

# <sup>31</sup>P Nuclear Magnetic Resonance Chemical Shielding Tensors of Phosphorylethanolamine, Lecithin, and Related Compounds: Applications to Head-Group Motion in Model Membranes<sup>†</sup>

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**ABSTRACT:** <sup>31</sup>P nuclear magnetic resonance (NMR) powder spectra have been used to obtain the principal values of the chemical shielding tensors of dipalmitoyllecithin (DPL), dipalmitoylphosphatidylethanolamine, and several related organophosphate mono- and diesters. In addition, the principal values and orientation of the phosphorylethanolamine shielding tensor were determined from <sup>31</sup>P NMR spectra of a single crystal. In all compounds studied the shielding tensors were clearly nonaxial. The monoester spectra are typified by the spectrum of phosphorylethanolamine with principal values of -67, -13, and 69 ppm relative to H<sub>3</sub>PO<sub>4</sub>. The diesters have a larger total anisotropy, as indicated by the DPL values of -81, -25, and 108 ppm. These data as

well as the orientation of the phosphorylethanolamine shielding tensor are correlated with the electron density distribution as determined by the bonding pattern of the phosphate. The spectrum of a DPL-water (1:1) mixture at 52 °C has a shift anisotropy of 30 ppm and displays a shape characteristic of an axial tensor. This change from the rigid lattice DPL pattern is explained in terms of motional narrowing, and the shielding tensor data are used to interpret the motion of the phospholipid head group. Simple rotation about the P-O(glycerol) bond is excluded, and a more complex motion involving rotation about both the P-O (glycerol) and glycerol C(2)-C(3) bonds is postulated.

The polar head groups of phospholipids have been shown to be determinants of the properties of both phospholipid-water systems and actual biological membranes. The mobility of both the acyl tails and ethanolamine head groups of phosphatidylethanolamine bilayers is modulated by the pH of the medium, and hence the charge on the head groups (Michaelson et al., 1974). Similarly it has been shown that the thermal phase transitions of a variety of phospholipids are dependent on the pH of the medium and the presence of Ca<sup>2+</sup> ions (Verkleij et al., 1974), and that Ca<sup>2+</sup> ions are capable of causing phase separations in mixed bilayers (Jacobson and Papahadjopoulos, 1975). Work with membranes of *Staphylococcus aureus* has shown that the head-group composition is largely responsible for the nature of the permeability barrier of the membrane (Haest et al., 1972), and the overall importance of phospholipids in membrane function is indicated by the large number of membrane-bound enzymes which have specific phospholipid head-group requirements for proper function (Steck and Fox, 1972).

<sup>31</sup>P nuclear magnetic resonance (NMR) is an attractive technique for studying the nature of the phospholipid head groups due to the high natural abundance of <sup>31</sup>P, its relatively large gyromagnetic ratio, and the fact that the problem of perturbing the system with a bulky probe group is obviated. Previous <sup>31</sup>P NMR studies of unsonicated lipid

dispersions and biological membranes have been hampered by the slow motion of the molecules, resulting in unaveraged <sup>31</sup>P-<sup>1</sup>H dipolar interactions which broaden the resonances sufficiently to obscure the chemical shift information (Davis and Inesi, 1972; Horwitz and Klein, 1972). Deuteration of the sample reduces the dipolar interactions (Kohler et al., 1972; Kroon et al., 1976). Alternatively, use of sufficiently high magnetic fields will permit the chemical shielding anisotropy to dominate the dipolar broadening. This is possible because the chemical shift is linearly dependent on the field, while the dipolar interaction is field independent. It is also possible to remove the <sup>31</sup>P-<sup>1</sup>H dipolar broadening by <sup>1</sup>H decoupling, either in a simple free induction decay experiment (Gally et al., 1975) or in a cross-polarization experiment (Pines et al., 1973). The two latter approaches were taken in this work. Removal of the dipolar interactions can reveal the tensorial information contained in the chemical shielding anisotropy of rigid or slowly tumbling molecules; such anisotropy data are averaged to the trace in rapidly tumbling molecules.

The chemical shift is interpretable only if sufficient information is known about the magnitude and orientation of the shielding tensor with respect to the molecular axis system. This paper is the first report in a long range project to obtain chemical shielding tensor data for organophosphates related to phospholipids and apply those data toward an understanding of the function and organization of phospholipid head groups in membranes and model membrane systems. Determinations have been made of all six components of the symmetric part of the chemical shielding tensor of phosphorylethanolamine and of the three principal elements of the shielding tensors of a variety of organophosphate powders including L- $\alpha$ -dipalmitoyllecithin (DPL)<sup>1</sup> and L-

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<sup>1</sup> Abbreviation used is: DPL, dipalmitoyllecithin.

