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Thermotropic Phase Behavior of Model Membranes Composed of Phosphatidylcholines Containing *dl*-Methyl Anteisobranched Fatty Acids. 1. Differential Scanning Calorimetric and ^{31}P NMR Spectroscopic Studies[†]

Ruthven N. A. H. Lewis, Brian D. Sykes, and Ronald N. McElhaney*
Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

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ABSTRACT: The thermotropic phase behavior of aqueous dispersions of nine *dl*-methyl branched anteisoacylphosphatidylcholines was studied by differential scanning calorimetry and ^{31}P nuclear magnetic resonance spectroscopy. The calorimetric studies demonstrate that these compounds all exhibit a complex phase behavior, consisting of at least two minor, low-enthalpy, gel-state transitions which occur at temperatures just prior to the onset of the gel/liquid-crystalline phase transition. In addition, at still lower temperatures, anteisobranched phosphatidylcholines containing fatty acyl chains with an odd number of carbon atoms show a major, higher enthalpy, gel-state transition, which was assigned to a conversion from a condensed to a more loosely packed gel phase. No such transition was observed for the even-numbered compounds in aqueous dispersion, but when dispersed in aqueous ethylene glycol, a major gel-state transition is clearly discernible for two of the even-numbered phospholipids. The major gel-state transition exhibits heating and cooling hysteresis and is fairly sensitive to the composition of the bulk aqueous phase. ^{31}P NMR spectroscopic studies indicate that the major gel-state transition is accompanied by a considerable change in the mobility of the phosphate head group and that, at temperatures just prior to the onset of the gel/liquid-crystalline phase transition, the mobility of the phosphate head group is comparable to that normally exhibited by the liquid-crystalline state of most other phospholipids. The temperatures at which the gel/liquid-crystalline phase transition occurs and the enthalpy change associated with this process are considerably lower than those of the saturated *n*-acyl-PC's of comparable acyl chain length. The calorimetric and ^{31}P nuclear magnetic resonance data thus indicate that, although the methyl anteisobranched phosphatidylcholines can form a fairly condensed gel state at sufficiently low temperatures, these phospholipids normally adopt a more loosely packed gel state, and possibly a more ordered liquid-crystalline state, than do phosphatidylcholines containing linear saturated fatty acids of comparable chain length.

Fatty acids containing a single methyl branch near the methyl terminus of the hydrocarbon chain are abundant and wide-spread constituents of the membrane lipids of a number of genera of eubacteria. In those microorganisms the methyl iso- and anteisobranched fatty acids usually account for 65-95% of the acyl chains in the membrane lipids, and they are believed to be the functional equivalents of the linear saturated and unsaturated fatty acids, respectively, of eucaryotic cell membranes [for a review and discussion, see Kaneda (1977), Kannenberg et al. (1983), and Lewis and McElhaney (1985a)]. In addition to being abundant and widely distributed in a variety of microorganisms, these branched-chain fatty acids can also support the growth of several procaryotic microorganisms in which they do not

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naturally occur (Rodwell & Peterson, 1971; Silbert et al., 1973). In the case of the cell wall-less procaryote *Acholeplasma laidlawii* B, methyl iso- and anteisobranched fatty acids can support normal growth and function under conditions where the membrane lipids are essentially acyl chain homogeneous (Silvius & McElhaney, 1978). These studies thus indicate that the methyl iso- and anteisobranched fatty acids possess all the physical and chemical properties needed to support the normal function of a natural biomembrane.

Despite the above, there have been relatively few studies on the physical properties of lipids containing branched-chain fatty acids. Of those, the model membrane systems composed of the 1,2-diisoacylphosphatidylcholines have been the most thoroughly studied so far. For those compounds, DSC[1] (Lewis & McElhaney, 1985a) and differential scanning dilatometric studies (Yang et al., 1986) have shown that, when fully hydrated, their thermotropic phase behavior involves two major structural changes which were assigned to a gel-state packing rearrangement and the gel/liquid-crystalline phase transition by X-ray diffraction (Church et al., 1985) and by FT-IR spectroscopic and ^{31}P NMR spectroscopic techniques (Mantsch et al., 1985). The above studies demonstrated that at low temperatures the isoacyl-PC's form a "condensed" phase similar in some respects to the "subgel" phase of the linear saturated acyl-PC's and that there are structural differences between the condensed phases of the odd- and even-numbered members of that homologous series of compounds. In addition, these studies indicated that branched-chain lipids adopt a loosely packed gel-state conformation prior to the melting of the acyl chains. The latter was also inferred from studies on monolayer films composed of branched-chain phosphatidylcholines (Kannenberg et al., 1983; Suzuki & Cadenhead, 1985) and observations on biological membranes that were highly enriched in iso- and anteisobranched-chain fatty acids (Haest et al., 1974; Legendre et al., 1980; Silvius & McElhaney, 1980a; Bouvier et al., 1981; Macdonald et al., 1985). Indeed, some studies have also hinted at the possibility that, in the liquid-crystalline state, branched-chain lipids may be more ordered than straight-chain lipids at comparable reduced temperatures (Macdonald et al., 1983; Suzuki & Cadenhead, 1985).

All studies on branched lipids have indicated that the physical properties of such lipids are very different from those of saturated, straight-chain lipids of comparable chain length. However, most of the ideas on the effect of the methyl branching have been developed from the more thoroughly studied methyl isobranched systems. The few studies specific to the methyl anteisobranched-chain systems have indicated that those lipids have lower chain-melting transition temperatures than do linear saturated lipids or the methyl isobranched lipids of comparable chain length [see Silvius and McElhaney (1980b)], observations that supported the expectations from earlier studies on biological membrane systems (Blume et al., 1978). DTA studies on some anteisobranched PC's have also hinted at the possibility of gel-state polymorphism (Silvius & McElhaney, 1980b), and a single-crystal X-ray study has shown that, in fatty acid crystals, the presence of the anteiso methyl branch can disrupt the packing of the acyl chains (Abrahamsson, 1958). However, an understanding of the behavior of the methyl anteisobranched hydrocarbon chain in a lipid bilayer is far from complete, and some recent studies have highlighted the inherent risks in extrapolating from the few systems that have been thoroughly studied [see Lewis and McElhaney (1985b)]. Consequently, we have synthesized a series of *dl*-methyl anteisobranched fatty acids and their respective PC's for a thorough study of their physical properties in model and biological membranes. The results of some DSC and ^{31}P NMR spectroscopic studies are presented here.

