coordinates which were elements of the reaction coordinate.

Two recent surveys[14,15] of the influences of internal–internal coordinate coupling on calculated kinetic isotope effects suggest that inclusion of an element of the reaction coordinate (especially a bond stretch) in such a couple could lead to effects substantially larger than those of bend–external coordinate interaction described here. First, the larger size of diagonal stretching force constants would increase the magnitude of the coupling force constant; second, modification of the restrictions on type I and type II calculations, to retain the preselected zero eigenvalue but permit additional, though minor, elements in the reaction coordinate eigenvector, would result in shifts in $F_{i,i}^{\neq}$ or $F_{i,j}^{\neq}$, which would affect $L(k/k')$ through terms $G_\alpha - G_\beta$ which are intrinsically large. (With or without these embellishments, anharmonicity corrections to $L(k/k')$ may be neglected.[16])

There are other attractive possibilities for the description and calculation of such strong medium interactions. One of these, the structured medium model, is under active investigation in our laboratory.

Acknowledgment is made to Donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

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[15] R. W. Kidd and P. E. Yankwich, in preparation.

[16] Corrections for anharmonicity properly made[17] have resolved discrepancies between theory and experiment in a number of H/D isotopic exchange equilibria;[18] the same principles would be expected to apply here (see eq 6 and Table V). Such corrections decrease rapidly with increasing principal moments of inertia and should be entirely negligible for ^{13}C isotope effects which have a dynamic as well as an equilibrium component (as here). For, as Van Hook has said,[19] "In the case of isotope rate effects, the inclusion of anharmonic correction terms seems a little pointless for the usual calculation where the parameters defining the transition state are essentially guessed." In the context of this report the isotope rate effect is primary and may be large, the medium effects are secondary and very small, and anharmonicity corrections to either may safely be neglected.

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Cis Double Bonds in Liquid Crystalline Bilayers[1]

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Abstract: The structure of lipid bilayers composed of cis unsaturated fatty acids is investigated by means of spin labels. It is found that the hydrocarbon chains assume a bent configuration and impose a certain degree of order even on the central part of the bilayer. Furthermore the spin labels indicate a rapid anisotropic motion of the bent lipid molecules with a correlation time of approximately 3×10^{-10} sec, the axis of motional averaging being perpendicular to the bilayer normal in the range of 30 to 80°. Below 30° the axis of motional averaging changes and the hydrocarbon chains are tilted with respect to the bilayer normal. From the flexibility gradient of the hydrocarbon chains a parameter characteristic of the interaction energy can be extracted. In bilayers with cis double bonds the molar interaction energy is found to be 300 cal per CH_2 unit smaller as compared to bilayers with saturated chains. Spin label data and X-ray diffraction measurements reveal a remarkable temperature adaptability of the unsaturated lipid bilayers. The linear expansion coefficient amounts to only $-0.5 \times 10^{-3}\,°K^{-1}$.

Lyotropic liquid crystals are suitable model systems for investigating structural properties of lipid membranes. Spin label studies have provided information about the structure of the various lyotropic phases and about the mobility in these phases.[3–8] In earlier communications we reported spin label results of lyotropic mesophases containing lipid molecules with saturated hydrocarbon chains. In this work we present results pertinent to the influence of cis double bonds using multilamellar systems of potassium oleate or potassium petroselinate and water, which we investigate with spin labeled fatty acids to probe the conformation of the hydrocarbon chains. The lipid layers of these systems are thus composed of identical molecules, all having a cis double bond between carbon atoms 9 and 10 (oleic acid) or 6 and 7 (petroselinic acid) which facilitates the molecular interpretation of the experimental results. Experimentally these systems have the further advantage that they can be oriented homogeneously which simplifies the spectra and allows an accurate quantitative evaluation. Since cis unsaturated fatty acids are essential elements for the proper functioning and the structure of many biological membranes, the purpose of this investigation is to contribute to a better understanding of the role of cis double bonds in biological systems.