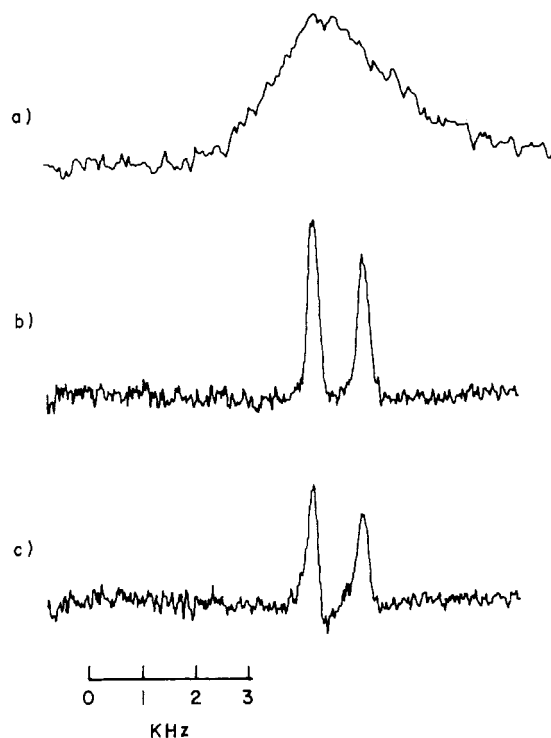


FIGURE 1:  $^{31}\text{P}$  NMR spectra (24.3 MHz) of a single crystal of phosphorylethanolamine at 25 °C in an arbitrary orientation. (a) The 250-pass FID with a repetition rate of 30 s. (b) As (a) but in the presence of an 8.6 G  $^1\text{H}$  decoupling field. (c) The 250-pass cross-polarization experiment with  $\tau_c = 1$  ms and a repetition rate of 5 s.

$\alpha$ -dipalmitoylphosphatidylethanolamine. The data from these shielding tensors were used to interpret the spectrum of the phosphate in the lecithin head group in lecithin-water mixtures in terms of its motion.

#### Materials and Methods

**Materials.** Crystals of  $\text{H}_3^+\text{NCH}_2\text{CH}_2\text{OPO}_3^-\text{H}$  (phosphorylethanolamine) were grown by slow evaporation at room temperature from an aqueous solution originally containing 5 g of phosphorylethanolamine in 20 ml of distilled water. The crystals grew as long rods and were harvested after approximately 10 days. A piece measuring  $4 \times 4 \times 5$  mm was cut from a longer rod and used in the NMR experiments. The orientation of the crystal was determined by the Laue precession method and the unit cell dimensions were measured as  $a = 8.97$ ,  $b = 7.72$ , and  $c = 8.82$  Å, in agreement with the reported values of 9.04, 7.75, and 8.86 Å (Kraut, 1961).

The powders were obtained from commercial sources and used without further purification. Approximately 150 mg was used for each NMR sample. L- $\alpha$ -Dipalmitoyllecithin bilayers were prepared by mixing DPL and distilled water in a ratio of 1:1 (w/w). This mixture exists in the bilayer phase above 41 °C (Chapman et al., 1967).

**Phosphorus Magnetic Resonance Spectrometer.** The spectrometer used for the  $^{31}\text{P}$  NMR experiments is a home-built instrument described in detail elsewhere (Kohler et al., 1976). It operates at 24.3 MHz for  $^{31}\text{P}$  detection and at 60 MHz for  $^1\text{H}$  decoupling, and is equipped with a deuterium field-frequency lock. To make it suitable for work with solids, the probe was specially designed to operate at high rf power and to recover quickly after rf pulses; the spectrometer was equipped with a computer driven goniometer to facilitate sample rotation. Temperature control was achieved

by regulating the flow of either warm or cold  $\text{N}_2$  gas past the sample. The temperature was measured with a thermocouple inserted in place of the sample in the probe and was found to fluctuate no more than 5 °C over the course of an experiment.

**Data Collection.** Two Fourier transform techniques were used to record the  $^{31}\text{P}$  NMR spectra. The spectra of the single crystal were taken using the cross-polarization nuclear enhancement technique used widely for  $^{13}\text{C}$  measurements and described in detail elsewhere (Pines et al., 1973). A single contact experiment was used with a cross-polarization time of 1 ms and an  $^1\text{H}$  decoupling field of 8.6 G in the rotating reference frame. The  $^{31}\text{P}$  powder spectra were obtained by recording the free induction decay using quadrature phase detection in the presence of continuous wave (cw) irradiation of the protons. The  $^{31}\text{P}$   $\pi/2$  pulse length was approximately 2  $\mu\text{s}$ , providing a field of 75 G in the rotation reference frame. The  $^1\text{H}$  decoupling field was 8–10 G in the rotating frame.

**Obtaining Chemical Shielding Tensors.** The powder spectra were used to obtain the three principal elements of the chemical shielding tensors by matching the powder patterns to those of computer-simulated spectra. The six components of the symmetric shielding tensor of phosphorylethanolamine were obtained from plots of resonance position as a function of rotation angle for three different orientations of the phosphorylethanolamine crystal. The method of sample orientation and details of the data analysis are presented elsewhere (Kohler et al., 1976).

#### Results

**Comparison of Cross-Polarization and Free Induction Decay Experiments.** Spectra of a single crystal of phosphorylethanolamine taken by three different methods are shown in Figure 1. Figure 1a is from a simple free induction decay (FID) experiment without  $^1\text{H}$  decoupling, while Figure 1b is from a FID experiment with a  $^1\text{H}$  decoupling field of 8.6 G. Figure 1c is from a cross-polarization experiment on the same sample.

