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Relative Roles of Cyclopropane-Containing and Cis-Unsaturated Fatty Acids in Determining Membrane Properties of *Acholeplasma laidlawii*: A Deuterium Nuclear Magnetic Resonance Study†

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ABSTRACT: Dihydrosterculic acid (*cis*-9,10-methyleneoctadecanoic acid, 19:cp,cΔ^9), specifically deuterated at several positions along the chain, has been incorporated biosynthetically into the membrane lipids of *Acholeplasma laidlawii* B. A detailed study of acyl chain order was carried out with deuterium nuclear magnetic resonance. The transition from the gel to the liquid-crystalline phase was determined to occur from −15 to 0 °C, a range somewhat narrower than, but with a midpoint similar to, that found for membranes enriched in oleic acid. The acyl chains of 19:cp,cΔ^9-containing membranes are less mobile in the gel state than those of oleic acid containing membranes. Above 0 °C, the lipids are in the liquid-crystalline phase and give rise to powder spectra characteristic of axially symmetric motion. The C^2H_2 segments near the cyclopropane ring gave rise to a quadrupolar powder pattern indicative of inequivalence of the two deuterons. The orientational fluctuations of the fatty acid chain segments in the membrane lipids are described in terms of deuterium order parameters. The overall ordering is greater everywhere than that in the case of oleoyl chains and features a maximum at the cyclopropyl moiety, in sharp contrast to the plateau found with saturated chains. Detailed analysis of the data for the cyclopropane ring indicates that the C-9–C-10 bond is inclined at 89° relative to the director of motional averaging, in sharp contrast to the 3° estimated for oleic acid in the same membranes. The effect of incorporation of cholesterol at 35 mol % lipid was examined. This had little effect on the breadth of the gel to liquid-crystal transition but did result in a gel state with lipid that is rotationally more rigid.

Biological membranes consist primarily of a lipid matrix in which other membrane components are organized. The lipid molecules may contain saturated, unsaturated, branched-chain, and cyclopropane fatty acids. Lipids containing saturated and/or unsaturated fatty acyl chains have been extensively studied while those containing other classes of fatty acids have received little attention. Fatty acids containing cyclopropyl rings are found in a variety of organisms and are common components of the membrane lipids of microorganisms (Christie, 1970). Although fatty acids containing cyclopropane rings have been postulated as replacements for unsaturated fatty acids (Christie, 1970; Cronan & Vagelos, 1972), their precise biochemical role remains obscure. An antioxidant role (Law et al., 1963) does not appear to be tenable since both anaerobes and aerobes possess these acids. Since the rate of cyclopropane ring biosynthesis is not influenced by the level of *S*-adenosylmethionine (SAM)[1] (Cronan et al., 1974), the formation of the cyclopropane ring is an unlikely mechanism

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[1] Abbreviations: SAM, *S*-adenosylmethionine; NMR, nuclear magnetic resonance; 19:cp,cΔ^9, *cis*-9,10-methyleneoctadecanoic acid; 18:1cΔ^9, *cis*-9-octadecenoic acid; PDSPC, 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

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for controlling the levels of SAM. Fatty acids containing cyclopropane rings are formed at the expense of their cis-unsaturated homologues (Cronan & Vagelos, 1972) and can be present at high levels, for example, when *Escherichia coli* enters the stationary phase (Peypoux & Michel, 1970; Cullis & de Kruijff, 1978). In the case of *Lactobacillus plantarum*, these fatty acids are present (Buist, 1980) and may reach levels of >90% of the total fatty acids (R. A. Byrd, A. Joyce, and I. C. P. Smith, unpublished results). Phospholipids containing fatty acids having an olefinic function or a cyclopropane ring have been examined recently by thermal analysis (Cronan et al., 1979; Silvius & McElhaney, 1979). In general, the results indicated that both types of acyl chain behave similarly in a membrane bilayer and suggested that they play some subtle role in the cell membrane (Silvius & McElhaney, 1979; Chopra & Eccles, 1977). In a recent study (Souzer, 1982), *E. coli* membrane stability was related to cell growth phase; membranes from cells cultured to stationary growth phase exhibited greater organizational stability. One might speculate that the occurrence of cyclopropane-containing fatty acids in the stationary phase of *E. coli* is associated in some way with membrane stability.

Since the thermotropic behavior of aqueous lipid dispersions, as monitored by calorimetric techniques, reflects cooperative properties such as acyl chain melting, noncooperative changes in acyl chain structural and dynamical properties remain unprobed. Deuterium nuclear magnetic resonance (^{2}H NMR) is particularly well suited to the monitoring of molecular structure and dynamics within membranes (Seelig, 1980; Smith, 1983; Davis, 1983). The incorporation of ^{2}H-labeled fatty acids into membrane lipids provides a convenient method of selectively examining lipid acyl chain organization at any desired depth in the membrane.

The purposes of the present study are to elucidate the effects of the cyclopropane ring on the properties of lipid acyl chains in a biological membrane and to correlate these effects with those observed for the olefinic function. ^{2}H NMR is used to probe the structure and dynamics of a cyclopropane-containing fatty acid, namely, dihydrosterculic acid, in the lipids of membranes derived from *Acholeplasma laidlawii* B. This microorganism was chosen for study because, while it does not produce cyclopropane-containing fatty acids, it will incorporate them into its membrane lipids (Silvius et al., 1977, 1980). Since the organism has only a single plasma membrane, relatively homogeneous membrane preparations are readily prepared and have been used in a number of other ^{2}H NMR studies (Smith, 1983; Jarrell et al., 1982; Rance et al., 1982; Davis et al., 1980; Smith et al., 1979). In addition, ^{2}H NMR studies have been reported recently on *A. laidlawii* membranes containing oleic acid (Rance et al., 1980, 1982), providing a parallel study for comparison with the present study. Finally, the ^{2}H NMR results are compared with those obtained for an analogous pure phospholipid system (Dufourc et al., 1983). These results are expected to form the basis for the interpretation of similar studies on more complex organisms that produce cyclopropane-containing fatty acids biosynthetically.

Materials and Methods

Specifically labeled oleic acids (Tulloch, 1979) were converted to the corresponding *cis*-9,10-methyleneoctadecanoic acids (dihydrosterculic acid) according to standard procedures (Christie et al., 1968). [19,19-$^2H_2$]Dihydrosterculic acid was prepared from oleic acid (Sigma Chemical Co., St. Louis, MO) and [$^2H_2$]methylene iodide (Winstein et al., 1966). The fatty acids were ≥95% pure, containing 0–3% of the trans isomer as determined by gas chromatography of the methyl esters (Table I). The deuterium content of the fatty acids was determined by mass spectroscopy (Christie et al., 1968) to be >90%.