Table I: Precursors to the Synthesis of Anteisobranched Fatty Acids

fatty acid[a]	carboxyl fragment	alkyl terminal fragment
$C14_{ai}$	azelaic acid monomethyl ester[b]	1-bromo-2-methylbutane
$C15_{ai}$	1,10-decanedioic acid	1-bromo-2-methylbutane
$C16_{ai}$	1,10-decanedioic acid	1-bromo-3-methylpentane
$C17_{ai}$	1,12-dodecanedioic acid	1-bromo-2-methylbutane
$C18_{ai}$	1,12-dodecanedioic acid	1-bromo-3-methylpentane
$C19_{ai}$	1,14-tetradecanedioic acid	1-bromo-2-methylbutane
$C20_{ai}$	1,14-tetradecanedioic acid	1-bromo-3-methylpentane
$C21_{ai}$	1,14-tetradecanedioic acid	1-bromo-4-methylhexane
$C22_{ai}$	1,14-tetradecanedioic acid	1-bromo-5-methylheptane

[a] Fatty acids are denoted by their carbon number (C*n*) and the subscript "ai" to designate anteisobranching. [b] This monomethyl ester was obtained from Aldrich Chemical Co.

MATERIALS AND METHODS

The sources of comercially available chemicals, solvents, and chromatographic adsorbents and their pretreatment and/or purification were the same as previously used in this laboratory (Lewis & McElhaney, 1985a). 1-Bromo-2-methylbutane was synthesized by the bromination of 2-methyl-1-butanol (Aldrich). 1-Bromo-3-methylpentane was synthesized from 2-methyl-1-butanol by the chain-elongation procedure used by Silvius and McElhaney (1980b). 1-Bromo-4-methylhexane and 1-bromo-5-methylheptane were synthesized from 1-bromo-2-methylbutane and 1-bromo-3-methylpentane, respectively, by the ethylene oxide elongation procedure used by Lewis and McElhaney (1985a). The *dl*-methyl anteisobranched fatty acids were synthesized from the precursors listed in Table I by the chemical reactions used by Lewis and McElhaney (1985a) and were recrystallized from 70% ethanol. The procedures for synthesis and purification of the PC's used in this study were the same as those used for the preparation of highly purified samples of 1,2-diisoacyl-PC's (Lewis & McElhaney, 1985a).

The thermotropic phase behavior of fully hydrated samples of these phospholipids was studied with a Microcal MC-1 high-sensitivity differential scanning calorimeter and a Perkin-Elmer DSC-2C differential scanning calorimeter equipped with a thermal analysis data station. Samples were hydrated in either distilled water or 50% aqueous ethylene glycol (v/v). The methods of sample preparation and quantification were the same as previously used in this laboratory (Lewis & McElhaney, 1985a). ^{31}P NMR spectra were recorded on a Bruker HXS-270 spectrometer or a Nicolet NT-300WB spectrometer. The details of sample preparation and data acquisition have been described previously (Mantsch et al., 1985). When the spectra of the gel-state conformation of these lipids were acquired the sample was held in the thermally equilibrated probe for at least 2 h before data acquisition, so as to ensure that the sample had obtained equilibrium.

RESULTS

Thermotropic Phase Behavior in Water. Aqueous dispersions of the *dl*-methyl anteisobranched PC's containing fatty acyl chains of 17 or more carbons exhibited phase transitions above 0 °C and were studied in the high-sensitivity calorimeter, while the shorter chain PC's were studied in the low-sensitivity

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[1] Abbreviations: PC, phosphatidylcholine; DSC, differential scanning calorimetry; DTA, differential thermal analysis; NMR, nuclear magnetic resonance; FT-IR, Fourier transform infrared; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

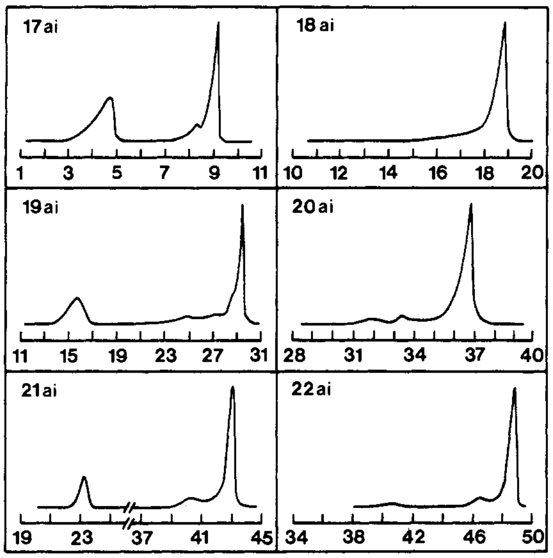

FIGURE 1: High-sensitivity DSC heating endotherms of aqueous dispersions of the anteisoacyl-PC's. The thermograms shown were recorded at scan rates that were sufficiently slow so as to avoid any distortion of the observations.

instrument. Figure 1 shows the high-sensitivity DSC heating endotherms recorded for the longer chain PC's studied. It is clear that there are significant differences in the behavior of the odd- and even-numbered members of this homologous series of compounds. The thermotropic behavior of the odd-numbered anteisobranched PC's was characterized by two main endothermic events: a relatively broad, low-temperature transition and a sharp, high-temperature transition that was preceded by at least one relatively low-energy, minor endothermic event. The two major transitions were progressively separated on the temperature scale with increasing acyl chain length. Infrared spectroscopic studies (Mantsch et al., following paper in this issue) have enabled the assignment of the two main events to a gel-state packing rearrangement (lower temperature event) and the gel/liquid-crystalline phase transition or chain-melting transition (higher temperature event). The latter is a relatively cooperative process that shows little or no hysteresis, while the low-temperature, gel-state transition was subject to considerable hysteresis, which increased with the increasing length of the acyl chain. Thus, while a 0.5-h incubation at 0 °C was sufficient to enable the full manifestation of the low-temperature phase of the 17_{ai} species, the low-temperature phase of the 21_{ai} species was formed only after incubation at 0–4 °C for at least 4 days. In contrast to the odd-numbered compounds, the even-numbered anteisobranched PC's studied in the high-sensitivity calorimeter showed no evidence of a major gel-state transition comparable to that observed for their odd-numbered counterparts, even after prolonged annealing at 0–4 °C for more than 12 months. However, in all other respects, their behavior appeared to be similar to that of their odd-numbered counterparts.