The most obvious feature of these spectra is the effectiveness of the  $^1\text{H}$  decoupling field in removing the heteronuclear broadening in both the cross-polarization and FID experiments. The line width was reduced from a value of 5.5 kHz to 500 Hz by the decoupling. Doubling the decoupling field produces no further line narrowing, and since the P–P distance in phosphorylethanolamine is only 4.275 Å (Kraut, 1961), it is likely that the residual broadening is due to homonuclear dipolar interactions. As a comparison, the P–P distance in  $\alpha\text{-Ca}_2\text{P}_2\text{O}_7$  is 2.897 Å (Calvo, 1968) and the line widths observed in undecoupled  $^{31}\text{P}$  spectra of this compound are on the order of a kilohertz (Kohler et al., 1976). Based on the  $1/r^3$  dependence of the dipolar interaction, it would be expected that the contribution of line width from the P–P dipolar interaction in phosphorylethanolamine should be on the order of 300 Hz. Since this is a rough estimation neglecting the orientation dependence of the dipolar interaction, assigning at least most of the remaining line width in the phosphorylethanolamine spectrum to P–P dipolar interactions is quite reasonable. The latter interactions should be reduced with a combined WA-HUHA-cross-polarization experiment.

A comparison of Figure 1b and c indicates that the cross-polarization and FID experiments with  $^1\text{H}$  decoupling give essentially the same result. An advantage of the cross-polarization technique is that the pulse repetition rate may be

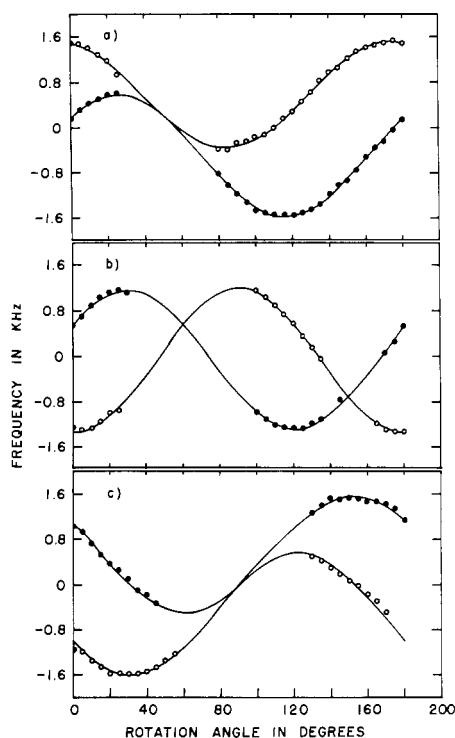


FIGURE 2: Angular dependence of  $^{31}\text{P}$  resonance frequency for three orientations of a crystal of phosphorylethanolamine. These plots include data points and theoretical curves calculated from least-squares analyses. The symbols are used consistently for the three sets of plots. The frequency is expressed relative to the resonance frequency of  $\text{H}_3\text{PO}_4$ .

much faster than for a FID experiment. In the present case, the cross-polarization experiment required only  $\frac{1}{6}$  as much time as the FID experiment. This results from the fact that between FID passes it is the  $^{31}\text{P}$  nuclei which must reestablish thermal equilibrium, while between cross-polarization passes it is the more abundant, less isolated  $^1\text{H}$  nuclei which must reestablish equilibrium. These relaxation times differ by at least a factor of six, as manifested by the acquisition times of the two spectra.

A disadvantage of the cross-polarization experiment can be the occurrence of baseline artifacts which make the spectra quite difficult to phase properly, as seen in Figure 1c. This problem is especially significant in the case of powder spectra where proper phasing is critical. The artifacts are probably caused by the  $^{31}\text{P}$  rf pulses and might be removed by phase alternation experiments (Stejskal and Schaefer, 1975). However, in the present case, the problem was avoided by taking the powder spectra by the simple FID method.

**Phosphorylethanolamine Chemical Shielding Tensor.** A typical cross-polarization spectrum from a single crystal of phosphorylethanolamine is shown in Figure 1c. The presence of two lines results from two magnetically inequivalent orientations of phosphorylethanolamine in its  $P2_1/c$  unit cell (Weil et al., 1973). Figure 2 contains plots of resonance frequencies as a function of rotation angle for three different orientations of the phosphorylethanolamine crystal. From these data two chemical shielding tensors are obtained. The tensors have the same principal elements but differ in orientation corresponding to the two different orientations of the phosphorylethanolamine molecule in the crystal unit cell. This allows two possible assignments of the orientation of the tensor on the molecule. The assignment shown in Figure 3 has no correlation with the bonds of the phosphate moiety, while that shown in Figure 4 shows definite correlation. The latter assignment, reproduced in greater detail in Figure 5, was chosen as the more probable orientation. Further support of this choice of orientation comes from the fact that the orientation of shielding tensors for  $\alpha\text{-Ca}_2\text{P}_2\text{O}_7$  (Kohler et al., 1976) and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  (Kohler and Klein, 1976) may also be correlated with the simple picture of phosphate bonding described in the discussion. Values of the principal elements of the shielding tensor appear in Table I.

