was the only one of a number of nitroxides with a five-membered ring and a small substituent group that exhibited a well-established and unique orientation with the molecular plane perpendicular to the long axis of the mesogen molecules of the Aerosol-OT-water and the decanol-decanoate-water systems. The presence of the double bond in position 3 of the ring, together with the carboxamide group, seems to be essential for this orientation, as only a very small orientation was observed with the corresponding cyano (IV) and carboxylic (I) derivatives, and with the saturated carboxamide derivative (III). The orientation of TMPO-CONH₂ (II) might be connected with the presence of two polar groups, the nitroxide and the carboxamide groups, suitably oriented with respect to each other and situated at a convenient distance so that the molecule will fit into the structure of the mesophase.

The orientation of TMPO-CONH₂ (II) with the molecular plane perpendicular to the long axis of the mesogen molecules in the Aerosol-OT-water and the decanol-decanoate-water systems is believed to be connected with a localization of this label in the water phase between the bilayers and a fixation of TMPO-CONH₂ to polar groups of the mesogen. If TMPO-CONH₂ had been located in the region of the hydrocarbon chains of the mesogen of the Aerosol-OT-H₂O, or the decanol-decanoate-H₂O systems, the molecular plane of this spin label would be expected to be oriented parallel to the long axis of the mesogen molecules as was shown to be the case in the smectic A phase of 4-*n*-butyloxybenzylidene-4'-acetoaniline with the small cyclic labels I, II, III, and IV. The instantaneous disappearance of the ESR signal upon the addition of ascorbic acid seems

also to support the supposition of a localization close to the water phase.

The steric arrangement, or the configuration formed by the polar groups of the mesogens, seems to be more important for the unique orientation of TMPO-CONH₂ than the nature of the polar group itself, as the polar group is SO₃⁻ in the AOT-water system, but COO⁻ and OH in the decanol-decanoate-water systems. Furthermore, no interaction between TMPO-CONH₂ and SO₃⁻ groups leading to a fixation could be detected when a water solution of TMPO-CONH₂ was added to the cation exchanger AG 50Wx4 containing SO₃⁻ as exchange groups.¹⁸ The finding that no orientation of TMPO-CONH₂ could be observed in the lamellar phase of β,γ -dipalmitoyl-DL- α -glycerylphosphatidylcholine also indirectly supports the importance of a special steric arrangement of polar groups for the unique orientation of TMPO-CONH₂ in the Aerosol-OT-water and the decanol-decanoate-water systems.

An almost instantaneous disappearance of the ESR signal upon the addition of ascorbic acid was observed also in the case of spin label III, i.e., the saturated analog to TMPO-CONH₂ (II). This finding indicated that the nitroxide group of III was situated very close to the boundary between the water phase and the bilayers, a localization of the nitroxide group which was similar to that suggested for TMPO-CONH₂ (II). However, the orientation of spin label III was relatively small and was quite different from that obtained with TMPO-CONH₂ (II) both in the Aerosol-OT-water and the decanol-decanoate-water systems.

The very different results obtained with TMPO-CONH₂ (II) and spin label III were evidently connected with the steric arrangement in these two labels. The carboxamide group is attached to the five-membered ring by a sp² bond in TMPO-CONH₂, an arrangement which locates this substituent group in the ring plane, whereas the sp³ hybridization on the carbon atom carrying the carboxamide group brings the substituent group out from the ring plane in spin label III.

TMPO-CN (IV) seems also to be located close to the boundary between the water phase and the bilayers as evidenced by the instantaneous disappearance of the ESR signal upon the addition of ascorbic acid. Both TMPO-CN (IV) and TMPO-CONH₂ (II) contain a pyrroline ring. Therefore the orientation quite different from that of TMPO-CONH₂ must be connected with the presence of the cyano group in III in place of the carboxamide group in II.

The Aerosol-OT-water system seems to be a unique mesophase with respect to the orientation of 12-doxylstearic acid (VI), 5-doxylstearic acid (VIII), and their methyl esters (VII, IX). The orientation of the $2p \pi$ orbital of these spin labels indicated that the part of the hydrocarbon chain carrying the doxyl group was oriented parallel with the lamellar bilayers in the case of 12-doxylstearic acid (VI) and its methyl ester (VII), but perpendicular to the bilayers in the case of 5-doxylstearic acid (VIII) and its methyl ester (IX).

These findings implied that the hydrocarbon chains of the doxylstearic acids and their methyl esters were bent somewhere between the carbon atoms 5 and 12, so that the polar ends were located perpendicular to the bilayers, and the hydrocarbon ends were aligned parallel to the bilayers between the two halves of the bilayers.

The bending of the hydrocarbon chains is considered to be due to the branching and the relatively short length of the alkyl groups, i.e., the 2-ethylhexyl groups of the Aerosol-OT molecules. In order to be contained inside the bilayers of Aerosol-OT, the hydrocarbon chains of the stearic acids had to be folded. A similar folding due to an unequal length of the molecules of the mesophase and the spin label

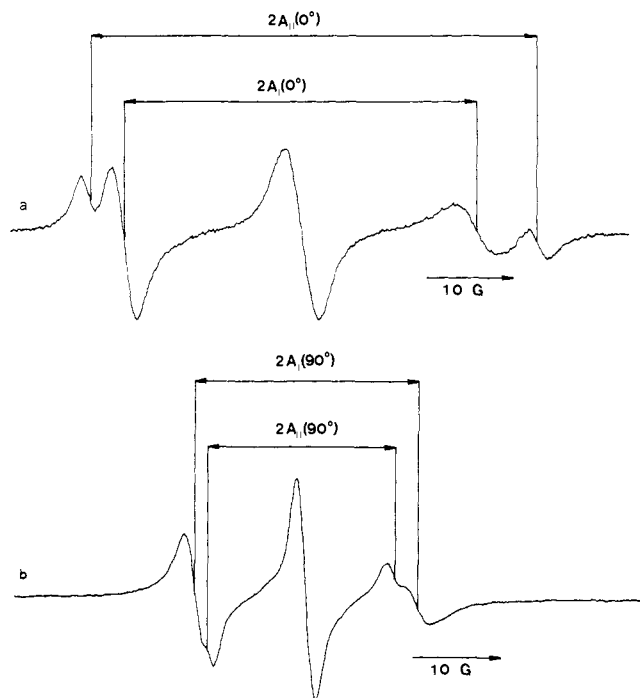


Figure 5. ESR spectra of the spin label 5-doxylstearic acid (VIII) in the Aerosol-OT-H₂O (50 wt %) system in a thin layer between parallel quartz plates at room temperature. Orientation of the sample with the angle ϕ between the normal to the bilayers of the mesophase and the magnetic field: (a) $\phi = 0^\circ$, (b) $\phi = 90^\circ$.

has been observed for some doxylstearic acids when contained in bilayers of sodium caprylate-caprylic acid-water.^{4c}

The presence of two signals, I and II, obtained with 5-doxylstearic acid (VIII) (Figure 5) is believed to be connected with two species of different orientations. The carboxylic groups of the species which gave rise to signal II seem to be anchored directly onto the water phase as evidenced by the rather high degree of orientational order, i.e.,

$S_{33} = 0.56$, a value close to $S_{33} = 0.60$ observed for the decanol-decanoate-water system. The carboxylic groups of the spin label species that gave rise to signal I are believed to be located at a small distance away from the water phase, a position which led to the somewhat smaller value of the orientational order, i.e., $S_{33} = 0.27$.

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