

Raman and X-Ray Studies of Single Crystals of Dipalmitoylphosphatidylcholine †‡

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(Received August 5, 1980)

Large single crystals ($7 \text{ mm} \times 2 \text{ mm} \times 5 \mu\text{m}$) of dipalmitoylphosphatidylcholine have been crystallized at low water concentration from organic solution. A combination of X-ray diffraction and Raman spectroscopy has been used to examine lipid molecular orientation in these crystals. The polarizability tensors of the Raman active vibrational modes of the hydrocarbon chains have been determined. The data indicate that the hydrocarbon chains are all-trans and tilted away from the normal to the lipid bilayers. This hydrocarbon chain tilt contributes to the biaxiality of these low-water lipid crystals. These crystals can be hydrated by exposing them to water partial pressures above 70% relative humidity. Upon hydration, the biaxiality dramatically decreases and the 49.3 \AA long spacing increases to 58 \AA , suggesting a decreased hydrocarbon chain tilt. The hydrated crystals appear to remain optically homogeneous, suggesting that they remain single crystals.

INTRODUCTION

Lecithins such as dipalmitoylphosphatidylcholine (DPPC) form lyotropic smectic liquid crystals at temperatures above the hydrocarbon chain melting points and at a variety of water concentrations. These lipid- H_2O samples are

† Invited lecture, presented at Eighth International Liquid Crystal Conference, Kyoto (Japan), June 30-July 4, 1980.

‡ This work has been sponsored in part by the National Institute of Health under grant GM-24081-02, by the National Science Foundation under grant DMR-77-24295 and by the Joint Services Electronics Program (U.S. Army, Navy, and Air Force) under Contract N00014-75-0648.

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interesting both because of their liquid crystalline properties and because they are considered useful as model systems for examining the physical properties of the lipid constituents found in biological membranes.

The principle feature that has distinguished liquid crystal research on lyotropic systems, such as the lipids, from thermotropics has been the absence of the well oriented, monodomain lyotropic samples that have been so fundamentals to the progress on thermotropics. The availability of such samples for thermotropics has facilitated a wide variety of measurements. Fundamental information has been obtained concerning optical properties, defect structures, elastic constants, critical phenomena, viscosities, etc. Similar studies on lipids and other lyotropic liquid crystals will require macroscopically aligned samples.

In a series of papers, Powers and Clark,¹ Powers and Pershan,² and Asher and Pershan^{3,4} demonstrated that high-quality, oriented, smectic liquid crystalline lipid samples 100 to 200 microns thick and 1 cm² in area could be formed by either a thermal or mechanical alignment process. The thermal process was useful at low water concentrations (2% to 7%) and required annealing temperatures between 100°C and 140°C. Alignment could be maintained in the smectic A phase on slow cooling; however, once cooled the sample temperature could not be raised without causing an "undulation instability"⁵ associated with a dilation of the sample due to the negative coefficient of thermal expansion of the bilayer thickness.⁶ Small temperature rises cause modest misalignments that subsequently anneal away in a matter of minutes. Slightly larger temperature rises cause a more complicated type of defect array known as the PFC or "parabolic focal conic".^{3,4} This type of array had previously been produced and characterized in thermotropic smectic-A liquid crystals by Rosenblatt *et al.*⁷ and is one example of the manner in which thermotropic and lyotropic liquid crystals exhibit similar features in well-aligned samples. A second example is the existence of anisotropic elastic constants that can be determined from Brillouin studies on thermotropic⁸ and lyotropic lipid-water⁹ smectic liquid crystals.