The room temperature spectra of a variety of organophosphate mono- and diester powders appear in Figure 6. All the spectra were taken in the presence of an  $^1\text{H}$  decoupling field, and have the characteristic powder pattern shapes for nonaxial chemical shielding tensors. The principal elements of the shielding tensors obtained from these

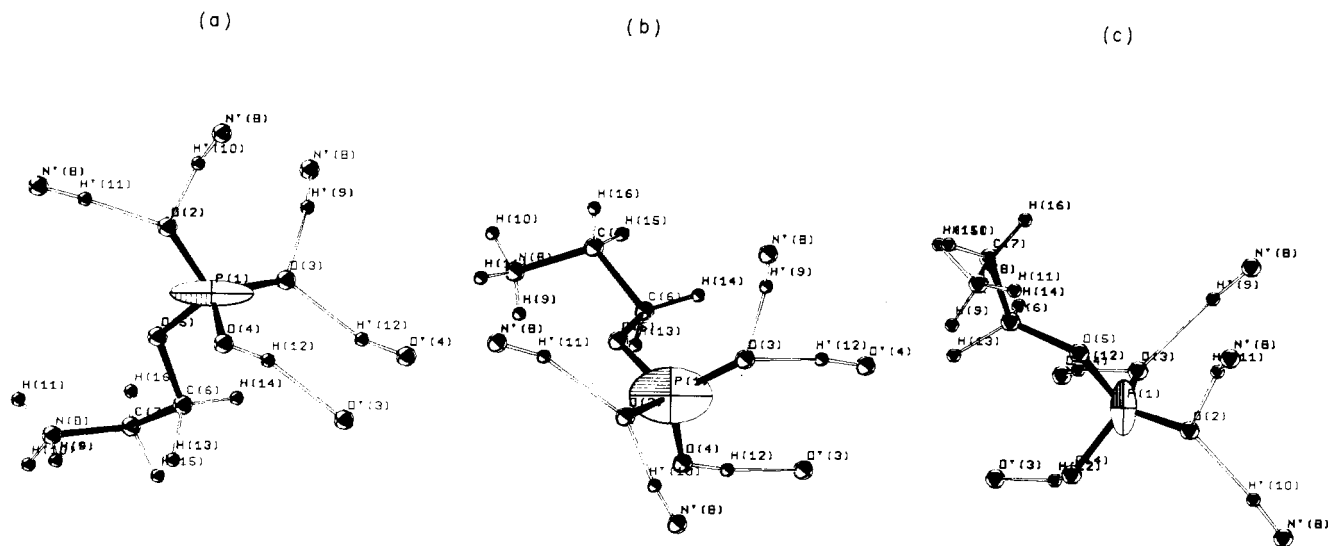
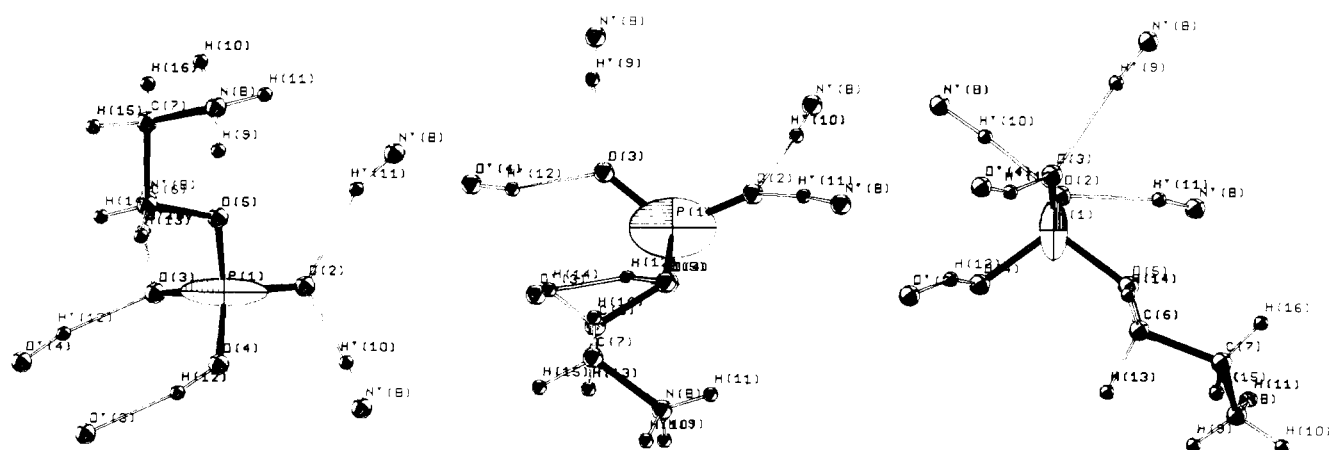


FIGURE 3: Chemical shielding tensor of phosphorylethanolamine: assignment A. These are projections of the molecule in the principal axis system of the shielding tensor of assignment A. The shielding tensor is shown as an ellipsoid, with the shortest axis representing the most downfield tensor component. The numbering of atoms corresponds to that of the original x-ray work (Kraut, 1961), with primed letters indicating atoms from neighboring molecules.