Illustrated in Figure 2 are the heating endotherms and cooling exotherms of the entire series of the *dl*-anteisobranched PC's used in this study. The thermograms shown in Figure 2 were all recorded by the lower sensitivity instrument and, with the exception of the 14_{ai} species, they were all recorded at the slowest scan rate possible with that instrument (18.75 °C h^{-1}). Although the thermograms do not show the detail offered by the high-sensitivity instrument, it is apparent that the behavior of the three shorter chain compounds is, in general, similar to that of their longer chain counterparts. However, there appeared to be a slight discontinuity in the behavior of the 15_{ai} species, since the separation between the two main endothermic events was considerably greater than what would be expected from the trends set by their longer chain, odd-numbered counterparts. The cooling exotherms shown in Figure 2 also show that, with the exception of the 14_{ai} species, the transitions assigned to the acyl chain melting process were observed at the same temperatures in both the heating and cooling modes, while there was considerable hysteresis in the other events, which were broadened and shifted to lower temperatures on cooling. In particular, the main gel-state transitions of all of the odd-numbered anteisobranched PC's (except the 17_{ai} species) were not calorimetrically observable in the cooling mode. For the 17_{ai} species the said process was observed, by DSC, at temperatures lower than that observed in the heating mode. For the 14_{ai} species, its behavior was apparently anomalous with respect to the trends set by the longer chain counterparts, since it exhibited a single calorimetrically resolvable transition at temperatures that differed in the heating and cooling modes. Like some short-chain isoacyl-PC's (Lewis & McElhaney, 1985a) and the odd-numbered ω-cyclohexyl-PC's (Lewis & McElhaney, 1985b), the observed thermotropic phase behavior was found to be an intrinsic property of the anteisobranched PC's studied, being dependent only upon the thermal history of the sample and not upon the heating or cooling rates utilized.

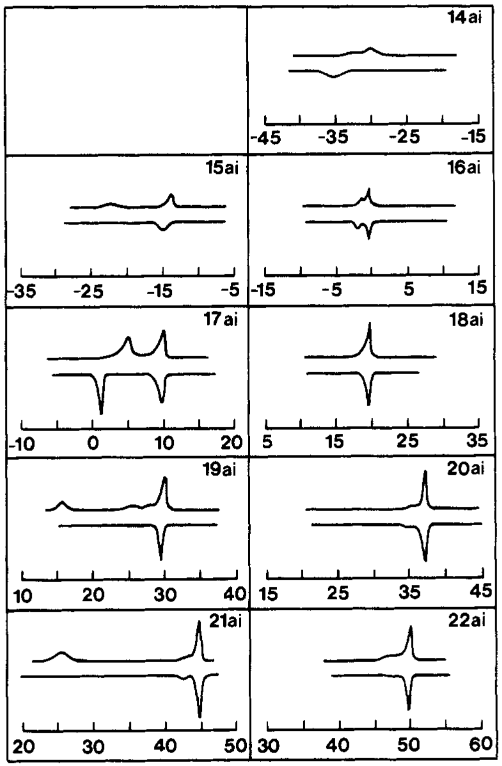

FIGURE 2: Low-sensitivity DSC thermograms of aqueous dispersions of the anteisoacyl-PC's. The heating endotherms and cooling exotherms shown were all recorded at a scan rate of 18.75 °C h^{-1}.

Thermotropic Phase Behavior in Aqueous Ethylene Glycol. These studies were prompted by the widespread use of aqueous ethylene glycol as an antifreeze solvent for studying the physical properties of lipids at temperatures below 0 °C and by recent studies showing a perturbation of the gel-state behavior of the isoacyl- and ω-cyclohexyl-PC's by that dispersant [see Lewis and McElhaney (1985a,b)]. The heating and cooling thermograms of these anteisoacyl-PC's in aqueous ethylene glycol (see Figure 3) were recorded by the Perkin-Elmer DSC-2C calorimeter at the slowest scan rates possible

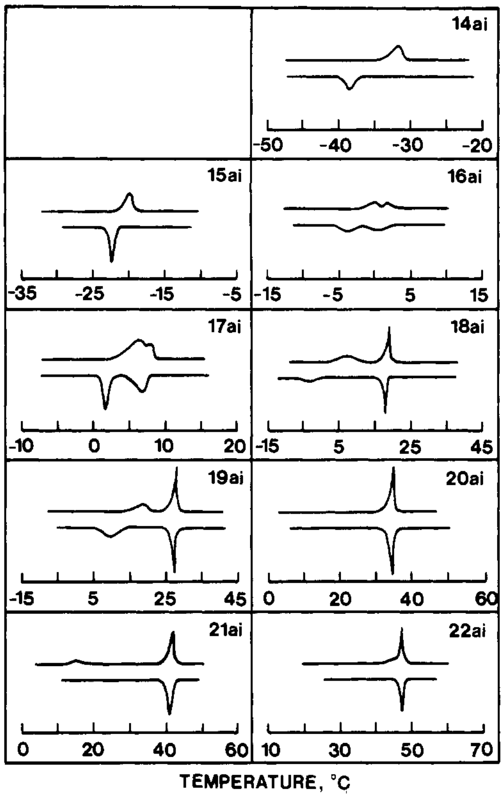

FIGURE 3: Low-sensitivity DSC thermograms of dispersions of the anteisoacyl-PC's in aqueous ethylene glycol. The heating endotherms and cooling exotherms shown were all recorded at a scan rate of 18.75 °C h^{-1}.

Table II: Thermodynamic Characterization of the Heating Endotherms of the *dl*-Methyl Anteiso PC's

	low-temperature transition[a]		high-temperature transition[c]		
PC	T_m[b] (°C)	ΔH (kcal mol^{-1})	T_m (°C)	ΔH (kcal mol^{-1})	ΔH_{total} (kcal mol^{-1})
14_{ai}[d]			−30.7		0.13; 2.6[e]
15_{ai}[d]			−13.9		2.8; 8.5[e]
16_{ai}	−5.0[e]	6.9[e]	−0.4	2.7; 4.3[e]	11.2[e]
17_{ai}	4.8; 7.4[e]	4.5	9.2	5.4	9.9
18_{ai}	6.9[e]	1.6[e]	18.9	6.4	8.0[e]
19_{ai}	15.6; 18.0[e]	2.9	29.5	7.9	10.8
20_{ai}			36.7	10.5	
21_{ai}	24.3; 26.7[e]	4.0	43.4	12.3	16.3
22_{ai}			48.7	13.8	

[a] This refers to the low-temperature, gel-state transition. [b] The maximum rate of heat absorption during the transition was recorded at these temperatures. [c] This refers to all thermotropic events except the main gel-state transition. [d] There was poor resolution of the thermotropic properties of these lipids. It is assumed that the events observed include at least the high-temperature endothermic events. [e] These refer to the values recorded when the samples were dispersed in aqueous ethylene glycol when they were the only values available or differed significantly from that of an aqueous dispersion.