Materials and Methods

Liquid Crystalline Phase. The phase diagram of the binary mixture potassium oleate–water (KOW) at 20°

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[2] Submitted in partial fulfillment of the requirements for the degree of Docteur d'Etat, University of Paris.

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Table I. Chemical Composition and Dimensions of the Bilayer Phases

Abbr used in text	Chemical comp (wt %) Potassium oleate[a]	Water	Bragg spacings, Å d_1	d_2	Thickness of lipid bilayer, Å	Area per polar group, Å^2
	71.5	28.5	39.1		26.9	39.1
KOW (75-25)	75	25	37.8		28.3	37.2
	81	19	38.1		30.9	34.1
	Potassium petroselinate[b]					
KPW (75-25)	75	25	40.5	20.1	~30.3	~34.6
KPW (80-20)	80	20	43.9	22.0	~35	~30.0

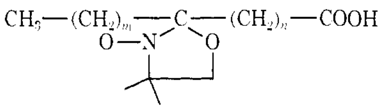
[a] Data taken from ref 9. [b] Measurements of D. Schneider, Biocenter, Department of Cellbiology, University of Basel.

has been described by Ekwall, *et al.*[9] A lamellar mesophase exists in the range of 18 to 30 wt % water. The phase diagram of the potassium petroselinate–water (KPW) mixture is unknown, but it is reasonable to assume similar properties as for the KOW system. This was verified for the lamellar region by X-ray diffraction and polarization microscopy and also by using spin labels.

Oleic acid (>96% purity; Fluka Ltd., Buchs, Switzerland) and petroselinic acid (>99% purity; Hormel Institute, Minnesota) were dissolved in absolute methanol and neutralized with a methanolic solution of potassium hydroxide. The solvent was removed under reduced pressure. The dry soaps were mixed with the appropriate amount of water in a glass ampoule; the ampoule was sealed under argon and the mixture heated several hours until a homogeneous liquid crystalline phase was formed (heating temperature <90°). The material was then left a few hours at room temperature to equilibrate.

The lamellar phases are viscous and of white, translucent appearance. Yellow samples, probably due to partial oxidation of the unsaturated fatty acids, were discarded. The homogeneity of all samples was examined by polarization microscopy. A texture characteristic of the lamellar D phase[9] was found for all KOW and KPW samples used in this work. The KPW preparations were further analyzed by means of X-ray diffraction. A diffraction pattern typical for a bilayer phase (Bragg spacing ratio 1 : $^1/_2$) was found. The chemical composition and the X-ray data are summarized in Table I.

Spin Labels. The spin label measurements were performed with spin labeled stearic acids I(*m*,*n*).[3,5] A small amount of a concentrated spin label solution was



I(*m*,*n*)

dried on a glass plate and the smectic phase added, and they were mixed with a spatula. In order to avoid dipolar broadening the spin label concentration in the phase was less than 0.1 wt %.

The material was then sucked into a rectangular esr quartz cell (50 × 4.5 × 0.02 mm). Due to shearing forces and surface effects the lipid bilayers were thus oriented homogeneously on a macroscopic scale between the two flat surfaces. To improve ordering the sample was eventually cycled through the gel–liquid crystal phase transition a few times. Oriented samples were also made by pressing the spin labeled liquid crystals between two single quartz plates. That a good orientation had been achieved was checked in both cases by the observation of almost complete pseudoisotropy under the polarization microscope.

Samples with a random distribution of bilayers were made with the same material in a spherical cell. From a comparison of the random samples with the oriented samples an eventual "tilt" of the fatty acid chains can be deduced.[7,10,11]

The measurements were made with a Varian E-9 epr spectrometer equipped with a variable temperature unit. The temperature in the epr resonance cavity was measured by means of a thermistor. The uncertainty in the temperature is ±1°. The temperature was varied between −10 and 80°.

Analysis of the Spin Label Spectra. Epr spectra were recorded with the magnetic field H_0 applied parallel and perpendicular to the normal of the large cell surface. The spectral differences between the two orientations indicated that the motions of the lipid molecules in the bilayer were anisotropic.