Table I: Composition of *A. laidlawii* Membranes Grown in the Presence of Labeled Dihydrosterculic Acid

position labeled	mol % fatty acid						mol % cholesterol
	14:0	16:0	18:0	18:1	19:cp	other	
2	5.3	37.8	8.0	1.0	48.9		0
	1.9	34.1	4.5	2.5	48.6	8.4	35 ± 5
5	6.0	36.4	6.6	2.7	49.7[a]		0
	4.5	36.0	6.3	2.5	50.0[a]		35 ± 5
8	6.0	39.7	7.4	1.6	45.3[a]		0
	4.7	40.9	5.9	1.1	46.9[a]		35 ± 5
9 and 10	6.4	36.8	6.4	0.2	50.6[a]		0
	5.2	33.1	4.0	1.0	57.7[a]		28 ± 5
11	1.0	33.3	5.5	trace	55.1	5.1	0
16	5.5	36.8	7.5	14.4	34.4	1.5	0
19	8.4	44.2	7.9	1.4	34.6	3.4	0
unlabeled	6.0	37.4	6.0	1.2	49.5		0

[a] Contains ≤2% of trans isomer of 19:cp.

The specifically deuterated fatty acids ([2,2-$^2H_2$]-, [5,5-$^2H_2$]-, [8,8-$^2H_2$]-, [9,10-$^2H_2$]-, [11,11-$^2H_2$]-, [16,16-$^2H_2$]-, [19,19-$^2H_2$]dihydrosterculic acid) were incorporated biosynthetically into membranes of *A. laidlawii* as described previously (Stockton et al., 1975). The growth medium was enriched with either the labeled fatty acid (20 mg/L) alone or the fatty acid (20 mg/L) and cholesterol (20 mg/L). The acyl chain distribution and the cholesterol content as determined by gas chromatography (Rance et al., 1980, 1982) are given in Table I. Lipid extracts were prepared according to procedures described previously (Jarrell et al., 1982).

The NMR samples consisted of ~200–300 mg of freeze-dried membranes, hydrated with deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a weight ratio of 1:3. Lipid samples for NMR were prepared as described above but were cyclically heated at 37 °C and freeze–thawed to homogeneity.

^{2}H NMR spectra were obtained at 46.063 MHz on a Bruker CXP-300 spectrometer by using a home-built probe (R. A. Byrd, unpublished data). NMR spectra were acquired by the quadrupolar echo technique (Davis et al., 1976) with full-phase cycling of the radio-frequency pulses. The spacing between the two $\pi/2$ pulses of the basic echo sequence was 50–60 μs, the $\pi/2$ pulse length was 5.5 μs (10-mm sample coil), and the recycle delay was 0.1–0.2 s. T_1 studies (H. C. Jarrell and I. C. P. Smith, unpublished results) indicated that the spectra were fully relaxed with respect to T_1. The frequency of the spectrometer was set at the center of the symmmetric powder pattern. Spectra were acquired with quadrature detection but were folded so that the two halves were superimposed, resulting in an increase in the signal-to-noise ratio by a factor of $2^{1/2}$. In all cases the folded and nonfolded spectra were compared to ensure that no distortions were introduced by the folding procedure.

The sample was enclosed in a glass dewar and the temperature regulated electronically to within approximately ±0.5 °C. After a temperature change, the sample was allowed 15 min to equilibrate. The temperature gradient across the sample was measured to be ≤0.1 °C.

The NMR data were transferred from the Bruker Aspect-2000 computer to a Nicolet 1280 data processor via an RS-232 line (R. A. Byrd and M. Rance, unpublished data). Spectral de-Paking calculations (Bloom et al., 1981) were done on the Nicolet 1280 computer by using ~300–500 data points with three iterations, giving good convergence.

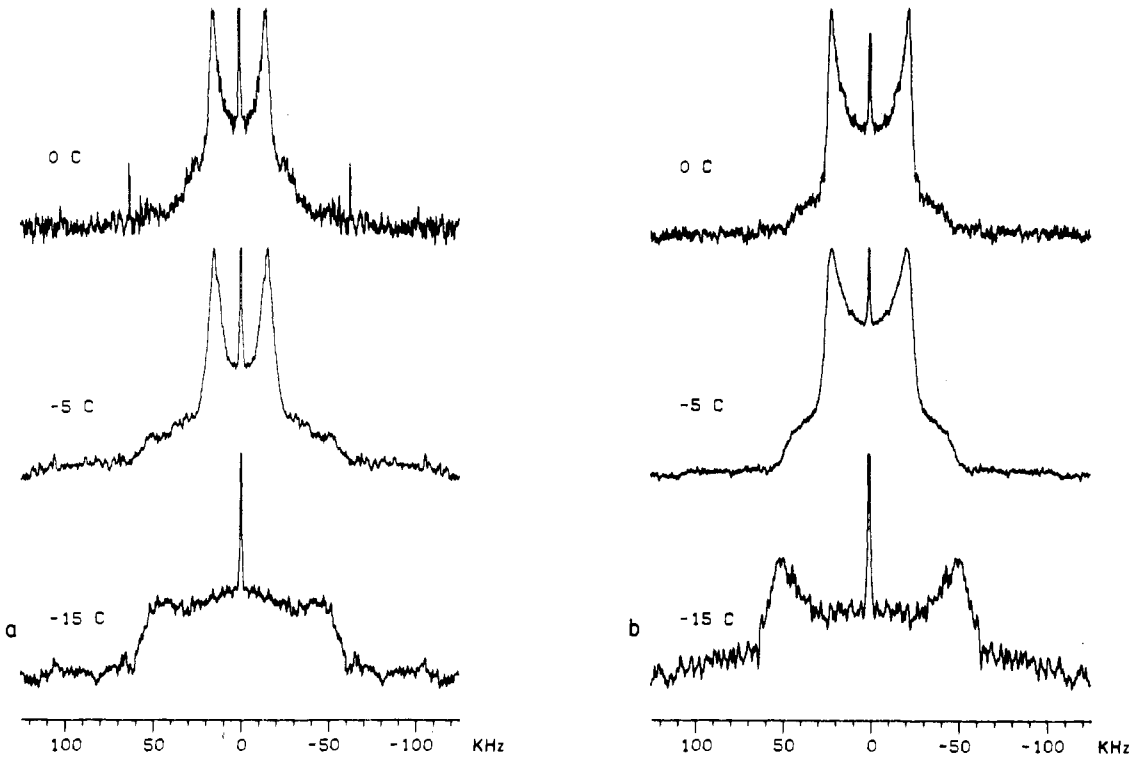

FIGURE 1: ^2H NMR spectra (46.063 MHz) of [5,5-$^2H_2$]dihydrosterculoyl chains in membranes of *A. laidlawii* (a) without and (b) with cholesterol at the indicated temperatures. The $\pi/2$ pulse duration was 5.75 μs, the pulse spacing was 60 μs, the recycle time was 0.1 s, and there were 64 000 accumulations.