An important difference between thermotropic and lyotropic liquid crystals is that whereas the thermotropics are usually pure one component systems, the lyotropics have two components and, as a result, have more complex phase diagrams. For example, DPPC shows a phase transition to a smectic A liquid crystalline phase at a temperature T_g which depends strongly upon sample water concentration.^{10,11} The phases existing below T_g also depend upon water concentration, and have not yet been well characterized as to whether they are liquid crystalline or true crystals. These phases are distinguished from the L_α or smectic A phase in that sharp in-plane peaks occur in their x-ray diffraction patterns¹⁰⁻¹² similar to the sharp peaks observed in thermotropics that have been identified as smectic B.¹³ There is also con-

siderable evidence that the lipid hydrocarbon chains are relatively fluid-like in the higher temperature L_α phases and rigid in the lower temperature phases.¹¹

For thermotropic liquid crystals the transition from the higher temperature smectic A phase to the lower temperature smectic B or crystalline B phase is easily accomplished without any significant deterioration in the macroscopic sample alignment. Unfortunately, neither the Powers,² nor the Asher^{3,4} techniques obtained well-aligned samples below T_g . Probably this is because the large changes in the bilayer thickness that occur at the phase transition^{11,12} causes stresses within the sample. This results in misalignments which are unable to heal due to kinetic problems.

We have succeeded in obtaining single crystals of dipalmitoylphosphatidylcholine (DPPC) at low water concentration and are exploring the lipid phase diagram by varying the temperature and water content of these samples. Albon *et al.*¹⁴ have previously reported the growth of different single crystals of DPPC¹⁴ and more recently Craievich *et al.* have carried out an X-ray study of single crystals of dipalmitoyl-glyceride.¹⁵ Further, Pearson and Pascher reported an X-ray molecular structure of the dihydrate of dimyristoylphosphatidylcholine.¹⁶ In this report, we will describe the conditions for crystal growth, the crystal optical properties, an X-ray determination of lattice parameters and a polarized Raman study of the hydrocarbon chain orientation.

I CRYSTAL GROWTH

The essential criteria for growing the DPPC crystals are the high purity DPPC samples obtained from Sigma Chemical Company (St. Louis, MO). Crystallization was obtained by slowing cooling a 2 mg/ml solution of DPPC from a CHCl_3 and acetone solution (3/2:V/V). The solution is cooled from 25° to 18°C over 4 days. Crystals of the type shown in Figure 1 form spontaneously at 18°C and grow to $\sim 5 \text{ mm} \times 2 \text{ mm} \times 5 \mu\text{m}$. The photographs in Figure 1(a) and (b) are taken between crossed polarizers at different orientations to the crystallites. It is obvious that the crystallites are uniformly biaxial.

Using a Soleil-Babinet compensator we determined that the index of refraction along the long axis of the crystal n_L is larger than that along the width n_W , $n_L - n_W \simeq 0.05$. On comparing this value with the biaxiality ($n_y - n_x \sim 1.5 \times 10^{-3}$) observed by Powers *et al.*¹ in a DPPC monohydrate sample that was aligned by thermal annealing in the L_α phase and then cooled to 25°C, it is apparent that the crystals are more birefringent. Further, the biaxiality in the crystals is homogeneous throughout the sample, unlike those cooled from the L_α phase.