The quantitative analysis of such anisotropic spectra proceeds in two steps. The first is an analysis of the motion of the nitroxide group alone; the second relates the movement of the label group to the overall motion of the hydrocarbon chain. This allows conclusions about the bilayer structure and bilayer fluidity.

A rigorous treatment of the first problem has been published elsewhere.[7] The rate of the motion of the NO group determines the line width of the resonance lines, while the degree of anisotropy of the motion is responsible for the position of the resonance lines and the hyperfine splittings. A convenient description of the average orientation of the NO group in the bilayer can be given in terms of order parameters S_{ij}, which are elements of the so-called order matrix.[12,13] This formalism, which is also used widely for the nmr spectra of molecules dissolved in nematic liquid crystals, allows an unambiguous analysis of the tensorial averages observed in the epr experiment. The order matrix, a symmetrical and traceless second rank tensor, may contain up to five independent elements. In the case of the nitroxyl radical this number is reduced to two (S_{11} and S_{33}), because the principal axes of the g tensor and the

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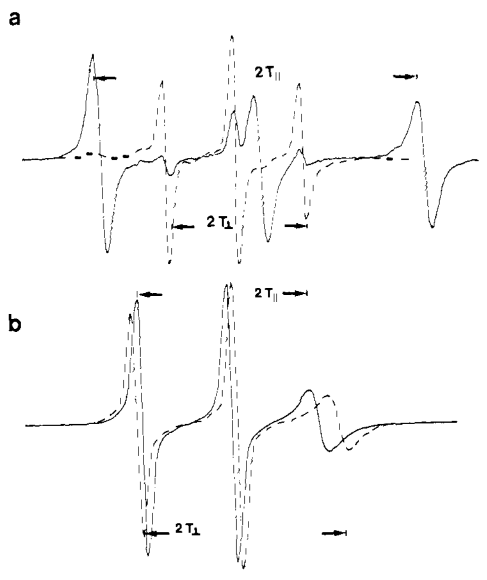

Figure 1. Spin label spectra of oriented potassium petroselinate (75 wt %)-water (25 wt %) bilayers at 40°: (a) spin label I(13,2); (b) spin label I(3,12). Solid line: magnetic field H_0 parallel to bilayer normal. Broken line: magnetic field perpendicular to bilayer normal.

hyperfine tensor in the molecular frame of the NO group are already known from single crystal studies. The order parameters S_{ii} are defined according to eq 1.

$$S_{ii} = (1/2)(3\langle\cos^2 \Theta_i\rangle - 1) \qquad (1)$$

Θ_1 and Θ_3 denote the angles between the NO bond (x axis of the molecular reference frame[7]) and the nitrogen $2p\pi$ orbital (z axis), respectively, with the axis of motional averaging. The angular brackets indicate a time average. In lipid bilayers the axis of motional averaging is in general identical with the normal on the bilayer surface, but other possibilities have also been observed.[10] The order parameter S_{33} can be determined from the hyperfine splittings $T_{\parallel}$ and $T_{\perp}$ (*cf.* Figure 1) according to[3]

$$S_{33} = (T_{\parallel} - T_{\perp})/(T_{zz} - T_{xx}) \qquad (2)$$

T_{zz} and T_{xx} are the maximal and minimal separation of the resonance lines as observed in single crystals. T_{zz} and T_{xx} must be corrected for the appropriate polarity of the environment. The determination of S_{11} requires the additional measurement of the g factor. A correct description of the spin label spectra must be based on these two order parameters or quantities related to these.