(c)

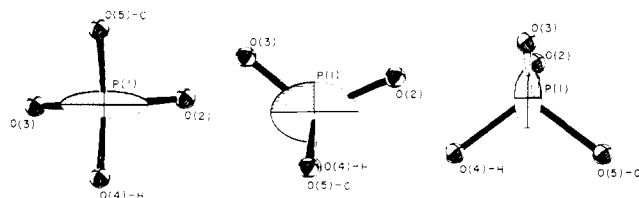


Table I: Principal Values of the  $^{31}\text{P}$  Shielding Tensors of Some Organophosphates.<sup>a</sup>

	$\sigma_{11}$	$\sigma_{22}$	$\sigma_{33}$	Trace <sup>b</sup>	$\sigma_{\text{soln}}$	Temp <sup>c</sup> (°C)
<b>Monoesters</b>						
Phosphorylethanol-amine						
Crystal	-67	-13	69	-3	-3.7 <sup>c</sup>	RT
Powder	-63	-8	65	-2		RT
	-63	-8	72	0		-72
Serine phosphate	-46	-3	52	1	-3.7 <sup>c</sup>	RT
	-42	1	61	7		-110
Glycerol phosphate	-63	5	44	-5		RT
	-63	5	44	-5		-100
<b>Diesters</b>						
L- $\alpha$ -Glycerylphosphorylcholine	-68	-16	87	1	+0.1 <sup>c</sup>	RT
	-68	-16	83	-1		-98
DPL	-81	-25	108	1	0.8 <sup>c</sup>	RT
Dipalmitoylphosphatidylethanolamine	-85	-14	87	-4	0.1 <sup>d</sup>	RT

<sup>a</sup> All values are in ppm relative to  $\text{H}_3\text{PO}_4$ , taking downfield as negative. The values from the powder spectra were obtained by matching computer simulated spectra and have approximately  $\pm 5$  ppm uncertainty. The spectra of serine phosphate and glycerol phosphate were difficult to simulate; the deviations from the simulated spectra may be due to P-P or P-Na dipolar interactions. This problem should be resolved by single crystal studies and combined WAHUHA-cross-polarization experiments now in progress. <sup>b</sup> Trace =  $\frac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33})$ . This is the value expected for  $\sigma_{\text{soln}}$  if there are no changes in the structure or magnetic susceptibility of the sample. <sup>c</sup> From Henderson et al., 1974. <sup>d</sup> Value for distearoylphosphatidylethanolamine (Henderson et al., 1974). <sup>e</sup> RT = room temperature and is  $25 \pm 5^\circ\text{C}$  depending on the rate of gas flow used.

A spectrum of a 1:1 DPL-water mixture taken at 55 °C is shown in Figure 7. This spectrum resembles previously reported spectra for similar systems (Sheetz and Chan, 1972; Gally et al., 1975; McLaughlin et al., 1975). A spectrum taken at  $24 \pm 2$  °C, below the transition temperature, was of poorer quality, but definitely showed an increased anisotropy from 30 ppm to approximately 70 ppm.

**Orientation of the Chemical Shielding Tensor in Phosphorylethanolamine.** The bonding pattern in a molecule or group establishes the electron density distribution which in

turn determines the chemical shielding tensors of the nuclei within the group. In general, the chemical shielding will have contributions depending on the electron distribution in both the ground state and excited states (Ramsey, 1952). Quantitative predictions of chemical shielding, therefore, must involve molecular orbital calculations and are beyond the scope of this paper. However, it has been empirically observed that the principal axes of the  $^{31}\text{P}$  chemical

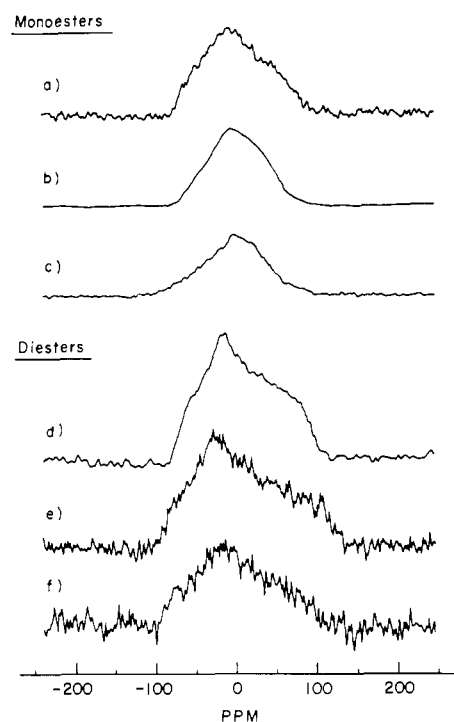


FIGURE 6:  $^{31}\text{P}$  NMR spectra of phosphate powders. These spectra were taken at 25 °C in the presence of an  $^1\text{H}$  decoupling field. The scale is in ppm relative to  $\text{H}_3\text{PO}_4$  and assigns the lower field resonances negative values. (a) Phosphorylethanolamine; (b) serine phosphate; (c) glycerol phosphate; (d) L- $\alpha$ -glycerylphosphorylcholine; (e) dipalmitoyllecithin (DPL); (f) Dipalmitoylphosphatidylethanolamine.

shielding tensor in phosphorylethanolamine relate simply to the electron density distribution of the ground state of the molecule.