Results and Discussion

Phase Transition. In order to make a valid comparison of the structural and dynamical properties of *A. laidlawii* membranes enriched in *cis*-9,10-methyleneoctadecanoic acid (dihydrosterculic acid, 19:cp,cΔ^9) with those of membranes enriched in oleic acid, the temperature over which the gel to liquid-crystalline phase transition occurs must be known. Recently, the thermotropic properties of a number of synthetic phosphatidylcholines with unsaturated or cyclopropane-containing fatty acyl chains were examined by differential thermal analysis (Silvius & McElhaney, 1979). In general, the replacement of cis double bonds by *cis*-cyclopropyl groups in the acyl chains was reported to raise the gel to liquid-crystal phase transition by ca. 15 °C. However, since bacterial membranes generally contain ≤50% of the total lipid acyl groups as unsaturated fatty acids, conversion of all unsaturated acyl moieties to cyclopropane-containing acyl groups would not be expected to elevate the midpoint temperature of the phase transition by more than a few degrees. Low-temperature ^2H NMR spectra of membranes containing [5,5-$^2H_2$]-19:cp,cΔ^9 are shown in Figure 1a. At 0 °C, in addition to the spectrum associated with lipid in the liquid-crystalline phase, a broad spectral component is visible. Below 0 °C, the broad component increases in intensity, dominating the spectrum by −15 °C with a separation of the shoulders of ca. 100 kHz. Typically, ^2H NMR spectra of membranes at temperatures just below that at which only gel-state lipid is present have broad rounded features with a separation at the shoulders of ca. 63 kHz (Smith et al., 1979; Rance et al., 1980; Jarrell et al., 1982). Similar spectra have been interpreted in terms of asymmetric rotational isomerization of the acyl chains (Huang et al., 1980). This does not appear to be the case with membranes containing dihydrosterculic acid at −15 °C, where the acyl chain appears to be undergoing motions that are slow relative to the inverse of the quadrupolar interaction. The relatively low signal-to-noise ratios of the spectra in Figure 1 preclude any further interpretation of the spectral shape. The transition from fast to relatively slow motion, as reflected in Figure 1, has also been observed for aqueous dispersions of 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine (PDSPC; Dufourc et al., 1983) as well as for membranes containing cholesterol (vide infra). The presence of a cyclopropyl moiety results in a relatively narrow temperature range for the transition from fast to relatively slow motion about the long molecular axis. This contrasts with results reported for *A. laidlawii* membranes containing oleic acid where considerable acyl chain motion persisted even at −73 °C (Rance et al., 1980), some 51 °C below the temperature at which only gel-state lipid is evident.

The gel to liquid-crystal phase transition for *A. laidlawii* membranes containing 19:cp,cΔ^9 appears to occur between −15 and 0 °C. The corresponding membranes containing 18:1cΔ^9 have been reported to undergo the transition between −30 and 0 °C with the transition centered at ca. −12 °C (Rance et al., 1980). The similarity in transition temperatures for the 19:cp,cΔ^9 and 18:1cΔ^9 membranes indicates that results obtained for the two systems at a given temperature may be compared directly.

Liquid Crystalline Phase Spectra. Representative ^2H NMR spectra for hydrated membranes enriched in labeled dihydrosterculic acid at 25 °C are shown in Figure 2. For temperatures above 0 °C, where all of the lipid is in the liquid-crystalline phase, most of the membranes (with the exception of those enriched in [5,5-$^2H_2$]- and [16,16-$^2H_2$]-19:cp,cΔ^9) gave rise to multiple quadrupolar powder patterns.

Molecular motion that occurs on a timescale shorter than, or on the order of, the quadrupolar interaction ($\sim 10^5$ s^{-1}) will reduce the quadrupolar splitting, $\Delta\nu_Q$, from its rigid lattice value of ca. 126 kHz. The angular fluctuation of the C–^2H bond relative to the bilayer normal can be expressed in terms of the C–^2H bond order parameter, S_{CD} (Seelig, 1977):

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

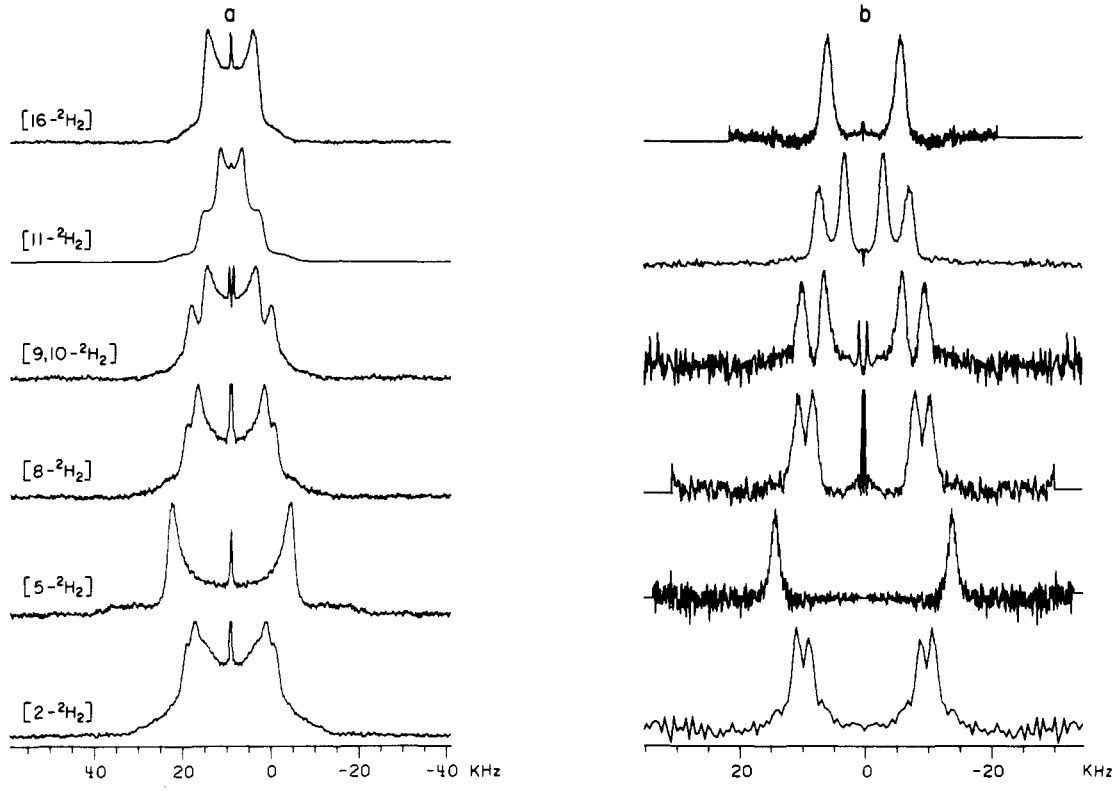

FIGURE 2: ^2H NMR spectra (46.063 MHz) of labeled dihydrosterculoyl chains in membranes of *A. laidlawii* at 25 °C. The $\pi/2$ pulse was 5.0–5.75 μs, the pulse spacing was 60 μs, and the recycle time was 0.1–0.25 s. (Left) Regular spectra; (right) de-Paked to 90° spectra.