with that instrument (18.75 °C h^{-1}). A close inspection of Figure 3 shows that the behavior of all the compounds save the 20_{ai} and 22_{ai} species was responsive to the change in the properties of the bulk solvent phase, especially when examined in the heating mode. For the odd-numbered compounds, the resolution of the gel/gel and gel/liquid-crystalline transitions in the heating mode was less than that observed in water, since, unlike the chain-melting phase transition, the gel/gel transition was calorimetrically resolvable at higher temperatures in the aqueous ethylene glycol solvent system. However, in the cooling mode, there appeared to be considerably less hysteresis in the lower temperature, gel-state transition, since when compared with an aqueous dispersion, the formation of the condensed, low-temperature phase appeared to be faster in the aqueous ethylene glycol solvent system used. Thus, unlike the aqueous dispersion, the gel-state transition of the 19_{ai} species was calorimetrically observable, and a partial formation of the condensed, low-temperature phase of the 21_{ai} species was calorimetrically detectable after an overnight incubation at 0–4 °C and was complete in 2 days. In addition, in the cooling mode, the gel/gel transition of the 17_{ai} species was obseved at temperatures near 1 °C when dispersed in water, while the same process was observed at temperatures near 3 °C when dispersed in aqueous ethylene glycol.

The behavior of two of the even-numbered members of this homologous series of anteisobranched PC's was significantly altered by the change in the solvent system. Aqueous ethylene glycol dispersions of both the 16_{ai} and 18_{ai} species showed evidence of an additional thermotropic event when cooled to temperatures near −10 °C. This low-temperature event showed the heating–cooling hysteresis characteristic of the main gel/gel transition of the odd-numbered compounds and was tentatively assigned to a gel-state packing transition by ^{31}P NMR spectroscopy (see the ^{31}P NMR section below). Like their odd-numbered counterparts, there was an increased temperature separation of the gel/gel and gel/liquid-crystalline transitions as the chain length increased. No such transition has as yet been resolved for the longer chain, even-numbered anteisoacyl-PC's, even after prolonged incubation in aqueous ethylene glycol at temperatures between −20 and 4 °C.

There was also evidence that, when dispersed in aqueous ethylene glycol, the thermotropic phase behavior of the shortest members of the odd- and even-numbered anteiso PC's was different from that of an aqueous dispersion and also discontinuous from the trends set by their longer chain counterparts. When dispersed in aqueous ethylene glycol, the thermotropic phase behavior of the 14_{ai} species was, in general, similar to that of an aqueous dispersion with respect to the transition temperatures and the heating–cooling reversibility characteristics. However, the transition enthalpy was considerably lower when the lipid was dispersed in water. In the case of the 15_{ai} species, the transition enthalpy of the aqueous dispersion was also lower than that of the aqueous ethylene glycol dispersion, and the thermotropic events were all recorded at temperatures lower than that of the aqueous dispersion. The DSC thermograms illustrated in Figure 3 also show that for the 15_{ai} species there was poor temperature resolution of the gel state from the chain-melting transition and that the heating–cooling reversibility characteristics appeared to be similar to that of the 14_{ai} species.

Thermodynamic Characterization. The thermodynamic parameters characteristic of the thermotropic phase behavior of the anteiso PC's are listed in Table II. The parameters of the main gel-state transition are listed separately, and no attempt was made to separate the data for the gel/liquid-crystalline phase transition from the minor gel-state events that precede it, since they are poorly resolved from the main chain-melting transition and their molecular basis is currently unknown. With the exception of the main gel-state transitions and the data described for the 14_{ai} species, the values reported are the same whether measured in the heating or cooling modes. The gel/liquid-crystalline phase transition temperature increases with increasing acyl chain number and is described by a smooth function of acyl chain carbon number (see Figure 4) with no evidence of odd–even alternation. Odd–even alternation in the chain-melting transition temperatures has been

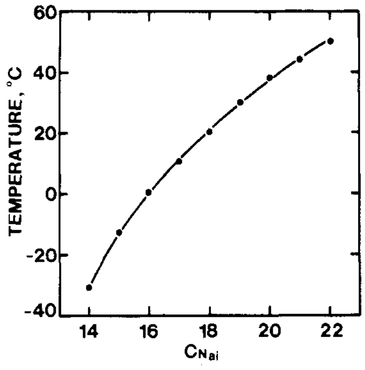

FIGURE 4: Acyl chain-length dependence of the main heating endothermic transition temperatures of the anteisoacyl-PC's.

observed in the heating behavior of the short-chain isoacyl-PC's and the ω-cyclohexyl-PC's (Lewis & McElhaney, 1985a,b), and in both instances there was evidence of the involvement of a major gel-state transition as a determinant of the chain-melting transition temperature. For these anteisoacyl-PC's the chain-melting process is clearly separated from the main gel-state phenomena on the temperature scale, and, as expected, no odd–even alternation in the chain-melting temperatures is observed.

The enthalpy values associated with the chain-melting process limited in Table II include some contributions from the minor gel-state transitions that precede it. In spite of this, these values are considerably lower than those exhibited by the *n*-acyl-PC's and isoacyl-PC's of comparable chain length [see Silvius (1982) and Lewis and McElhaney (1985a)]. In addition, the gel/liquid-crystalline phase transition temperatures of these compounds are some 20–40 °C lower than those of the *n*-acyl-PC's and some 10–20 °C lower than those of the isoacyl-PC's [see Silivus (1982) and Lewis and McElhaney (1985a)]. These observations may be indicative of the disruptive effect of the anteiso methyl branch on the packing and ordering of the acyl chains in bilayers containing such structures (see Discussion).

The main gel-state transitions of some anteisobranched PC's exhibit hysteresis and are affected by the composition of the bulk aqueous phase. Thus the transition temperature values listed in Table II are almost certainly not true equilibrium temperatures, and this precluded an evaluation of whether or not those temperatures exhibited odd–even discontinuities. There were no clear trends apparent from the enthalpy changes associated with those transitions and, given the above kinetic considerations, we cannot be certain that the conversion to the condensed gel phase was complete under the experimental conditions. Consequently, the gel-state transition enthalpy values listed in Table II should perhaps be regarded as minimum values. Nonetheless, those values appear to be in the range of the subtransition enthalpies of the saturated *n*-acyl-PC's (Chen et al., 1980) and of the gel-phase transitions of the odd-numbered isoacyl-PC's (Lewis & McElhaney, 1985a). However, the range of these values is somewhat lower than that of the even-numbered isoacyl-PC's (Lewis & McElhaney, 1985a) or the mixed-chain saturated *n*-acyl-PC's (Serrallach et al., 1984; Stumpel et al., 1983). Finally, it is also clear that there is some discrepancy between the enthalpy values recorded for aqueous and aqueous ethylene glycol dispersions of the shorter chain compounds. Given the sensitivity of the gel-state transition of these compounds to the composition of the bulk aqueous phase, we suspect that a major component of this discrepancy may be related to those effects.