The interpretation of the order parameters in terms of a statistical model is less straightforward, since the S_{ii} represent tensorial averages. As can be seen from eq 1, the S_{ii} may assume values in the range of $1 \geqslant S_{ii} \geqslant -0.5$; *i.e.*, they may even become negative. This clearly shows that the order parameters are not directly connected with any random walk model, where "negative" order is meaningless. Negative values of the order parameters are possible because the elements S_{ii} are not only dependent on the randomness of the movement but also on the orientation of the probing group with respect to the axis of motional averaging. Let us illustrate this problem for S_{33} in the case of spin labels I(m,n). If the hydrocarbon chain is frozen in the all-trans configuration the nitrogen $2p\pi$ orbital is extended along the hydrocarbon chain. If the latter is furthermore oriented exactly parallel to the bilayer normal (axis of motional averaging), then $S_{33} = 1$. Let us now consider the hypothetical situation where we leave the motion of the hydrocarbon chain unaltered but change the orientation of the orbital by 90°. The nitrogen $2p\pi$ orbital would then be oriented perpendicular to the axis of rotation, yielding $S_{33} = -0.5$. Similarly an inclination of the probing $2p\pi$ orbital by 54.5° (so-called magic angle) would yield an order parameter $S_{33} = 0$. Thus even if the motion of various carrier molecules has the same statistical order, different values of S_{33} can be observed depending on the orientation of the probing orbital with respect to the axis of rotation. This orientation effect must be borne in mind in the discussion of the results of unsaturated bilayers.

The second parameter which determines the absolute value of the order parameter is the anisotropy of the motion. Leaving the orientation of the NO group unchanged with respect to the molecular axis of the carrier molecule it follows that the more random the motion of the molecule the smaller the absolute value of the order parameter. A totally isotropic tumbling of the molecule thus leads automatically to order parameters $S_{ii} = 0$. Due to the above mentioned orientation effect the reverse conclusion is not allowed, however. An experimental result $S_{33} = 0$ may also be due to a combination of a not completely random motion and a change in the orientation of the probing group due to a bent conformation of the hydrocarbon chain. In the latter case the bent molecule as a whole must rotate rapidly around the bilayer normal.

Results and Discussion

Figure 1 contains the epr signals of two different stearic acid spin labels in oriented bilayers of potassium petroselinate. The magnetic field H_0 is applied parallel and perpendicular to the bilayer normal. For those spin labels where the nitroxyl group is positioned close to the polar surface of the bilayer (Figure 1a), $T_{\parallel}$ is larger than $T_{\perp}$, and S_{33}, according to eq 2, becomes positive. In the central part of the unsaturated bilayer $T_{\parallel}$ is smaller than $T_{\perp}$ (Figure 1b) and S_{33} is negative. Spectra similar to those shown in Figure 1 are observed in the whole temperature interval from 30 to 80°. The corresponding order parameters are plotted in Figures 2 and 3.

The most prominent features of Figures 2 and 3 are the negative order parameters observed in the bilayer interior. In all KOW systems regardless of the water content S_{33} becomes negative for $n > 8$. For the petroselinic acid bilayer this is observed only for the system with the higher water content, whereas in the KPW system with the smaller water content S_{33} is positive for all n and all temperatures.

Negative order parameters are also observed when the same stearic acid spin labels are incorporated into a sodium caprylate–caprylic acid bilayer.[5] In this case the bilayer is shorter than the stearic acid molecule in the extended configuration. In order to keep the hydrocarbon chain in a hydrophobic environment the molecule is therefore forced into a bent configuration, leading to a more or less perpendicular orientation of the nitrogen $2p\pi$ orbital with respect to the axis of molecular rotation.

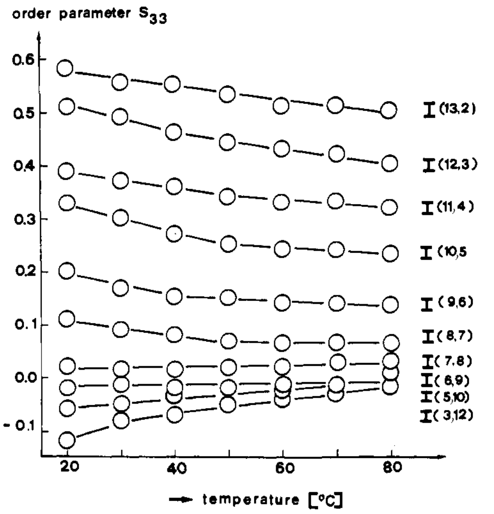

Figure 2. Order parameter S_{33} as a function of temperature for various spin labels I(*m*,*n*). Potassium oleate (80 wt %)–water (20 wt %).