This equation assumes that the electric field gradient at the deuteron nucleus is axially symmetric about the C–^2H bond axis and that motion of the C–^2H bond is axially symmetric. The static quadrupole coupling constant (e^2qQ/h) is about 170 kHz for aliphatic C–^2H bonds (Burnett & Muller, 1971) and 183 kHz for the cyclopropyl C–^2H bonds (Burnett & Muller, 1971; Dufourc et al., 1983). The value of S_{CD} is determined by the average orientation of the C–^2H bond with respect to the bilayer normal and by the amplitude of the angular fluctuations about this average orientation.

Membranes containing [2,2-$^2H_2$]-19:cp,cΔ^9 give rise to ^2H NMR spectra that exhibit at least three overlapping powder patterns (Figure 2). In model membrane systems, the ^2H spectra of phospholipids labeled at the C-2 position of both acyl chains are characterized by three components (Seelig & Seelig, 1975), one attributable to the *sn*-1 chain and two associated with the *sn*-2 chain. Membranes containing oleic acid labeled at the C-2 position also give rise to ^2H spectra having multiple components (Rance et al., 1980). A detailed analysis of the ^2H spectra of membranes containing 19:cp,cΔ^9 and 18:1cΔ^9 labeled at the C-2 position is reported elsewhere (Rance et al., 1983). The analysis revealed that there are four spectral components that are attributable to conformational differences at the C-2 position of the *sn*-2 chain and are associated with the lipid head group classes present in the membrane. No conformational differences were visible at the C-3 position. The specificity of dihydrosterculic acid for the *sn*-2 position of glycero lipids is greater than that of oleic acid (Saito et al., 1977). Since 19:cp,cΔ^9 comprises $\leq$50% of the total fatty acid composition of the *A. laidlawii* membrane lipids, spectral contributions due to the fatty acid at the *sn*-1 position are expected to be small. In the case of membranes labeled with [2,2-$^2H_2$]-19:cp,cΔ^9, less than 10% of the fatty acid is associated with the *sn*-1 position (Rance et al., 1983). ^2H spectra of membranes enriched in [9,10-$^2H_2$]-19:cp,cΔ^9 consist of two overlapping powder patterns of equal integrated intensity, Figure 2, suggesting that the deuterons are motionally inequivalent.

In spectra in which poorly resolved overlapping patterns are present, it is difficult to obtain accurate values of bond order parameters and relative spectral intensities directly from the experimental spectrum. Recently, Bloom and co-workers (Bloom et al., 1981) have reported a method of extracting oriented-sample spectra accurately from powder spectra, the oriented-sample spectrum being that for a single orientation of the director of motional averaging with respect to the external magnetic field direction (in the present discussion, the 90° orientation). The "de-Paked" spectra calculated for the C-9–C-10 segment, as well as for the other labeled positions, are shown in Figure 2. The de-Paked spectra shown in Figure 2 facilitate the determination of S_{CD} values and relative spectral intensities without resorting to time-consuming spectral simulations.

If the C-9–C-10 bond of dihydrosterculic acid were parallel to the director of motional averaging, the deuterons at these positions would be motionally equivalent. Because the bonds in the cyclopropane system are rigid, on the ^2H NMR timescale, the average angle between the C-^2H bonds at positions 9 and 10 and the director would be the same. Since two quadrupolar splittings are observed, the C-9–C-10 bond cannot be parallel to the director. In the case of membranes containing oleic acid labeled at the double bond, ^2H NMR spectra reflect the inequivalence of deuterons at these positions. Seelig & Waespe-Šarčevič (1978) have shown that a relatively small angle of 7–8° between the double bond and the director is sufficient to account for the two quadrupolar splittings observed for the double-bond positions in oleic acid containing phospholipid systems. A similar tilting of the double bond of oleic acid in *A. laidlawii* membranes was assumed to be the source of the inequality of the deuterons at the C-9 and C-10 positions (Rance et al., 1980). In the case of model membranes composed of 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-

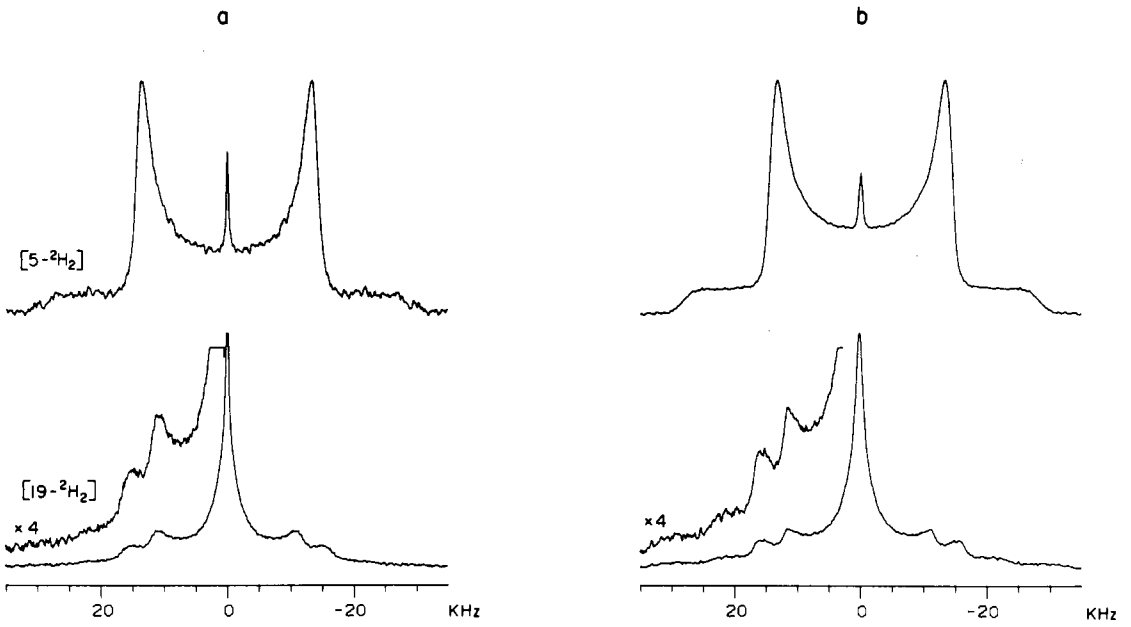

FIGURE 3: ^2H NMR spectra (46.063 MHz) of labeled dihydrosterculoyl chains in (a) membranes and (b) aqueous dispersions of lipid extracts of (a) at 25 °C. Spectra were acquired as in Figure 2.

phosphocholine (PDSPC), the deuterons in [9,10-$^2H_2$]-19:cp,cΔ^9 were nonequivalent (Dufourc et al., 1983). The C-9–C-10 bond was determined to be tilted with respect to the director at an angle of 89°. The similarity of the quadrupolar splittings observed for model and *A. laidlawii* membranes containing [9,10-$^2H_2$]dihydrosterculic acid (Figure 4) suggests that the cyclopropane ring system assumes comparable orientations with respect to the director in both systems.