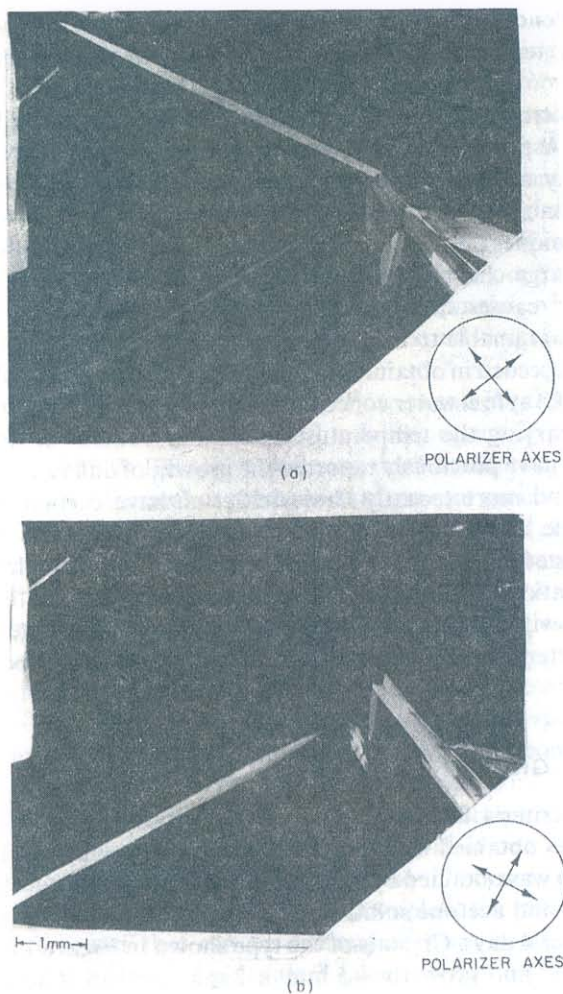


FIGURE 1 Crossed polarizer photomicrograph of DPPC crystals for different orientations of polarizers. Note that when crystal axes lie along polarizer axes the light remains linearly polarized and is not transmitted through the analyzer.

We have systematically observed these crystals optically over periods as long as one to two weeks at varying partial pressures of water and found that no change occurs below approximately 70% relative humidity of water between 15–25°C. Above 70% relative humidity the birefringence drops precipitously at the sample edges. Equilibrium is reached within the crystals in one to two days and they become optically uniform with only small biaxiality ($\Delta n < 0.002$). This appears to be an irreversible change since evacuat-

ing the sample for a number of days at 25°C does not restore the initial large birefringence.

Unless specified, all samples were maintained in sealed containers under relative humidities of water considerably less than 10%. Thus, we are confident that there was no significant change in the water content of the samples during the measurements to be described below.

II X-RAY DIFFRACTION

Figure 2 shows the results of a $\theta - 2\theta$ scan on single crystals oriented with the scattering vector normal to the broad flat faces of the crystals (i.e., along the thin direction). The data were taken with a General Electric model XRD5 diffractometer using $\text{CrK}\alpha$ radiation ($\lambda = 2.2909 \text{ \AA}$). The indicated orders of Bragg peaks correspond to a periodicity of 49.3 Å. This might be compared with 58.1 Å reported by Powers and Clark¹ for a monohydrate sample formed by thermal annealing or values obtained by Tardieu *et al.*¹² for dimyristic-PC (DMPC) of 47.6 Å and for distearic-PC (DSPC) of 56.8 Å. The series DMPC, DPPC and DSPC only differ in the number of carbons in the hydrocarbon chains, 14, 16 and 18 respectively. The average of Tardieu *et al.*'s values for DMPC and DSPC, 52.2 Å, is 2.9 Å larger than for our crystals. In contrast, Albon reported X-ray diffraction results for anhydrous single crystals of DPPC in which the long spacing was 97.0 Å, but the structure factor was such that the odd orders were missing. For his crystals a $\theta - 2\theta$ scan,