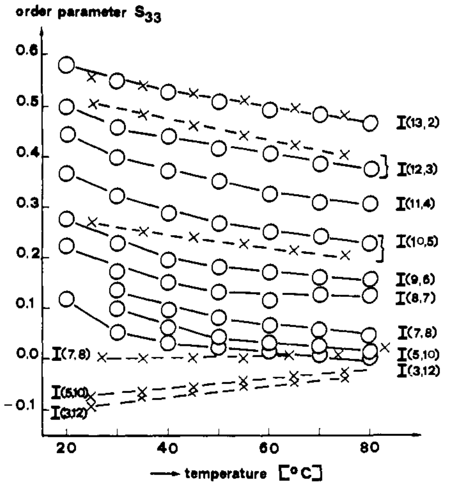

Figure 3. Order parameter S_{33} as a function of temperature for spin labels I(*m*,*n*): (○) potassium petroselinate (75 wt %)–water (25 wt %); (×) potassium petroselinate (80 wt %)–water (20 wt %).

This argument cannot be applied to the KOW or the KPW system, since the unsaturated fatty acids have the same chain length as the spin labels. Here the decisive factor is the cis double bond. As is obvious from molecular models the introduction of a cis double bond into a straight hydrocarbon chain leads to a bent configuration of the molecule. Negative order parameters occur for $n > 8$. According to our nomenclature a spin label with bond number $n = 8$ corresponds to stearic acid with the nitroxyl group attached to the carbon atom 10. Since the double bonds are located between C6 and C7 (KPW) or C9 and C10 (KOW), negative order parameters occur in the middle of the bilayer between the two unsaturated sites. This region is therefore not completely disordered; instead the spin label data reveal that the hydrocarbon chains in the bilayer are bent at a point below the position of the double bond. The chains exhibit an average motion which is biased to a direction parallel to the bilayer surface. From a comparison of spin label spectra arising from oriented and random distributions of bilayers we can further conclude that the axis of motional averaging is perpendicular to the bilayer surface in the range from 30 to 80°. The correlation time can be estimated to be approximately 3×10^{-10} sec, since the observed lines are rather narrow. This is a surprising result, because it means that all parts of the bent molecules are rotating rapidly around a common axis, namely the bilayer normal. One would expect that such a motion causes more friction between neighboring molecules than having the two parts of the bent molecules rotating around two different axes which are inclined to each other. However, the physical possibility of such a motion is also suggested by the X-ray data. It is found that the polar groups occupy a very large surface area of 37 Å^2 compared to 25 Å^2 for a saturated hydrocarbon chain. The introduction of a cis double bond increases the surface area per molecule by about 50%, leaving the molecules more room to move.

Below 30° the hyperfine splitting $T_{\parallel}$ as measured by I(13,2) is smaller in the oriented than in the random sample, indicating a tilt of at least the upper part of the fatty acid chains with respect to the bilayer surface. The axis of motional averaging deviates from the bilayer normal and the tilt angle increases with decreasing temperature. These results are corroborated by X-ray diffraction studies of oleic acid single crystals, where a tilt angle of 38° has been found.[14]

The water content is also an important factor in determining the structure of the bilayer. In general, the effect of increasing the water content of the meso phase is to decrease the order parameter S_{33}. In the KOW systems this effect is smooth; *i.e.*, the general pattern of Figure 2 is preserved for all water concentrations. However, in the KPW system the change in the water content induces a shift from positive to negative order parameters in the central part of the bilayer (*cf.* Figure 3). Since the amount of water is so small (the molar ratio of water : soap ranges from 4 at 18.4% water to 8 at 31% water), all water will probably be bound to the potassium ions and the carboxyl groups.[9,15] The decrease of the order parameter S_{33} may be related to an increase in the spacing of the polar groups due to increasing hydration.