^{31}P Nuclear Magnetic Resonance Spectroscopy. Figure 5 shows a series of ^{31}P NMR spectra of an aqueous dispersion of 19_{ai} PC, the behavior of which is, in general, typical of the longer chain, odd-number anteisoacyl-PC's. The spectra shown were acquired at a series of temperatures in a sequence that corresponded with the heating mode of the DSC experiment. At low temperatures, the compound exhibits a broad spectrum (basal line width ≅ 150 ppm), which is characteristic of slow, axially asymmetric motion of the phosphate head group on the ^{31}P NMR time scale (Seelig, 1978; Campbell et al., 1979). When the compound is heated to temperatures near 12 °C, there is a slight narrowing of the spectrum along with the appearance of a "peak" near 15 ppm upfield. Upon further heating to 16 °C, the peak sharpens and the spectrum narrows, adopting the form characteristic of axially symmetric motion of the phosphate head group in a phospholipid bilayer. However, the width of the spectrum (basal line width ≅ 100 ppm) suggests that the motion of the phosphate was probably in the "slow" end of the range of axially symmetric motion feasible in a lipid bilayer and indicates that the lipid was still in a gel-state conformation. These changes are coincident with the main, gel-state transition of this lipid and are typical of the ^{31}P NMR spectral changes that occur upon warming the odd-numbered anteisoacyl-PC's through their major gel-state transitions. It is also apparent from the spectrum acquired at 12 °C that there is some formation of the higher temperature gel state, as indicated by the presence of the relatively sharp peak in the spectrum. Thus, although no changes were apparent from the DSC thermograms, the ^{31}P NMR data suggest that some structural changes are occurring at temperatures below those resolved by DSC. In fact, simulations using a spectral addition program indicate that the spectrum acquired at 12 °C could be constructed from those acquired at 4 and 16 °C, if it is assumed that there is a 30% conversion to the higher temperature gel state, and that there is a slow rate of exchange between both states. Given the length of time taken for the equilibration of the sample and the acquisition of the NMR spectra (≅6 h total), the above observations

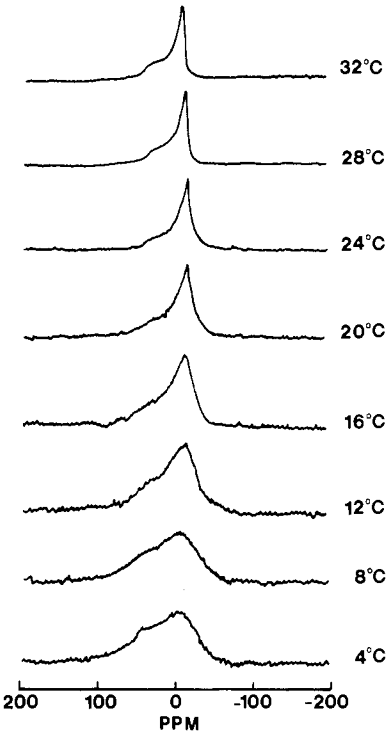

FIGURE 5: Proton-decoupled ^{31}P NMR spectra of an aqueous dispersion of 1,2-di-*dl*-anteisononadecanoyl-PC. The spectra shown were acquired at the temperatures indicated in a temperature sequence that corresponded with the heating mode of the DSC experiment.

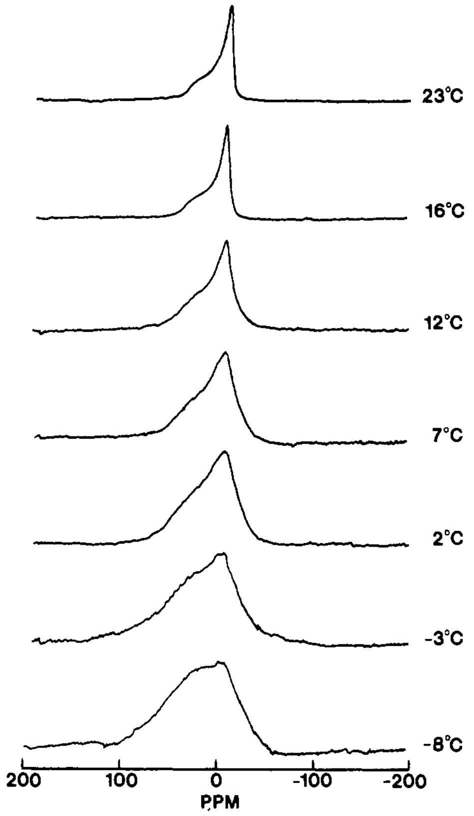

FIGURE 6: Proton-decoupled ^{31}P NMR spectra of an aqueous ethylene glycol dispersion of 1,2-di-*dl*-anteisooctadecanoyl-PC. The spectra shown were acquired at the temperatures indicated in a temperature sequence that corresponded with the heating mode of the DSC experiment.

indicate that the process under observation must have been equilibrating on a time scale considerably longer than those feasible in the DSC experiments. Upon further heating of the same compound to temperatures in the range of the other gel-state transitions (16 °C < T < 28 °C), there is a slight narrowing of the basal line width and the peak near 15 ppm upfield sharpens considerably. In those spectra, the sharpening of the peak near 15 ppm upfield was not accompanied by a narrowing of the basal line width until the temperature got very close to that at which the acyl chains began to melt. We also found that the ^{31}P NMR spectra acquired at these "intermediate" temperatures (16 °C < T < 28 °C) could be constructed by a simple spectral addition of the gel-state spectrum acquired at 16 °C and that typical of the liquid-crystalline state of this lipid. Since this lipid is still in a gel state at temperatures below 28 °C, the above observation suggests that there may be a gel-state conformation of this lipid for which the motion of the phosphate head group is fast and axially symmetric on the ^{31}P NMR time scale. This was confirmed by the gel-state spectrum acquired at 28 °C, which was virtually indistinguishable from that acquired at 32 °C when the compound is in the liquid-crystalline state. The above changes in the ^{31}P NMR spectra of the 19_{ai} species typified that of all the longer chain, odd-numbered anteisoacyl-PC's whether dispersed in water or aqueous ethylene glycol.