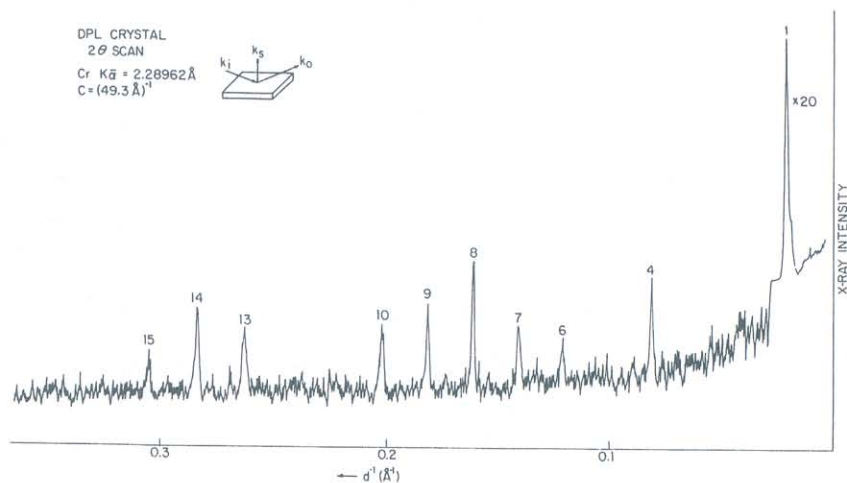


FIGURE 2 $\theta - 2\theta$ scan of DPPC single crystals using $\text{Cr K}\alpha$ radiation and scattering vector normal to the crystal faces.

like that in Figure 2, would result in a series of peaks identical to those of a crystal with spacing of $97.0/2 = 48.5 \text{ \AA}$. The two cases are only distinguished by the full (h, k, l) structure. In the crystals reported here we did not observe any peaks requiring a lattice spacing longer than 49.3 \AA .

Because of difficulties in orienting the crystal edges, powder patterns were measured to obtain the in-plane Bragg peaks. A large number of crystals were partially oriented in X-ray capillaries with their flat faces approximately parallel to one another. A powder pattern was then recorded for this partially-oriented, multicrystalline sample.

Table I lists the various peaks and the crystallographic assignments that are graphically displayed in Figure 3. The monoclinic crystals have in-plane spacings of $a = 8.02 \text{ \AA}$ and $b = 10.54 \text{ \AA}$ with an 85° angle between them. The $c = 49.3 \text{ \AA}$ axis lies normal to the lipid bilayer plane. These lattice parameter assignments result in a unit cell that is approximately 15% smaller than expected for DPPC on the basis of comparisons with measurements by Tardieu *et al.* on a series of phosphatidyl cholines.¹²

TABLE I

Comparison of calculated and observed Bragg spacings for DPPC crystals

| <i>h</i> | <i>k</i> | <i>l</i> | d^{-1} (obs.) | d^{-1} (calc.) | $\Delta(d^{-1})$ |
|----------|-----------|----------|---------------------|------------------|------------------|
| 0 | 0 | 1 | 0.0203 | 0.0203 | |
| 0 | 0 | 4 | 0.0811 | 0.0811 | |
| 0 | 1 | 0 | 0.094 ± 0.005 | 0.095 | 0.001 |
| 0 | 0 | 5 | 0.1014 | 0.1014 | |
| 0 | 0 | 6 | 0.1218 | 0.1217 | -0.0001 |
| 1 | 0 | 2 | 0.133 ± 0.002 | 0.1316 | -0.001 |
| 0 | 0 | 7 | 0.1419 | 0.1420 | 0.0001 |
| 1 | 1 | 0 | 0.151 ± 0.002 | 0.1505 | -0.0005 |
| 0 | 0 | 8 | 0.1622 | 0.1623 | |
| 1 | $\bar{1}$ | 2 | 0.170 ± 0.001 | 0.1687 | -0.001 |
| 1 | 1 | 4 | | 0.1710 | +0.001 |
| 1 | $\bar{1}$ | 3 | 0.176 ± 0.001 | 0.1747 | -0.001 |
| 1 | 0 | 6 | | 0.1746 | -0.001 |
| 0 | 0 | 9 | 0.1825 | 0.1826 | |
| 0 | 2 | 1 | 0.192 ± 0.0005 | 0.1916 | -0.0004 |
| 0 | 2 | 3 | 0.200 ± 0.002 | 0.2000 | |
| 0 | 0 | 10 | 0.2028 | 0.2028 | |
| 1 | 2 | 0 | 0.217 ± 0.0015 | 0.2186 | 0.0011 |
| 1 | 2 | 3 | 0.2262 ± 0.0006 | 0.2269 | 0.0007 |
| 1 | $\bar{2}$ | 0 | 0.237 ± 0.001 | 0.237 | |
| 2 | 0 | 0 | 0.2500 ± 0.0005 | 0.2503 | +0.0003 |
| 2 | 1 | 0 | 0.260 ± 0.001 | 0.2599 | -0.0001 |
| 0 | 0 | 13 | 0.2637 | 0.2637 | |
| 0 | 0 | 14 | 0.2840 | 0.2840 | |
| 0 | 0 | 15 | 0.3042 | 0.3042 | |