The electrostatic interactions between water dipoles, counterions, and polar groups of the fatty acids are only one factor which influences the structure of a lyotropic liquid crystalline phase. Other contributions arise from dispersive forces between different lipid layers,[16] from van der Waals attraction and steric repulsion between neighboring lipid molecules,[17] and also from intramolecular conformational energies of the individual hydrocarbon chains.[18] A specific structure of lyotropic liquid crystals, *e.g.*, a bilayer, a cylindrical rod, or an inverted cylindrical rod, is the result of a precarious balance of all these different energies. No comprehensive theory of lyotropic liquid crystals is as

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Table II. Energy Parameters q for the KOW (80–20) System

Spin label I(m,n)		Temp, °C 80	70	60	50	40	30	20
I(13,2)	S_{33}	0.50	0.51	0.51	0.53	0.55	0.56	0.58
	$-q^a$	2.44	2.43	2.36	2.40	2.44	2.42	2.45
I(11,4)	S_{33}	0.32	0.33	0.33	0.34	0.36	0.37	0.39
	$-q^a$	1.51	1.51	1.46	1.46	1.50	1.50	1.53
I(9,6)	S_{33}	0.13_5	0.13_6	0.14_1	0.14_7	0.15_5	0.16_7	0.20_4
	$-q^a$	0.66	0.64	0.65	0.65	0.65	0.69	0.81
I(3,12)	S_{33}	−0.02	−0.03	−0.04	−0.05	−0.07	−0.09	−0.12
	$-q^a$	−0.12	−0.15	−0.20	−0.25	−0.32	−0.43	−0.60

[a] kcal mol^{-1}.

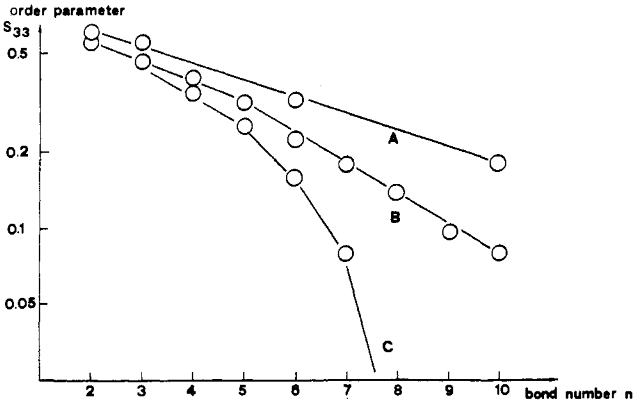

Figure 4. Flexibility profile of three bilayers at 30°: (A) sodium decanoate (28 wt %)–decanol (42 wt %)–water (30 wt %); (B) potassium petroselinate (80 wt %)–water (20 wt %); (C) potassium petroselinate (75 wt %)–water (25 wt %).

yet available. However, a preliminary analysis of the order parameter S_{33} in a bilayer may be attempted in two ways. One possibility is to describe the order parameter S_{33} analogous to the mean molecular field theory used for nematic liquid crystals,[19] In nematic phases the angular deviation of rod-like molecules from a parallel alignment of the molecular axes is given by the distribution function

$$p(\Theta) \propto \exp\{-(q/RT)\cos^2\Theta\} \tag{3}$$

from which the order parameter S_{33} is calculated according to

$$S_{33} = \frac{\int_0^{\pi/2} (^3/_2\cos^2\Theta_3 - {^1/_2})\exp\{-(q/RT)\cos^2\Theta_3\}\sin\Theta_3\, d\Theta_3}{\int_0^{\pi/2} \exp\{-(q/RT)\cos^2\Theta_3\}\sin\Theta_3\, d\Theta_3} \tag{4}$$