Membranes containing [8,8-$^2H_2$]dihydrosterculic acid, as well as those containing [11,11-$^2H_2$]-19:cp,cΔ^9, give rise to ^2H NMR spectra that are each composed of two overlapping powder patterns of equal integrated intensity (Figure 2). The corresponding membranes containing either [8,8-$^2H_2$]- or [11,11-$^2H_2$]oleic acid exhibit ^2H spectra in which only one quadrupolar splitting is manifest (Rance et al., 1983). In the case of the oleate-containing membranes, the tilting of the double bond with respect to the director also causes the C^2H_2 segments next to the double bond to be tilted, with the deuterons within each methylene group remaining magnetically equivalent (Seelig & Waespe-Šarčevič, 1978). The situation is clearly different for membranes containing dihydrosterculic acid. By analogy with the oleate system, the methylene segments adjacent to the cyclopropane ring are most likely tilted with respect to the director. According to eq 1, for the deuterons within each methylene segment to be motionally nonequivalent, the two C–^2H bonds are being averaged about different angles relative to the bilayer normal; this is comparable to the situation involving deuterons at the C-2 position of the *sn*-2 acyl chain (vide supra). The quadrupolar splittings of the C-8 and C-11 positions have values that are similar to those obtained for PDSPC model membranes (Figure 4), reflecting similar behavior of the acyl chains in both systems.

Membranes containing [5,5-$^2H_2$]dihydrosterculic acid give rise to a single axially symmetric powder spectrum at all temperatures above 0 °C, as do membranes containing [16,16-$^2H_2$]-19:cp,cΔ^9 (Figure 2). The values of the quadrupolar splittings or bond order parameters are larger than those reported for the corresponding oleate systems (Rance et al., 1980). As expected, the C-16 position is more disordered than the C-5 position, reflecting increased amplitudes and rates of motion at the methyl end of the chain relative to those near the carboxyl end (Smith, 1983; Davis, 1983).

The ^2H NMR spectrum obtained for membranes containing [19,19-$^2H_2$]dihydrosterculic acid at 25 °C is shown in Figure 3a. In addition to the presence of two quadrupolar splittings of 23.4 and 32.2 kHz, a relatively narrow component is present. The narrow feature does not appear to be associated with water since the same result was obtained with two membrane preparations and since the relative contribution of the narrow spectral component to the total spectrum did not change when the recycle time of the NMR experiment was increased from 0.1 to 2 s. ^2H NMR spectra of hydrated 1-palmitoyl-2-[19,19-$^2H_2$]dihydrosterculoyl-*sn*-glycero-3-phosphocholine exhibit two overlapping quadrupolar powder spectra of equal integrated intensity (Dufourc et al., 1983), but no narrow component was detected. The ^2H NMR spectrum of the lipid extract (Figure 3b) is nearly identical with that of the membranes, indicating that protein had little or no effect on the orientational order at the C-19 position. The origin of the narrow component remains unclear.

Figure 4a shows the bond order parameter, S_{CD}, as a function of label position at 25 °C for 19:cp,cΔ^9 and 18:1cΔ^9 membrane systems. Figure 4b shows the corresponding profiles for the lipids of the 19:cp,cΔ^9 membranes and for pure PDSPC. The S_{CD} values were calculated according to eq 1 with the assumption of a quadrupole coupling constant of 170 kHz for all labeled positions except C-9, C-10, and C-19. For the latter, a value of 183 kHz was used (Dufourc et al., 1983). Figure 4a suggests that at all positions monitored the membranes containing dihydrosterculic acid were more ordered than the corresponding oleic acid containing membranes. Figure 4b indicates that positions up to the cyclopropane ring are slightly more ordered in the natural membranes relative to the corresponding pure phospholipid system, whereas for the 16-position, the phospholipid system is slightly more ordered. These results are in general agreement with the trends observed for oleate systems (Rance et al., 1980).

The comparison of $\Delta\nu_Q$ or S_{CD} for the cyclopropane-containing and unsaturated fatty acid systems does not afford a straightforward evaluation of the relative behavior of the two systems. The bond order parameter S_{CD} contains the geometrical relationship between the C–^2H bond and the axis of

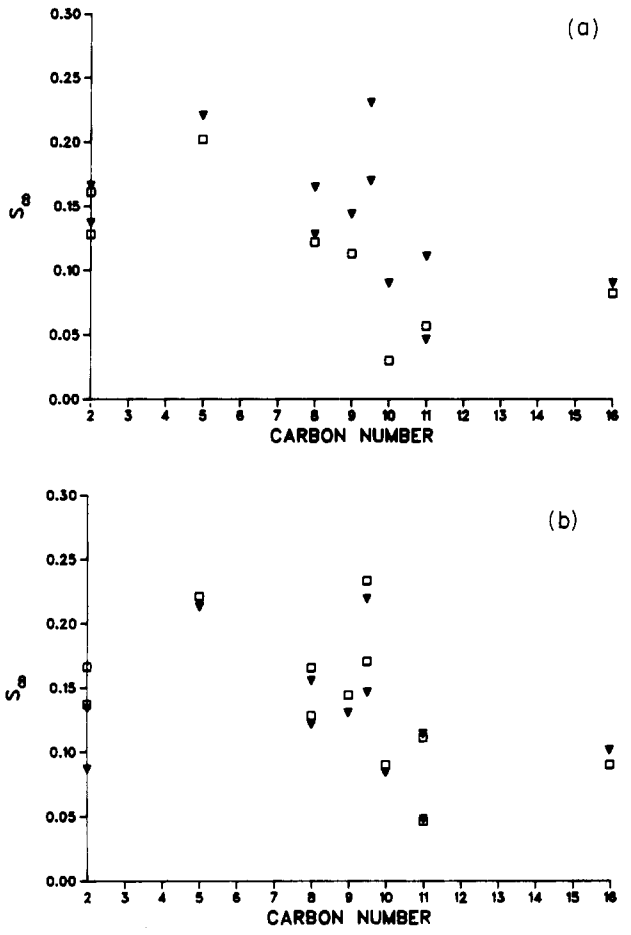

FIGURE 4: Variation of deuterium order parameter, S_{CD}, with labeled carbon atom. (a) (□) Oleate-labeled *A. laidlawii* membrane at 25 °C; (▼) dihydrosterculoyl chains in *A. laidlawii* membranes at 25 °C. (b) Dihydrosterculoyl chains in (□) *A. laidlawii* membranes and (▼) PDSPC at 25 °C. Data for oleate-labeled membranes are taken from Rance et al. (1980), and data for PDSPC are taken from Dufourc et al. (1983). The points for C-19 are placed between those for C-9 and C-10.