d^{-1} is defined from the Bragg formula $d^{-1} = 2 \sin \theta / \lambda$ cell parameters: $a = 8.02 \text{ \AA}$, $b = 10.54 \text{ \AA}$, $c = 49.3 \text{ \AA}$, $\gamma = 85^\circ$

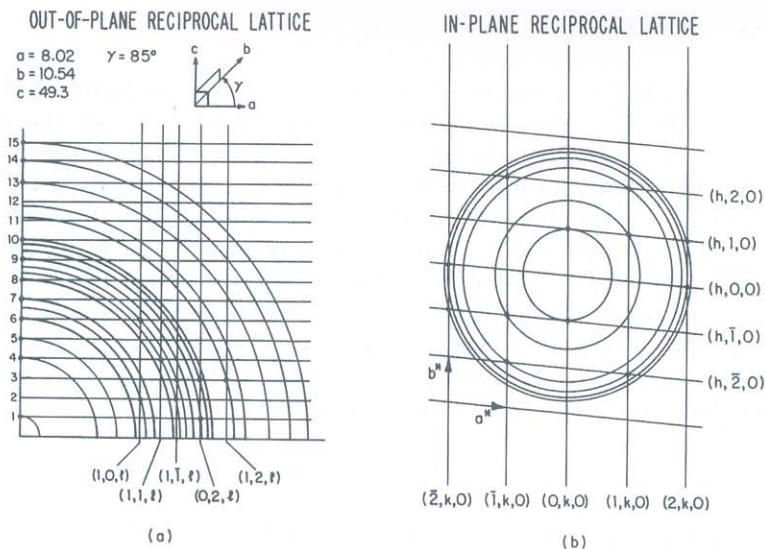


FIGURE 3 Indexing of DPPC crystal Bragg reflections on the reciprocal lattice: (a) in-plane and out of bilayer plane reciprocal lattice; (b) in-plane reciprocal lattice.

This discrepancy partially arises from the strong Bragg peak that we observe at $d = 4.0 \text{ \AA}$ and that Tardieu *et al.* observe from 4.2 to 4.3 \AA . Our lattice parameters result in a cross-sectional area for the hydrocarbon chains of 19.0 \AA^2 if we assume a chain tilt of 25 degrees (or 18.2 \AA^2 if we assume the tilt is 30 degrees). Tardieu *et al.* obtained areas of 21.3 \AA^2 for a tilt of 23° . These cross-sectional areas can be compared with Bunn's measurement of 18.2 \AA^2 for the cross-section of a hydrocarbon chain in single crystals of "normal paraffin".¹⁷ Similarly, Pearson and Pascher¹⁶ measure a cross-sectional area of 19.0 \AA^2 in single crystals of the dihydrate of DMPC. Thus part of the difference between our crystals and those studied by Tardieu *et al.* derives from a closer packing of the hydrocarbon chains in our crystals. It is interesting to note that although the hydrocarbon chain length in DPPC is 2 carbons longer than that in DMPC the unit cell volume that we measure for DPPC is 3% smaller than that determined by Pearson and Pascher for the dihydrate of DMPC. This could arise from a more compact packing of the polar head groups in our crystals. This could be due to a smaller water content. It should be noted that when our crystals are exposed to 70% relative humidity they undergo a phase change to a crystal whose long spacing of 58 \AA corresponds to the spacing reported by Powers and Clark¹ for DPPC samples which they identified as the monohydrate.