q is the orientational energy of the molecules, due to dispersive interactions. In a previous publication we assumed that the distribution function 3 is also applicable to a lipid bilayer, although q may vary with the distance from the water–lipid interface. This enabled us to give a quantitative analysis of the epr spectra of spin labels I(m,n) in a sodium decanoate–decanol bilayer.[7] The wider range of temperature stability of the KOW and KPW bilayers allows a more extensive test of the applicability of eq 3. Some representative results are shown in Table II. Considering spin label I(13,2), for example, we see that q remains constant between 30 and 80°, whereas the order parameter increases from $S_{33} = 0.50$ at 80° to $S_{33} = 0.56$ at 30°. At constant temperature the same change of the order parameter would require an increase of q by 0.4 kcal. On the other hand a continuous change of q is found for spin label I(3,12). From a comparison of the various bilayers and spin labels the following general conclusion can be drawn. For order parameters $S_{33} > 0.15$, the probability function 3 yields a remarkably good approximation of the temperature behavior of all unsaturated bilayers studied here. This may indicate that the same dispersive forces which are responsible for the alignment of nematic liquid crystals are also a major energy term for the ordering of the outer regions of a lipid bilayer. In the central part of the bilayer the segmental motion is more random, dispersive forces seem less important, and thus eq 3 fails to account for the observed temperature dependence.

A second approach is to evaluate the *incremental* change of the order parameter S_{33} as the nitroxyl group is attached at different positions of the hydrocarbon chain. The degree of order decreases toward the central part of the bilayer as is shown in Figure 4 for two KPW systems and also for a bilayer system containing no double bonds. In simple liquid crystalline bilayers as, for example, system A this behavior can be described by the following relation.

$$S_{33} = S_0 S_\alpha^{\,n} \tag{5}$$

S_0 is the rigid body motion of the lipid molecule, S_α denotes the incremental flexibility, and n is the number of methylene units between the polar group and the *N*-oxyloxazolidine ring. In Figure 4 a logarithmic scale has been used for S_{33} and the slope of the different curves is therefore a measure of the chain flexibility. Figure 4 reveals that the saturated system is distinctly more rigid than the unsaturated bilayers. The flexibility gradient method has the advantage that S_α can be interpreted in terms of a statistical model for the chain flexibility.[4] It is assumed that the flexibility S_α is caused by rotational isomerizations between trans and gauche conformations of carbon–carbon bonds. Cooperative interactions between adjacent segments of a hydrocarbon chain are taken into account by using a linear Ising model. Extending the formalism developed in ref 4 leads to the following simple equation for S_α

$$(^2/_3)(S_\alpha^{\,2} + 1) = [(1-\sigma)^2 + 8\sigma]^{-1/2} \tag{6}$$

where

$$\sigma = \exp(-E_\sigma/RT) \tag{7}$$

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The parameter E_σ may be called bond orientation potential, since it determines the average position of a hydrocarbon bond with respect to its foregoing neighbor. For a hydrocarbon chain in a normal liquid E_σ is identical with the bond rotation potential E_{rot} (500 cal mol^{-1}), which is also the energy difference between a trans and a gauche conformation of a hydrocarbon chain in the gaseous state. In the liquid crystalline state E_σ in addition also contains contributions from intermolecular interaction energies. Since the intramolecular bond rotation energy remains constant, E_σ may be used as a parameter to compare the interaction energies in lipid bilayers, at least in the region of positive order parameters; the smaller the E_σ values, the smaller the interaction forces and the more flexible the hydrocarbon chain. Figure 5 shows the E_σ parameter for various systems.

Three bilayers containing unsaturated hydrocarbon chains are compared with a simple bilayer containing no double bonds. In the case of the sodium decanoate–decanol bilayer E_σ is calculated from the average flexibility of ten carbon–carbon bonds; for the other three systems E_σ refers to the first five carbon–carbon bonds only. The bond orientation potential E_σ of the sodium decanoate–decanol bilayer is nearly constant and amounts to $E_\sigma \approx$ 1400–1500 cal mol^{-1} in the range of 50 to 10°, while the unsaturated systems KPW (75-25) and KOW (all water contents) have an E_σ parameter of 1300 to 1200 cal mol^{-1} in the interval of 80 to 30°. The incorporation of the double bonds lowers the interaction energy by approximately 200–300 cal mol^{-1}. This result is consistent with film balance measurements of monomolecular layers of stearic acid and oleic acid, where an energy difference of 300 cal mol^{-1} per CH_2 group has been found.[20] The KPW (80-20) system shows an exceptional behavior which we do not understand as yet.