Illustrated in Figure 6 is a comparable series of ^{31}P NMR spectra of an aqueous ethylene glycol dispersion of 18_{ai} PC. The low-temperature spectrum ($T \cong -8$ °C) exhibits the characteristics of axially asymmetric motion of the phosphate head group, and it narrows to that expected of axially symmetric motion upon warming to temperatures near 2 °C. A similar spectral change accompanied the transition through the low-temperature heating endotherm observed with a dispersion of 16_{ai} PC in aqueous ethylene glycol. This enabled a tentative assignment of the low-temperature heating endothermic transition of these even-numbered compounds to a gel-state packing transition that may be comparable to those exhibited by the odd-numbered member of this homologous series of compounds. It is also clear from the spectra shown in Figure 6 that significant structural changes are occurring at temperatures below that of the range of the gel-state packing transition observed by DSC. In this and other respects the behavior of the aqueous ethylene glycol dispersion of 18_{ai} PC seen by ^{31}P NMR appeared to be similar to that of the odd-numbered anteisoacyl-PC's.

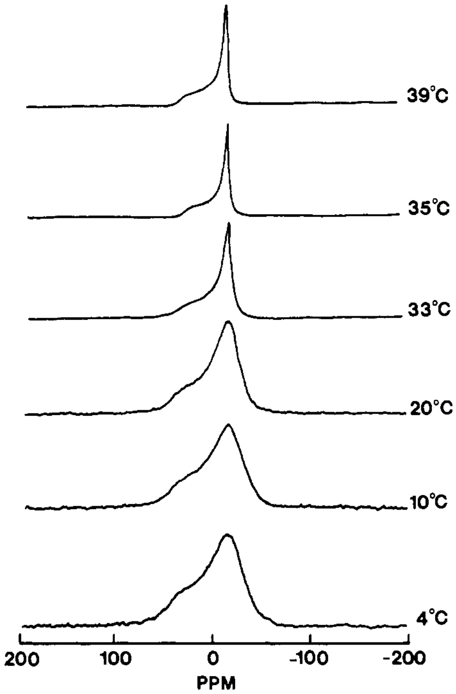

FIGURE 7: Proton-decoupled ^{31}P NMR spectra of an aqueous dispersion of 1,2-di-*dl*-anteisoeicosanoyl-PC. The spectra shown were acquired at the temperatures indicated in a temperature sequence that corresponded with the heating mode of the DSC experiment.

The ^{31}P NMR spectra shown in Figure 7 is of the 20_{ai} species, which typifies the behavior of the two longest even-numbered PC's studied. The spectra acquired at low temperatures (T < 28 °C) are typical of the gel state of phospholipid bilayers in which the motion of the phosphate head group is probably in the slow end of the range of axially symmetric motion feasible in a lipid bilayer. The spectra acquired at 4 °C show no changes with time and are essentially unchanged after annealing at 0–4 °C for 1 year. When the sample was warmed to temperatures in the range of the minor gel-state transitions, the spectral changes appear to be similar to those of the odd-numbered compounds, since the spectra obtained could be reconstructed by spectral addition as described for the odd-numbered compounds. However, we found that for the even-numbered compounds the transition through the first of the minor gel-state transitions resulted in a ^{31}P NMR spectrum which could be constructed if it is assumed that there is a 70% conversion to a state in which the ^{31}P NMR spectrum is similar to that of the liquid-crystalline state. This is in marked contrast to the odd-numbered compounds, for which the spectra obtained after transition through the first of the minor gel-state phenomena could be constructed if a 30–40% conversion is assumed. Like the odd-numbered compounds, the limiting spectral line shape characteristic of fast axially symmetric motion in a phospholipid bilayer structure is approached upon transition through the second of the minor gel-state transitions characterized by DSC.

DISCUSSION

A major feature of the thermotropic properties of these *dl*-methyl anteisoacyl-PC's is the complexity of their gel-state behavior. The thermotropic behavior of these compounds is considerably more complex than that of the *n*-acyl-PC's [see Chen et al. (1980)] and the methyl isobranched PC's (Lewis & McElhaney, 1985a). The *n*-acyl-PC's exhibit two gel-state transitions, the subtransition and the pretransition, which have been well characterized by a number of physical techniques [see Fuldner (1981), Cameron and Mantsch (1982), Ruocco and Shipley (1982), and Ranck (1983)], while the isoacyl-PC's exhibit a single gel-state transition that appears to be the structural equivalent of both the pretransition and the subtransition of the *n*-acyl-PC's (Mantsch et al., 1985; Church et al., 1985). Most of the *dl*-anteisobranched PC's exhibited at least two minor calorimetrically detectable transitions at temperatures just below the onset of the melting of the acyl chains. The studies described here, as well as some FT-IR spectroscopic studies (Mantsch et al., following paper in this issue), have not yet been able to assign those transitions to any defined physical process, and they appear not to have any resolved structural equivalents in the behavior of the *n*-acyl- and isoacyl-PC's. The FT-IR spectroscopic studies suggest that, although the minor gel-state transitions are accompanied by an increase in acyl chain mobility, they do not involve a major melting of the acyl chains, while the ^{31}P NMR data indicate that these events are accompanied by an increase in mobility of the phosphate head group such that the limiting spectral line shape characteristic of fast axially symmetric motion is approached before the melting of the acyl chains. In these respects there may be some similarity between the minor gel-state transitions of these anteisoacyl PC's and those of the even-numbered, ω-cyclohexyl-PC's [see Lewis and McElhaney (1985b)]. At lower temperatures the odd-numbered anteisoacyl PC's also exhibit a major gel-state transition, which appears to be a transition from a condensed gel phase to a more loosely packed gel phase by FT-IR spectroscopy (Mantsch et al., following paper in this issue). This transition was also accompanied by a change in the ^{31}P NMR spectra properties consistent with a change from slow axially asymmetric motion to axially symmetric motion of the phosphate on the ^{31}P NMR time scale. In these respects the main gel-state transition of the odd-numbered anteisoacyl-PC's appears to share some of the characteristics of the subtransition of the *n*-acyl-PC's (Fuldner, 1981) and the gel-state transition of the isoacyl-PC's (Mantsch et al., 1985), as well as the gel-state transition of some saturated, mixed-chain *n*-acyl-PC's (Lewis et al., 1984).