III RAMAN RESULTS

Jen *et al.* originally demonstrated the potential for using polarized Raman measurements to determine the orientational statistics of thermotropic liquid crystals.¹⁸ In order to determine the feasibility of similar studies in the lipid systems we examined the polarized Raman spectrum of these DPPC single crystals. Since the spectra only depend on the polarizations of the incident and scattered light, and not on the directions of light propagation, we will use the following compact notation. Let the subscript T refer to the direction normal to the flat face of the crystal, L to the in-plane direction along the long axis of the crystals (i.e., the slowest optical axis in the plane) and W to the in-plane direction along the width of the flat face. Then the spectra shown in Figure 4 correspond to polarizations (α, β) where $\alpha, \beta = T, L, W$.

The relative intensities for different polarization combinations were experimentally normalized in the following way. For each direction of incident radiation and polarization α , and for each direction of scattered radiation, two polarizations β and β' of the scattered radiation were recorded. The computer controlled spectrometer¹⁹ is preceded by a polarization scrambler such that the efficiency of detection of β and β' polarizations are identical. Thus, aside from small corrections associated with the light collection acceptance angle, sample shape and birefringence (less than 10%) the relative intensities of the $\alpha\beta$ and $\alpha\beta'$ spectra are directly measurable. The individual Raman spectra obtained for each of the possible incident and scattered polarizations are shown in Figure 4. The spectra are scaled to accurately display the relative Raman intensities observed for each polarization.

The Raman spectra from DPPC single crystals indicate the hydrocarbon chains are in an all-trans configuration.²⁰⁻²³ We restrict discussion of the Raman anisotropies to the 1131 cm^{-1} line, since we will present a separate, and more lengthy discussion of the general analysis elsewhere. This line has previously been identified as due to a C—C stretching mode of the hydrocarbon chains. Experimental studies of oriented fibres of polyethylene indicate the existence of a similar vibrational mode in which the Raman intensity for the 1131 cm^{-1} vibration is approximately 8 times more intense for incident and scattered polarizations along the hydrocarbon chains than for any other combination.²⁴ Table II displays the observed intensity matrix for 1131 cm^{-1} line in DPPC crystals. The most striking features is the absence of any intensity for the (WW) polarizations. If we assume the polarizability tensor for the 1131 cm^{-1} chain vibration in DPPC corresponds to that assigned to the 1131 cm^{-1} vibration in polyethylene by Gall *et al.*²⁴ then a model in which the hydrocarbon chains in the DPPC crystals are tilted in the L-T plane is