molecular diffusion. Proceeding in a manner suggested previously for rigid parts of the molecule (Seelig & Waespe-Šarčevič, 1978; Petersen & Chan, 1977)

$$S_{CD} = \left(\frac{\overline{3\cos^2\alpha - 1}}{2}\right)\left(\frac{\overline{3\cos^2\gamma - 1}}{2}\right) \qquad (2)$$

$$S_{CD} = S_{mol}S_{geo}$$

where α is the instantaneous angle between the director and the axis of molecular diffusion and γ is the angle between the C–^{2}H bond and the axis of diffusion. The first term of eq 2 applies to the motion of the segment as a whole, S_{mol}, and the second term relates the orientation of the C–D bond in the segment-fixed axis system. It is the geometrical (latter) factor that gives rise to the S_{CD}–position profile obtained for oleic acid containing systems (Seelig & Waespe-Šarčevič, 1978) (Figure 4). As a consequence of the rigidity of the cyclopropane unit, deuterons at positions C-9, C-10, and C-19 have the same segmental order parameter, S_{mol}, and differ only in their orientation with respect to the axis of diffusion of the cyclopropane ring as a whole. Two methods for obtaining molecular order parameters from S_{CD} values have been reported (Seelig & Waespe-Šarčevič, 1978; Taylor et al., 1981). Each method defines a segment-fixed coordinate system in which the axis of diffusion is located. The first method relates the observable S_{CD} order parameters to order matrices defined

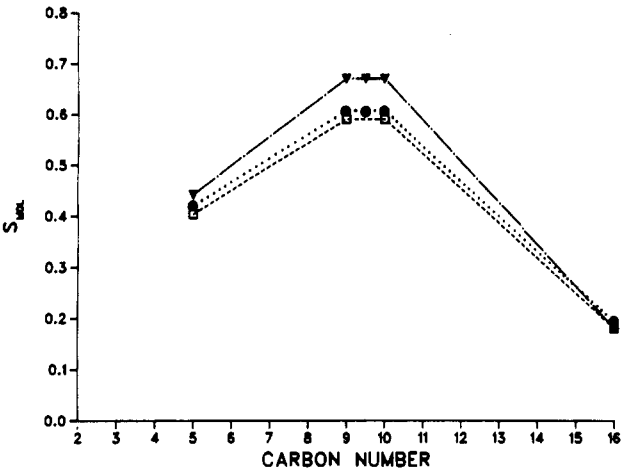

FIGURE 5: Variation of segmental order parameter, S_{mol}, with labeled carbon atoms at 25 °C: (□) oleoyl chain in *A. laidlawii* membranes; dihydrosterculoyl chains in (●) PDSPC and in (▼) *A. laidlawii* membranes. The data for C-19 lie between those for C-9 and C-10.

for the segment-fixed coordinate systems. These matrices are diagonalized to give an axially symmetric order matrix as required by the uniaxial properties of the fluid bilayer (Seelig & Waespe-Šarčevič, 1978; Dufourc et al., 1983). For the cyclopropane ring, at least three independent bond order parameters are required; four experimental parameters are available. The second method determines the orientations of the C–^{2}H bonds with respect to the axis of diffusion by comparison of calculated and experimental quadrupolar splitting ratios (Taylor et al., 1982). These methods have been extended to the dihydrosterculic acid systems and are described in detail elsewhere (Dufourc et al., 1983). In the case of PDSPC at 25 °C, an S_{mol} of 0.59 was calculated, with the C-9–C-10 bond of the cyclopropane-containing fatty acid tilted at an angle of 89° with respect to the director. A similar analysis for membranes containing 19:cp,cΔ^9 at 25 °C gives an S_{mol} of 0.67 ± 0.02 with the C-9–C-10 bond also tilted at an angle of 89° relative to the director. The analysis used the values of the two quadrupolar splittings observed for the deuterons at C-19. An attempt to account for the narrow component in the spectra of membranes containing [19,19-$^2H_2$]dihydrosterculic acid in terms of a lipid component having a different geometrical relationship with respect to the bilayer normal gave ambiguous results and was not pursued further. A plot of S_{mol} vs. position is shown in Figure 5. The values for S_{mol} at positions C-5 and C-16 were taken to be $-2S_{CD}$ (Huang et al., 1980). For comparison, the corresponding profiles for PDSPC and membranes containing 18:1cΔ^9 are plotted in Figure 5. The value of S_{mol} reported for positions C-9 and C-10 of the oleoyl chains was estimated from the ratio of the quadrupolar splittings that were reported for the deuterons at these positions (Rance et al., 1980). Since the ratio method of calculating S_{mol} can give a number of solutions (Taylor et al., 1981), particularly when only one ratio is available, there is some uncertainty in the choice of the correct S_{mol}. It has been suggested that the double bonds of the oleoyl chains in *A. laidlawii* membranes are tilted away from the director (Rance et al., 1980). Due to the similarity in results obtained for oleoyl chains in model and natural membranes, one can expect that the double bond is tilted away from the bilayer normal by similar angles in these systems. Seelig & Waespe-Šarčevič (1978) have calculated a tilt of 7–8° between the bilayer normal and the double bond for POPC. By use of the ratio of $\Delta\nu_Q$ for the C-9 and C-10 deuterons of POPC, a tilt angle of ca. 8 ± 1° and S_{mol} of 0.37 ± 0.01 were calculated, in good agreement with the values of

Table II: Temperature Dependence of Quadrupolar Splitting

carbon atom labeled	temperature coefficient of $\Delta\nu_Q$ (X100 kHz/K) without cholesterol	with cholesterol
5[a]	16.5	31.3
5[b]	15.4	26.0
8[a]	14.5	21.3
8[a]	17.0	31.8
8[b]	10.8	
9[a]	13.5	23.0
9[b]	8.8	15.1
10[a]	7.5	15.0
10[b]	6.9	8.2
11[a]	13.0	
11[a]	16.0	
11[b]	9.0	
16[a]	10.5	
16[b]	12.0	

[a] Data for 19:cp,cΔ^9. [b] Data for 18:1cΔ^9, from Rance et al. (1982).