Another interesting feature of these studies is the apparent difference in the gel-state behavior of the odd- and even-numbered members of the homologous series of anteisobranched PC's, especially when dispersed in water. In aqueous dispersion, the odd-numbered compounds form a condensed gel phase relatively easily, though, for the longer chain species, the full manifestation of that phase required some annealing at low temperatures. In the case of aqueous dispersions of the even-numbered compounds there was no evidence for the formation of such a phase even after annealing at 0–4 °C for periods in excess of 12 months. However, we were able to induce the formation of a condensed phase in two of the even-numbered compounds (16_{ai} and 18_{ai}) by cooling aqueous ethylene glycol dispersions of those compounds to temperatures near −15 °C. A similar treatment of the 20_{ai} and 22_{ai} species failed to induce the formation of a condensed phase even after prolonged annealing times. In principle, the observed differences in the gel-state behavior of the odd- and even-numbered anteisoacyl-PC's may be ascribable to kinetic considerations. The formation of a condensed gel phase of the type described here is usually a slow process [see Chen et al. (1980), Lewis et al. (1984), Serrallach et al. (1984), and Lewis and McElhaney (1985a)], and some studies on the *n*-acyl-PC's and the isoacyl-PC's have suggested that the kinetics of formation of the condensed gel phases of the odd- and even-numbered compounds may be different (Lewis & McElhaney, 1985a; Lewis et al., 1987). Furthermore, the data described here and elsewhere (Lewis & McElhaney, 1985a; Lewis et al., 1987) support the conclusion that the hysteresis in the transitions involved in the formation and decomposition of the condensed gel phase of some PC's increases with increasing acyl length; i.e., the formation of a condensed gel phase is more favorable with the shorter chain compounds. Thus, the absence of a gel-state packing transition in the DSC thermograms of the even-numbered PC's shown in Figures 1 and 2 may be the result of an exceedingly slow rate of equilibration in the case of the even-numbered PC's studied, and not a reflection of any thermodynamic considerations. The effects of aqueous ethylene glycol on the thermotropic phase behavior of the 16_{ai} and the 18_{ai} species could also be accommodated within such an interpretation, since the aqueous ethylene glycol solvent system appears to increase the rate at which the condensed phase forms (see below). The presence of the major gel-state transition in the DSC thermograms of the 16_{ai} and 18_{ai} compounds shown in Figure 3 could be ascribed to an improvement in the kinetics of formation of their condensed gel phases by the aqueous ethylene glycol solvent used. In the case of the 20_{ai} and 22_{ai} compounds, the above considerations would suggest that, in spite of the improvement in the kinetics of formation of their condensed gel phases by the aqueous ethylene glycol, the chain-length effect was still predominant and the process occurred on a time scale that is considerably greater than those undertaken in this study.

The possible involvement of the above kinetic considerations as determinants of the observed properties of these lipids complicates the evaluation of whether there were differences in the gel-state properties of the odd- and even-numbered members of this homologous series of anteisobranched PC's. Recent studies on PC's containing branched or alicyclic substituents near the methyl termini of the fatty acyl chain (Lewis & McElhaney, 1985a,b) have shown that there are structural and behavioral differences in the gel states of the odd- and even-numbered members of each homologous series. This odd–even effect is manifest in the solid-state behavior of paraffinic compounds generally, when their long chains are tilted to the end-group planes of their crystalline or quasi-crystalline lattices [see Broadhurst (1962)], and is usually apparent from an odd–even alternation in the solid-state transition temperatures and other physical properties of the solid state. In this case there was no odd–even alternation in the gel/liquid-crystalline phase transition temperatures. We were also unable to determine whether there was any odd–even alternation in the main gel-state transition temperatures, since the transition temperatures resolved by DSC were shown by ^{31}P NMR spectroscopy to be higher than the two equilibrium temperatures, and, in addition, the transition temperatures were not available for some of the longer chain, even-numbered PC's. However, there was some indication that there may be structural differences between the gel states of these odd- and even-numbered anteisoacyl-PC's. In the case of the even-numbered compounds, we found that the transition through the minor gel-state transitions was accompanied by a faster approach to the liquid-crystalline-like ^{31}P NMR spectrum when compared with the odd-numbered compounds, which suggests that there may be subtle differences in the structural changes which precede the chain-melting transition of the odd- and even-numbered PC's. Indeed, the above findings suggest that at temperatures in the range of the minor gel-state transitions the phosphate head group of the even-numbered compounds may be subject to fewer motional restrictions than their odd-numbered counterparts, and this could be a reflection of differences in the gel-state packing modes at those temperatures. In addition, we have also noted that the formation of the condensed gel phases of the odd-numbered compounds appears to be more favorable than those of the even-numbered compounds. Although it is unclear whether the observed behavioral differences between the odd- and even-numbered compounds have a kinetic or thermodynamic basis, it seems likely that they must be a reflection of differences in the structures of their respective gel states and/or condensed phases or differences in the processes of interconversion between them.

It is also clear that the composition of the bulk aqueous phase is a significant determinant of the observable behavior of these compounds. Previous studies on PC model membrane systems composed of isobranched acyl chains (Lewis & McElhaney, 1985a) or ω-cyclohexyl acyl chains (Lewis & McElhaney, 1985b) have shown that the gel-state behavior of aqueous ethylene glycol dispersions of many of those compounds is different from that of an aqueous dispersion and provided some evidence of their being a significant kinetic component to those differences. In these studies the use of the aqueous glycol antifreeze solvent as a dispersant also had significant effects on the gel-state behavior of the PC's studied. When in aqueous dispersion, the gel-state packing transition of the odd-numbered compounds was calorimetrically resolvable at temperatures higher than that of an aqueous dispersion. However, the ^{31}P NMR spectroscopic studies showed that, like the aqueous dispersion, there was a significant kinetic component to those observations, since there were structural changes occurring at temperatures well below the gel-state transition temperatures resolved by DSC. Thus, our observations suggest that the kinetic limitations responsible for the heating hysteresis in the gel-state properties of these compounds are more severe when they are dispersed in aqueous ethylene glycol. Our studies also show that, when dispersed in aqueous ethylene glycol, the formation of the condensed gel phases of these anteisoacyl-PC's appears to be more favorable than when dispersed in water. Thus, when compared with an aqueous dispersion, the effects of the aqueous ethylene glycol solvent system apparently increase the rate of formation of the condensed gel phase at low temperatures, and, at higher temperatures, it decreases the rate at which the condensed gel phase converts to the more loosely packed gel phase. Since, with the possible exception of the two shortest chain compounds studied, the energetics of the process is apparently unaffected, the above could only occur if the formation of the condensed gel phase and its decomposition to the more disordered gel phase proceeded via different mechanistic pathways. These results also hint at the possibility of hydration changes being an integral part of the complex processes involved in the formation and decomposition of the condensed gel phases of these PC's. Hydration changes have been associated with the subtransition of the *n*-acyl-PC's (Cameron & Mantsch, 1982) as well as the gel-state transition of the isoacyl-PC's (Mantsch et al., 1985), and such gel-state processes have been shown to be affected by the properties of the bulk aqueous phase [see Lewis and McElhaney (1985a,b)].