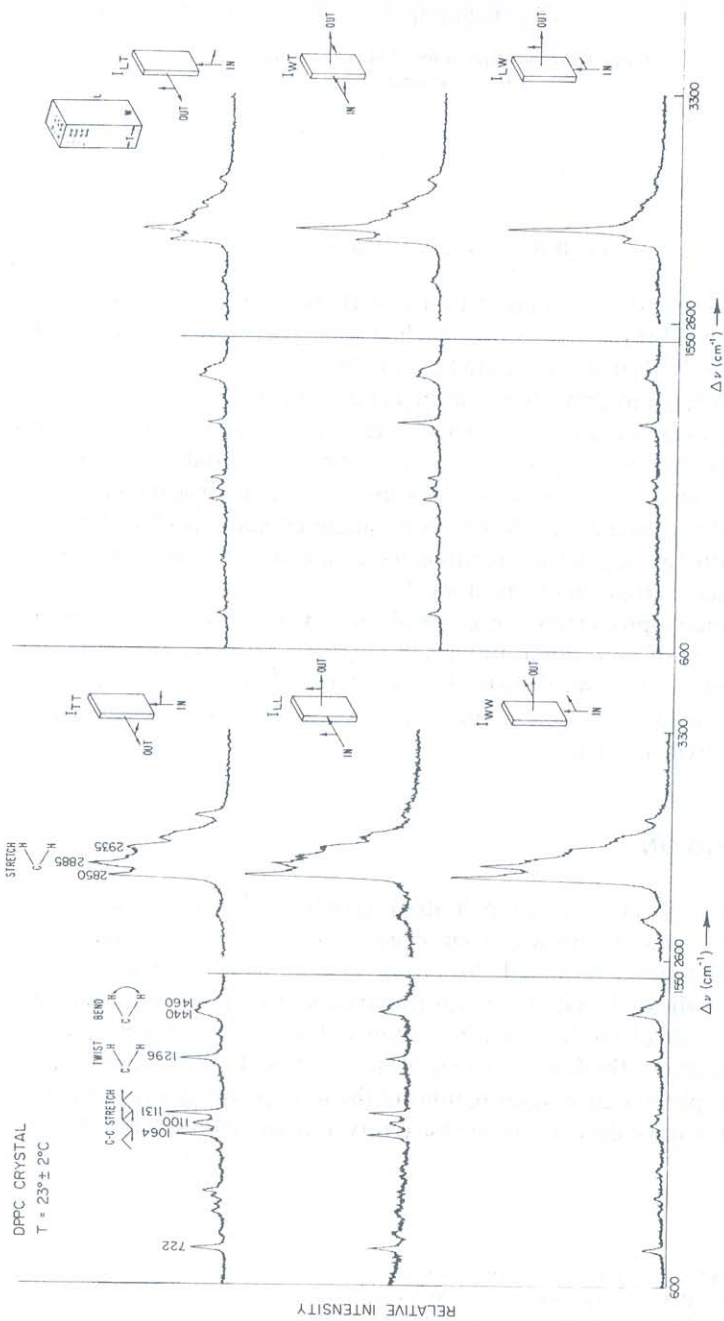


FIGURE 4 Polarized Raman spectra of DPPC crystals, Argon laser power 0.15 W, $\lambda = 5145 \text{ \AA}$. Multiple scans. Average slit width = 3 cm^{-1} . Crystal orientation is shown to the right of each spectrum. See text for labeling.

TABLE II
Raman intensity matrix for 1131 cm^{-1} Peak
of DPPC crystals

| | | | |
|----------|----------|----------------|----------|
| | <i>L</i> | <i>W</i> | <i>T</i> |
| <i>L</i> | 0.6 | 0.15 | 0.3 |
| <i>W</i> | | 0 ^a | 0.2 |
| <i>T</i> | | | 1.0 |

^a The *WW* intensity is less than 0.1.

consistent with the data displayed in Table II. In particular, if the planes containing the all-trans hydrocarbon chains lie perpendicular to the *W* direction of the *WW* intensity is predicted to be zero.

If we use the same arguments as Tardieu *et al.*^{1,2} the long spacing observed by our X-ray measurements (49.3 Å) should correspond to a tilt angle of approximately 30°. This is consistent with the polarizability tensor, the measured Raman intensity, and, if we assume that the hydrocarbons in our DPPC crystal are packed as closely as in single crystal paraffin, it is also consistent with the *a*, *b* lattice parameters assigned previously. The birefringence indicates that the tilt is along *L*.

Since symmetry properties are generally much more sensitive than frequencies as tests of vibrational models, a complete analysis of the Raman data continued in Figure 4 will be the best available proof that the vibrational properties of the hydrocarbon chains of DPPC can be modeled by those of infinite polyethylene chains.

IV CONCLUSION

Large biaxial single crystals of dipalmitoylphosphatidylcholine at low water concentrations can be grown from organic solution. X-ray diffraction measurements have determined the lattice crystal spacings while Raman studies have indicated that the extraordinarily short lipid bilayer spacing results from a tilt of the hydrocarbon chains. The hydrocarbon chains lie in a plane normal to the fast optic axis of the crystals. These crystals can be hydrated to explore higher water regions of the lipid phase diagram. Hydration results in a large decrease in the biaxiality and an increased interbilayer distance.

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