7–8° and 0.37 ± 0.04, respectively, reported previously (Seelig & Waespe-Šarčevič, 1978). The S_{mol} value reported in Figure 5 for membranes enriched in 18:1cΔ^9 corresponds to a tilt angle of 3° and an S_{mol} of 0.59 ± 0.02. A second possiblity, that the double bond is tilted by ~9° and has an S_{mol} of 0.36 ± 0.01, was rejected because the fit of the calculated quadrupolar splittings to the experimental values was poorer than that obtained with the first set of parameters. The value of S_{mol} shown in Figure 5 may be taken as a maximum limit for the order parameter for the C-9–C-10 segment of the oleoyl chains in the membranes. Figure 5 indicates that membranes enriched in the cyclopropane-containing fatty acid are slightly more ordered than the corresponding pure phospholipid system and more ordered than the corresponding oleic acid system. The effect of the cyclopropane ring appears to be an ordering of the membrane relative to that observed in membranes containing a cis double bond.

Above 0 °C, the spectra reflect the presence of lipid in a liquid-crystalline phase for all labeled positions. The temperature dependence of the quadrupolar splittings are given in Table II for membranes containing 19:cp,cΔ^9 and 18:1cΔ^9. Data present in Table II are the slopes obtained by approximating lines to $\Delta\nu_Q$ vs. temperature plots (not shown). The temperature variation of $\Delta\nu_Q$ for a given position in both membrane systems is similar. Differences in the temperature dependences between the two systems are difficult to interpret for positions C-8 to C-11 of the acyl chains since changes in the quadrupolar splittings may reflect changes in the segmental order parameters and/or variations in the geometrical factors. Table II indicates that although the cyclopropane ring may increase segmental order relative to that of the oleate system, it does not significantly affect the temperature response of the amplitudes of motion relative to those of the oleate-containing membranes. Results of relaxation studies suggest that the rates of acyl chain motion in membranes containing dihydrosterculic acid are less than those present in membranes enriched in oleic acid at the same temperature (H. C. Jarrell and I. C. P. Smith, unpublished results).

Previous ^{2}H NMR studies have indicated that protein has little or no effect on the ordering of lipid in *A. laidlawii* membranes (Kang et al., 1981; Jarrell et al., 1982). ^{2}H NMR spectra of lipid extracts from membranes enriched in dihydrosterculic acid labeled at C-5 and C-19 are shown in Figure 3; ^{2}H spectra of lipid extracts of [2,2-$^2H_2$]-19:cp,-cΔ^9-containing membranes are discussed elsewhere (Rance et al., 1983). In all cases, the lipid extracts and the corresponding membranes give rise to very similar ^{2}H spectra. The quadrupolar splittings are the same for both systems, within experimental error. The small differences between ^{2}H spectra of membranes and extracted lipids, Figure 3, may reflect slight increases in line widths associated with ^{2}H spectra of membranes relative to those present in spectra of lipid extracts (Kang et al., 1981; Jarrell et al., 1982; Rance et al., 1983). In general, replacement of a cis double bond with a cyclopropane ring does not appear to give rise to any significant changes in the weak lipid–protein interaction manifest in ^{2}H NMR spectra of labeled lipids.

Effect of Cholesterol. Cholesterol is a major component of many biomembranes and exerts a significant influence upon their physical characteristics. A recent study of *A. laidlawii* membranes in which perdeuterated palmitic acid and cholesterol were incorporated established that the presence of cholesterol affected the width and position of the gel to liquid-crystal phase transition (Davis et al., 1980). The general shape of the order–position profile of lipid in the liquid-crystalline phase was maintained, but the average degree of order was higher in the presence of cholesterol. A recent study of *A. laidlawii* membranes containing oleic acid and cholesterol established that the average order was also increased by the presence of cholesterol. However, the broad phase transition of the membrane lipids, which is characterisitic for samples lacking cholesterol, was only slightly altered by the presence of up to 27 mol % cholesterol.

Membranes containing labeled dihydrosterculic acid and cholesterol were prepared and examined by ^{2}H NMR in order to assess the effect, if any, of the cyclopropyl moiety on lipid–sterol interactons. Low-temperature spectra of membranes containing [5,5-$^2H_2$]-19:cp,cΔ^9 and 33 mol % cholesterol are shown in Figure 1b. At 0 °C, the ^{2}H NMR spectrum is attributable to lipid in the liquid-crystalline phase. Cooling to −15 °C results in a large increase in $\Delta\nu_Q$ to ~100 kHz, suggesting that the acyl chains are not moving fast enough to average the quadrupolar interaction. A comparison of spectra for membranes with and without cholesterol indicates that in the presence of the sterol, membrane lipids are able to pack more readily into a rigid lattice. At −15 °C, the ^{2}H spectrum of cholesterol-containing membranes resembles an axially symmetric rigid limit powder spectrum more closely than does that of cholesterol-free membranes. Calorimetric studies of phospholipids with cyclopropane-containing fatty acids have suggested that since the synthetic fatty acids are racemic, and hence the phospholipid is a diastereomeric mixture, packing of the acyl chains into a highly ordered gel state may be less efficient (Silvius & McElhaney, 1979). Cholesterol may act as a spacer molecule, thereby enabling the diastereomeric membrane lipids to pack more efficiently. Comparison of the ^{2}H spectra in Figure 1 indicates that the temperatures at which the gel to liquid-crystalline phase transition occur are very similar for both membrane systems.

The presence of cholesterol results in an increase in the quadrupolar splitting at each position studied and at each temperature. The cholesterol levels for membranes containing 19:cp,cΔ^9 are very similar (Table I) while those reported for the corresponding oleate membranes differed considerably (Rance et al., 1982). Observations on model systems have shown that the variaton of $\Delta\nu_Q$ with cholesterol concentraton is approximately linear up to ca. 30 mol % (Stockton & Smith, 1976; Jacobs & Oldfield, 1979; Taylor et al., 1982). In order to compare results on both membrane systems, the percentage increases in $\Delta\nu_Q$ relative to sterol content were calculated and

Table III: Influence of Cholesterol on Quadrupolar Splitting at 25 °C

carbon atom labeled	$\Delta\nu_Q$ (kHz) without	with	increase (kHz)	% increase in $\Delta\nu_Q$	% increase per mol % cholesterol
2[a]	12.6	12.4	−0.2	−1.6	−0.05
	17.6	20.2	2.6	15.0	0.43
	21.5	26.4	4.9	23.0	0.66
5[b]	28.2	38.2	10.0	35.0	1.05
5[b]	26.0	30.8	4.8	19.0	0.70
8[a]	16.3	23.6	7.3	44.7	1.28
	21.0	33.7	12.7	60.5	1.73
9[a]	19.3	25.2	5.9	30.6	1.09
9[b]	14.7	17.5	2.8	19.0	0.80
10[a]	12.3	15.4	3.1	25.2	0.90
10[b]	4.4	5.3	0.9	21.0	0.90