These studies also provide some evidence that the thermotropic properties of the short-chain compounds (14_{ai} and 15_{ai}) were discontinuous from the trends set by their longer chain homologues. These compounds show some evidence of anomalous heating–cooling reversibility characteristics, especially when dispersed in aqueous ethylene glycol. Such properties were also observed in the thermotropic phase behavior of the shorter chain isoacyl-PC's (Lewis & McElhaney, 1985a) and the odd-numbered ω-cyclohexyl-PC's (Lewis & McElhaney, 1985b), and a plausible physical basis of this behavior has been described previously (Lewis & McElhaney, 1985a). It is also clear that, unlike the longer chain compounds, the measured transition enthalpies of these two PC's are considerably greater when dispersed in aqueous ethylene glycol. An obvious difference between the 14_{ai} and 15_{ai} PC's and their longer chain homologues is the fact that, in aqueous suspension, the bulk solvent phase was frozen at the temperatures at which their DSC thermograms were recorded. A similar effect was observed in studies of the thermotropic phase behavior of the shorter chain, ω-cyclohexyl-PC's (Lewis & McElhaney, 1985b), for which the freezing of the bulk solvent phase may also have contributed to the observed effects. To evaluate the possible effects of the freezing of the bulk solvent phase on the behavior of the anteisobranched PC's, we compared the thermotropic phase behavior of 16_{ai} PC in frozen deuterium oxide (melting point ≅ 3.8 °C) with that shown in Figure 2, which was obtained in supercooled water. The results (data not presented) show that the freezing of the bulk aqueous phase had no significant effect upon the transition temperature, the measured transition enthalphy, or the heating–cooling reversibility characteristics. The only observable effect was an inability to resolve the fine structure of the DSC thermograms when the bulk aqueous phase was frozen. However, it should be noted that the physical processes resolved for an aqueous dispersion of the 16_{ai} compound do not include a gel-state packing transition. Thus, the above observations would suggest that the gel/liquid-crystalline phase transition and perhaps the minor gel-state transitions which precede it are not very sensitive to the composition or physical state of the bulk aqueous phase. But, given that the data described here clearly show that the interconversions between the gel state and condensed gel phases of these anteisoacyl-PC's are sensitive to the composition of the bulk aqueous phase, it is possible that the differences in the enthalpy values shown in Table II may have arisen from the inability of these short-chain compounds to form a condensed gel phase when the bulk solvent phase is frozen.

It is also clear from these studies that the presence of a methyl anteisobranch on the acyl chains must significantly affect the packing of the acyl chains in this model membrane system, since their gel/liquid-crystalline phase transition temperatures and enthalpy changes associated with the process are considerably lower than that reported for comparable *n*-acyl-PC's [see Silvius (1982) for a compilation of such data] and the methyl isobranched PC's (Lewis & McElhaney, 1985a). The disruptive effects of this type of chain branching on the packing of the hydrocarbon chains of a biological membrane have been inferred from some ^{19}F NMR studies [see Macdonald et al. (1983, 1985)], which suggested that when compared with the *n*-alkyl hydrocarbon chain structure both the methyl isobranched and methyl anteisobranched alkyl chain structures resulted in a more disordered (i.e., loosely packed) gel-state and perhaps, at comparable reduced temperatures, a more ordered liquid-crystalline state. The ^{31}P NMR data described here are consistent with such conclusions, since we have shown that in the gel-state conformation prior to the onset of the chain-melting process the mobility of their phosphate head groups becomes virtually indistinguishable from that of liquid-crystalline state. This is in marked contrast to the *n*-acyl-PC's (Seelig, 1978; Fuldner, 1981) and the isoacyl-PC's (Mantsch et al., 1985), for which the limiting spectral line shape consistent with fast axially symmetric motion of the phosphate head group on the ^{31}P NMR time scale is approached only when they are in their liquid-crystalline states. We also observed that the basal line widths of the ^{31}P NMR spectra of the condensed gel phases of these lipids ($\cong$130 ppm) are considerably narrower than that reported for DPPC and some mixed-chain, *n*-acyl-PC's (Fuldner, 1981; Lewis et al., 1984), for which the basal line widths of their ^{31}P NMR spectra are in the region of 180 ppm. This indicates that the anteisoacyl-PC's condensed gel phases are characterized by a greater mobility of their phosphate head group and suggests that the condensed gel phases of these anteiso PC's are probably more loosely packed than those of the *n*-acyl-PC's. It is interesting, however, that the ^{31}P NMR spectral line widths of these condensed gel phases were very similar to those of some isoacyl-PC's (Mantsch et al., 1985), even though single-crystal X-ray diffraction studies on *dl*-methyl anteisobranched fatty acids (Abrahamsson, 1958, 1959) have shown that the methyl anteisobranch is more disruptive of the packing of a hydrocarbon chain than a methyl isobranch. The following paper describes some infrared spectroscopic studies aimed at the assignment of the physical basis of some of the data described here.

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Registry No. 14_{ai}, 73651-81-7; 15_{ai}, 73641-09-5; 16_{ai}, 73641-10-8; 17_{ai}, 73641-11-9; 18_{ai}, 73641-12-0; 19_{ai}, 73641-13-1; 20_{ai}, 108320-55-4; 21_{ai}, 108320-56-5; 22_{ai}, 108320-57-6; $C14_{ai}$, 29709-05-5; $C15_{ai}$, 5502-94-3; $C16_{ai}$, 20121-96-4; $C17_{ai}$, 5918-29-6; $C18_{ai}$, 29709-08-8; $C19_{ai}$, 17001-28-4; $C20_{ai}$, 53254-53-8; $C21_{ai}$, 36332-93-1; $C22_{ai}$, 36332-94-2; $MeO_2C(CH_2)_7CO_2H$, 2104-19-0; $HO_2C(CH_2)_8CO_2H$, 111-20-6; $HO_2C(CH_2)_{10}CO_2H$, 693-23-2; $HO_2C(CH_2)_{12}CO_2H$, 821-38-5; $BrCH_2CH(Me)Et$, 10422-35-2; $Br(CH_2)_2CH(Me)Et$, 51116-73-5; $Br(CH_2)_3CH(Me)Et$, 71434-55-4; $Br(CH_2)_4CH(Me)Et$, 54089-01-9; $HO(CH_2)_2OH$, 107-21-1.

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