The electron density distribution of phosphorylethanolamine may be described as a perturbation of the distribution for a  $\text{PO}_4^{3-}$  ion. The  $\text{PO}_4^{3-}$  ion itself is a regular tetrahedron, having P-O bond lengths which are all approximately 1.54 Å. This value is less than the calculated single P-O bond length of 1.71 Å (Shomaker and Stevenson, 1941). The partial double bond character of all the bonds in  $\text{PO}_4^{3-}$  is explained in terms of bonding between the empty  $3d_{z^2}$  and  $3d_{x^2-y^2}$  orbitals of the phosphorus and the filled  $2p$  and  $2p'$  orbitals of the oxygens (Cruickshank, 1961). When the ion is perturbed by protonation or esterification, bonding will involve one of the  $2p$  orbitals of the participating oxygen, thus reducing the participation of that orbital in  $\pi$  bonding with the phosphorus atom. The resulting effect is that the double bond character of the P-O(R) bond is reduced and the double bond character of the bonds to the nonesterified oxygens is increased. This shifts the electron density away from the P-O(R) bond and toward the remaining P-O bonds, lengthening the former and shortening the latter. The effects of forming a (P)O-H bond are similar to those of forming a (P)O-R bond, although the magnitude of the perturbation is smaller. As an example, serine phosphate has its (R)O-P bond lengthened to 1.61 Å, the (H)O-P bond lengthened to 1.56 Å, and the remaining P-O bonds shortened to 1.52 and 1.50 Å (Cruickshank, 1961; McCallum et al., 1959).

The bond lengths and bond angles for phosphorylethanolamine are listed in Table II. One of the oxygens, O(5), is bonded to a carbon to form the ester, and O(4) is covalently bonded to H(12). The bond lengths to these two oxygens are longer than those for  $\text{PO}_4^{3-}$  indicating less  $\pi$  character

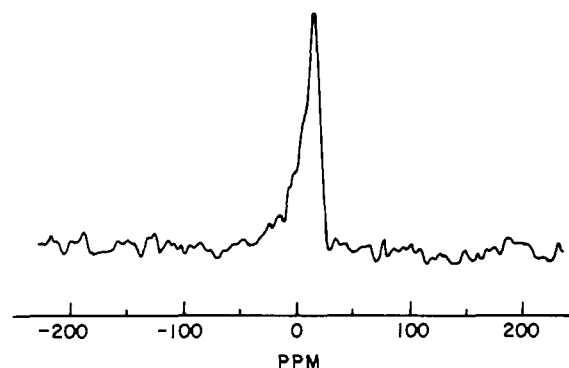


FIGURE 7:  $^{31}\text{P}$  NMR spectrum of a DPL-water mixture. The DPL-water ratio was 1:1 (w/w) and the spectrum was taken at 52 °C in the presence of an  $^1\text{H}$  decoupling field. The scale is in ppm relative to  $\text{H}_3\text{PO}_4$ , and assigns the lower field resonances negative values.

Table II: Structure Data for Phosphorylethanolamine.<sup>a</sup>

Bond Lengths (Å)		Bond Angles (deg)	
P(1)-O(2)	1.493	O(2)-P(1)-O(3)	117.4
P(1)-O(3)	1.503	O(2)-P(1)-O(4)	109.8
P(1)-O(4)	1.557	O(2)-P(1)-O(5)	103.9
P(1)-O(5)	1.591	O(3)-P(1)-O(4)	109.3
		O(3)-P(1)-O(5)	109.6
		O(4)-P(1)-O(5)	106.2

<sup>a</sup> Data from Kraut (1961).

and lower electron density. The remaining two P-O bonds are shortened due to the increased availability of the  $3d$  orbitals and resultant increased  $\pi$  character of the bonds. The fact that these two oxygens share in hydrogen bonds with protons from other molecules is presumed a much weaker perturbation. The electron density around the phosphorus nucleus is concentrated in the plane containing the short O(2)-P-O(3) bonds, leaving the phosphorus relatively deshielded in the perpendicular O(5)-P-O(4) plane. The most downfield component of the shielding tensor is found in this plane of low electron density. Within the high electron density plane containing the P-O(2) and P-O(3) bonds, the electron density would be concentrated along the bonds, and the minimum electron density would be expected to be along the direction bisecting the O(2)-P-O(3) bond angle. The more downfield of the two principal elements of the tensor in this plane is found to be along the bisector of the O(2)-P-O(3) bond angle. Thus the principal axes of the  $^{31}\text{P}$  chemical shielding tensor of phosphorylethanolamine correlate with the ground state electronic distribution around the phosphorus, with the most downfield tensor component in the plane of lowest electron density and the most upfield component along the direction of highest electron density. Similar correlations have been observed for the shielding tensors of  $\alpha\text{-Ca}_2\text{P}_2\text{O}_7$  (Kohler et al., 1976) and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  (Kohler and Klein, 1976).