[a] Membranes containing 19:cp,cΔ[9]. [b] Membranes containing 18:1cΔ[9], from Rance et al. (1982).

are given in Table III. The C-2 position appears to be the least sensitive of the dihydrosterculic acyl chain positions examined. A comparison of entries in the last column of Table III indicates that the cyclopropane fatty acid is at least as sensitive as oleic acid to the perturbing effects of cholesterol. Of interest is the relative change in $\Delta\nu_Q$ values for the deuterons at C-8. If the change in $\Delta\nu_Q$ were strictly associated with a change in the segmental order parameter, S_{mol}, eq 2 indicates that for the C-8 deuterons, the relative change in S_{CD} values should be the same for each deuteron. The results in Table III suggest that for the C-8 position, cholesterol also influences the orientations of the deuterons with respect to the axis of motion. A similar effect appears to be operating at the C-2 position although the changes in $\Delta\nu_Q$ appear to be attenuated relative to changes at other positions in the acyl chain. In general, cyclopropane and unsaturated fatty acyl chains have similar responses to the presence of cholesterol in the membrane.

In addition to causing an increase in the quadrupolar splittings, the presence of cholesterol causes $\Delta\nu_Q$ to have temperature dependences greater than those of cholesterol-free membranes. The temperature coefficients of the quadrupolar splittings are given in Table II. At each carbon position measured, cholesterol causes an increase in the temperature coefficient, in agreement with observations reported for oleic acid containing membranes (Rance et al., 1982). In addition, the effect of cholesterol may be slightly greater for the 19:cp,cΔ[9] membranes than for 18:1cΔ[9] membranes (Table II).

Conclusions

Previous studies have suggested that, for heterogeneous systems, the replacement of cis double bonds by *cis*-cyclopropane ring functions in lipid fatty acyl chains would have little effect on the temperature at which the gel to liquid-crystalline phase transition occurs. Replacement of oleic acid in *A. laidlawii* membranes with dihydrosterculic acid does not affect the temperature at which gel-state lipid appears, as monitored by ^2H NMR, but the temperature range of the phase transition is narrower. The average degree of order of membranes containing 19:cp,cΔ[9] is greater than that of membranes containing 18:1cΔ[9] for all positions measured and at all temperatures. The latter results suggest that the cyclopropane ring function gives rise to a less "fluid" lipid matrix. Relaxation studies should provide information on the relative chain dynamics in the two membrane systems.

The presence of cholesterol has little effect on the temperature range over which the gel to liquid-crystalline phase transition occurs, behavior similar to that reported for oleic acid containing membranes (Rance et al., 1982). The presence of cholesterol leads to an overall increase in the average degree of orientational order of the membrane lipids. Thus, although cyclopropane-containing fatty acids may provide a reasonable replacement for unsaturated fatty acids, they do not yield membranes whose properties are physically identical.

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Registry No. *cis*-9,10-Methyleneoctadecanoic acid, 4675-61-0; cholesterol, 57-88-5.

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Gadolinium(III) and Manganese(II) Binding by a Polyether Ionophore. Influence of Cation Charge and Solvent Polarity on the Binding Sites of Lasalocid A (X-537A)†

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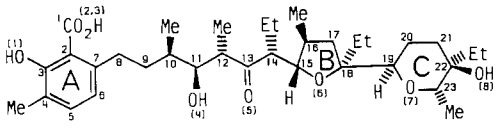
ABSTRACT: Gadolinium(III) and manganese(II) binding sites for the carboxylic polyether antibiotic lasalocid A (X-537A) were determined in a polar solvent, *N,N*-dimethylformamide, and in a relatively nonpolar solvent, chloroform-d_1, by carbon-13 NMR spin–lattice relaxation methods. The results show that binding sites used by the ionophore depend upon both the cation charge and the solvent polarity. In *N,N*-dimethylformamide, Gd(III) binds only at the anionic carboxylate moiety, whereas Mn(II) binds not only at this group but also at O_4 and O_7. In chloroform solution, both cations bind lasalocid via the carboxylate group, O_4, O_7, and O_8. There is some evidence for two modes of binding involving the carboxylate group in chloroform, and these appear to be in rapid exchange at ambient temperature. Methods are given for preparing $Gd(LAS)_3 \cdot XCHCl_3$, $La(LAS)_3 \cdot YCHCl_3$, and $Mn(LAS)_2 \cdot {}^1/_2CHCl_3$, where $X = {}^3/_2$ or ${}^5/_2$, $Y = 1$ or 2, and LAS is the anion of lasalocid A.

Lasalocid A (I),[1] an antibiotic of the polyether series, is



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well-known for its ability to transport metal cations and biogenic amines across natural and artificial membranes (Westley, 1975, 1982; Ovchinnikov & Kolosov, 1979; Poonia & Bajaj, 1979; Pressman, 1976). X-ray crystallographic studies have been carried out for Ba^{2+}, Ag^+, and Na^+ complexes of the lasalocid A anion (hereafter abbreviated LAS) (Johnson et al., 1970; Maier & Paul, 1971; Schmidt et al., 1974; Chiang & Paul, 1977; Smith et al., 1978). In all but one (Chiang & Paul, 1977) of these structures two molecules of LAS, each in a cyclic conformation stabilized by intramolecular hydrogen bonds, are found with the cation(s) sandwiched between them. Most of the oxygens are directed inward, resulting in a hydrophobic outer surface for the complex. If this structure is maintained in the solution phase, it could account for the high solubilities of the complexes in nonpolar solvents and in the interior of lipid bilayer membranes. In all of the crystal structures, the five oxygens O_4, O_5, O_6, O_7, and O_8 are involved in cation binding. In only two cases (Johnson et al., 1970; Schmidt et al., 1974) is a carboxylate oxygen bound to the cation, and in no case is O_1 involved in cation binding.

Relatively little is definitely known of the structures of LAS complexes in the biologically more relevant solution phase. Indeed, evidence is accumulating that solution-phase structures differ considerably from those found in the solid state and that they depend upon both cation charge and solvent polarity. For example, the ^{13}C NMR spectrum of the Tl^+ complex in $CDCl_3$ at low temperature shows $^{203,205}Tl$–^{13}C spin coupling indicative of Tl^+ binding at a carboxylate O, O_5, O_6, and O_8 (Lallemand & Michon, 1978). The effects of Cu^{2+} on LAS ^{13}C NMR

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[1] The numbering scheme used here is that proposed by Westley for polyether antibiotics (Westley, 1976). Oxygen numbers are shown in parentheses.

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