It has been mentioned above that the E_σ parameter is a measure of the chain flexibility. From eq 5 and 6 it is predicted that the smaller E_σ the less temperature dependent the chain flexibility should be. We have therefore measured the temperature dependence of the Bragg spacing d and determined the linear expansion coefficient α

$$\alpha = \frac{1\Delta d}{d\Delta t}$$

where Δd is the change in the Bragg spacing in the temperature interval Δt. For both KPW systems a linear decrease of d with increasing temperature was observed in the range of 30 to 70°. For the KPW (75-25) system with E_σ = 1200–1300 cal mol^{-1} the Bragg spacing decreases only by 0.9 Å yielding an extremely small expansion coefficient of $\alpha = -0.53 \times 10^{-3}$ °K^{-1}. For the KPW (80-20) system with E_σ = 1400–1500 the expansion coefficient amounts to $\alpha = -1.8 \times 10^{-3}$ °K^{-1}.[21] The diffraction experiments are thus at least qualitatively consistent with the bilayer flexibility as detected by the spin labels.

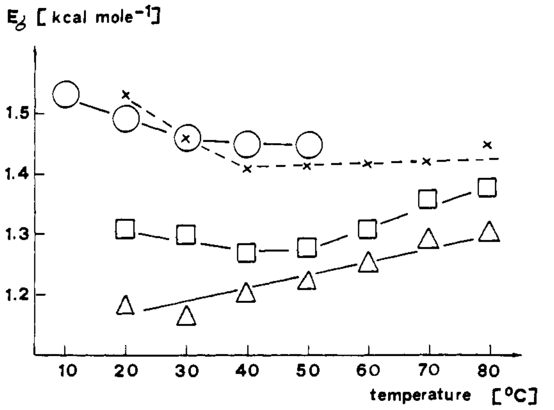

Figure 5. E_σ parameter as a function of temperature: (○) sodium decanoate (28 wt %)-decanol (42 wt %)-water (30 wt %); (×) potassium petroselinate (80 wt %)-water (20 wt %); (△) potassium oleate (80 wt %)-water (20 wt %); (△) potassium petroselinate (75 wt %)-water (25 wt %).

Conclusions

From spin label and X-ray data it can thus be concluded that the incorporation of cis unsaturated lipid molecules leads to an expanded bilayer structure with a large surface area per polar group. At the same time such unsaturated bilayers exhibit a remarkable temperature adaptability, the relative change in the bilayer thickness with temperature being distinctly smaller as compared to systems with saturated chains. This can be explained by differences in the interaction energies characteristic of the various systems. It is reasonable to assume that by a similar mechanism cis double bonds also regulate the temperature adaptability of living cells.

Probably the most interesting finding of this study is the fact that the lipid molecules assume a bent configuration in which they rotate around the bilayer normal of the model systems. It has been shown that the presence of specific phospholipids is an essential requirement for the activity of several membrane bound enzymes and that in some cases the enzyme activity depends on the percentage of cis unsaturation of the phospholipid chains.[22] The finding that the cis double bond can be replaced in these systems by a cis cyclopropane ring with almost equal efficiency supports the idea that unsaturation plays an important role not by its chemical nature but by the configurational properties it confers to the hydrocarbon chains. This suggests the possibility that the structure and chain motion observed in our model systems is of relevance for the functional properties of real membranes. Spin label experiments have already been carried out with membranes biosynthetically enriched in unsaturated fatty acids.[23] However, since the measurements were performed on isotropic distributions of membrane fragments, small negative and positive order parameters must lead to similar spectra. Only oriented membrane fragments and/or a precise line shape analysis would allow an eventual detection of bent chains in natural membranes.

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