**Shielding Tensors from Powder Pattern Data.** Based on the data from phosphorylethanolamine together with data from  $\alpha\text{-Ca}_2\text{P}_2\text{O}_7$  and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  not reported here, predictions may be made concerning the shielding tensors of other phosphate mono- and diesters. Monoesters would be expected to have chemical shielding tensors closely resembling that of phosphorylethanolamine in both orientation and magnitude since the distortion from the simple phosphate tetrahedron should be similar. Of course the orienta-

tions of the tensors cannot be compared until more single crystal data are available, but the values of the principal elements of the tensors may be compared from the data in Table I. All the monoester shielding tensors are similar in that  $|\sigma_{11}|$  and  $|\sigma_{33}| \leq 70$  ppm and  $\sigma_{22}$  is close to zero ppm (relative to  $\text{H}_3\text{PO}_4$ ).

Diester differ from phosphorylethanolamine in that they have a  $\text{R}(\text{O})\text{-P-O}(\text{R})$  structure rather than a  $(\text{R})\text{O-P-O}(\text{H})$  structure. Since protonation and esterification both involve the same basic perturbation, the electron density patterns of the two types of molecules should be similar and therefore the orientation of the shielding tensors ought to be equivalent to that of phosphorylethanolamine. The magnitudes of the diester tensor components would be expected to be different, however. The  $(\text{R})\text{O-P-O}(\text{R})$  plane would have a lower electron density than the  $(\text{R})\text{O-P-O}(\text{H})$  plane, and the low field component of a diester tensor should thus occur at lower field than that of phosphorylethanolamine. Similarly, the high field component would be expected to occur at a higher field for the diesters. This qualitative picture is substantiated by the data for diesters appearing in Table I. All the diesters have  $\sigma_{11}$  shifted downfield from the  $\sigma_{11}$  values for monoesters, and the  $\sigma_{33}$  values shifted upfield.

**Motional Narrowing.** If the phosphate group is moving on the NMR time scale, the  $^{31}\text{P}$  chemical shielding tensor will be subject to motional averaging and the observed chemical shift anisotropy will be reduced from its rigid lattice value. The nature of the averaging and the resultant line shape depend on the exact nature of the motion involved. If the motion is fast on the NMR time scale ( $\Delta\omega_{\text{anis}}\tau \ll 1$ , where  $\Delta\omega_{\text{anis}}$  is the rigid lattice shift anisotropy and  $\tau$  is the correlation time of the motion), then the remaining anisotropy does not depend on the *rate* of motion, but rather on the *type* of motion. For example, in the simple case of fast rotation about a fixed axis in the molecule, the rotation causes averaging of the chemical shielding elements in the plane perpendicular to the rotational axis. The original shielding tensor is replaced by an effective tensor which is axially symmetric and has the elements

$$\begin{aligned}\sigma_{\parallel} &= \sin^2 \beta \cos^2 \alpha \sigma_{11} + \sin^2 \beta \sin^2 \alpha \sigma_{22} + \cos^2 \beta \sigma_{33} \\ \sigma_{\perp} &= \frac{1}{2}(1 - \cos^2 \alpha \sin^2 \beta) \sigma_{11} + \frac{1}{2}(1 - \sin^2 \alpha \sin^2 \beta) \sigma_{22} + \frac{1}{2} \sin^2 \beta \sigma_{33} \\ \Delta\sigma &= \frac{1}{2}(3 \cos^2 \beta - 1) \left[ \sigma_{33} - \frac{1}{2}(\sigma_{11} + \sigma_{22}) \right] + \frac{3}{4}(\sigma_{11} - \sigma_{22}) \sin^2 \beta \cos 2\alpha\end{aligned}$$

where  $\sigma_{\parallel}$  and  $\sigma_{\perp}$  are the components parallel and perpendicular to the rotation axis and the angles  $\alpha$  and  $\beta$  are Euler angles between the rotation axis and the principal axes of the shielding tensor (Mehring et al., 1971). The anisotropy  $\Delta\sigma$  which is observed depends only on the orientation of the rotation axis and the values of  $\sigma_{ii}$ , and not on the rate of motion.

In more complicated types of motion the shielding tensor may be thought of as averaged over some solid angle. This may be caused by "wobble" of the rotation axis, the presence of more than one reorientation axis, or by some type of jumping between different orientations. With these types of compound motion a simple mathematical treatment is not possible, but the observed anisotropy may be qualitatively related to the solid angle over which the tensor is averaged. The larger the angle, the smaller the anisotropy, until the limiting case of isotropic motion and zero shielding anisotropy is reached with

$$\sigma_i = \frac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33})$$

If the motion is slow on the NMR time scale, the averaging process is incomplete and the resultant line shapes are quite different and may bear little resemblance to the normal powder patterns (Spiess, 1975).

The absence of axially symmetric powder patterns and the fact that the spectra do not change appreciably when the temperature is lowered to approximately  $-100^\circ\text{C}$  indicate that the phosphate groups of the powders studied are probably rigid on the NMR time scale at room temperature. This is not surprising since, unlike the recently studied nonionic carbon compounds where motional narrowing has been seen at room temperature (Mehring et al., 1971; Pines et al., 1972; Kaplan et al., 1973), the phosphate compounds each have several hydrogen bonds which would have to be broken to allow rotation.

A very different situation is seen when the DPL-water spectrum is compared to that of DPL powder. The observed reduction in the shift anisotropy indicates the presence of motion. The previously reported spectra of similar DPL-water systems (Sheetz and Chan, 1972; Gally et al., 1975; McLaughlin et al., 1975) also show evidence of motional narrowing. One of these spectra was taken at a high field strength where  $\Delta\omega_{\text{anis}} = 153 \text{ kAv}^2$ . Since narrowing was observed, the correlation time of the motion was less than  $6.5 \mu\text{s}$ . Therefore, in our low field case where  $\Delta\omega_{\text{anis}} = 28.9 \text{ kAv}$ ,  $\Delta\omega\tau \ll 1$ , and the motion is indeed fast on the NMR time scale.

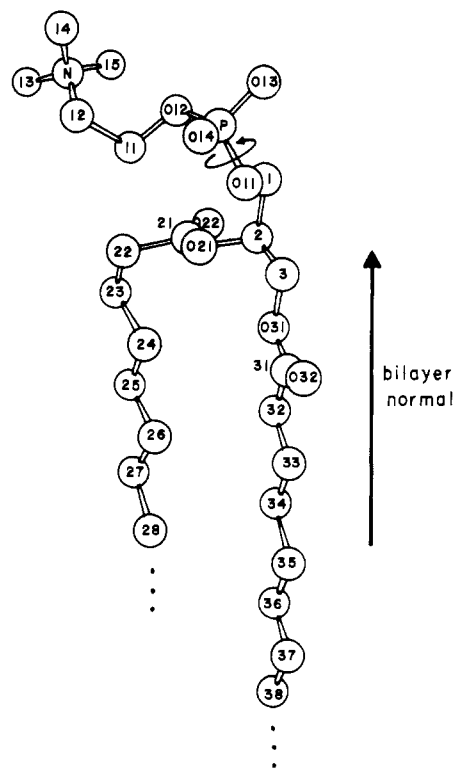


FIGURE 8: Schematic representation of DPL. The truncated molecule is shown in a probable conformation based on the x-ray crystallographic structure of dilauroylphosphatidylethanolamine (Hitchcock et al., 1974). The simplest mode of phosphate rotation is indicated by the arrow. As explained in the text, this motion alone will not produce the spectrum in Figure 7.

<sup>2</sup> 1 Av =  $2\pi$  Hz.

If it is assumed that the orientation of the shielding tensor in DPL approximates that of the phosphorylethanolamine tensor, something may be said about the possible modes of motion. The simplest motion for the phosphate group is an unhindered rotation about the P-O(11) bond, as shown in Figure 8. This rotation axis has Euler angles of  $\alpha = 36^\circ$  and  $\beta = 90^\circ$  and would predict a  $\Delta\sigma$  of approximately  $-95$  ppm. This is not in accord with the observed spectra. Further, no single rotational axis was found which would generate the observed spectrum. These data suggest that the phosphate is experiencing a more complicated motion. One possibility is that combined rotations about the P-O(11) bond and the glycerol C(2)-C(3) bond contribute to the motion of the phosphate group. This interpretation of the motion entails rotation of the entire head group about an axis perpendicular to the bilayer plane. This is in agreement with the results from deuterium magnetic resonance studies of the choline head group (Gally et al., 1975) which indicate that the choline rotates about an axis perpendicular to the bilayer plane, and indicate that the glycerol C(3) with an order parameter of  $S = 0.44$  is not rigid.

An additional motional component often invoked is the tumbling of the phospholipid multilayers. The minimum radius for the multilayers in a 1:1 DPL-H<sub>2</sub>O mixture is on the order of  $1 \mu$ . The correlation time for multilayer reorientation calculated from the Stokes equation for particles of this size is  $\tau = 1$  s (using  $\eta = 0.01$  P,  $T = 293^\circ\text{C}$ ), and such long correlation times are clearly incommensurate with those evaluated above.

Many other models of the motion may be formulated as well. The goal of this discussion is not to state the definitive model of motion in the phospholipid head groups, but rather to give an indication of how the shielding tensor data may be used to understand this motion. The data have shown that the simple model of rotation about the P-O(11) bond is inadequate, and led to other models as more plausible. An in-depth analysis of the head-group motion in the DPL-water system is in progress and will be reported at a later date. Further, it should be reiterated that the statements about the motion in DPL are based upon the crucial assumption that the orientation of the DPL shielding tensor is the same as that for phosphorylethanolamine. This is reasonable, but nevertheless it is an assumption. Data from crystals of phosphate diesters as well as from oriented bilayers are necessary for elucidating the details of the <sup>31</sup>P chemical shielding tensor in DPL, and studies directed toward this goal are now in progress.

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